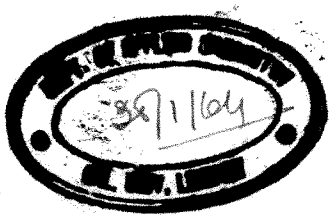
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BRITISH RAILWAYS AND THE PROBLEM OF REDUNDANCY

AN important section in the report of the National Economic Development Council, *Conditions Favourable to Faster Growth**, is that dealing with mobility and redundancy. This is of particular interest in view of the extent to which redundancy is proving a major obstacle to the acceptance of the basic recommendations of the Beeching Report† on *The Reshaping of British Railways*, though it is the comparative scale of redundancy involved rather than its actual necessity that sharpens the issue. It is clear enough from the Council's Report that redundancy is inevitable if the development necessary for the growth of Britain's economy is to be carried out, that measures to facilitate mobility of labour and avoid the hardship of redundancy will be essential. To some extent redundancy can be avoided by forward planning of labour requirements, and the inquiry carried out by the Council suggested that it would be feasible to invite industries to make regular long-term forecasts of their manpower requirements by types of skill to provide a basis for planning apprenticeship, adult training and redeployment programmes.

This essentially is what is done in the Beeching Report, and this section of the Report on the growth of the economy indicates the many factors which affect the geographical mobility of labour, some of which, like housing, lie outside the scope of industry itself. Here local authorities could help considerably. It is suggested that a special 'industrial mobility' housing subsidy scheme might be worked out to provide a stronger financial incentive for local authorities to build houses for transferred workers, and to extend the system of granting certificates of housing needs to 'key workers' to cover suitable unemployed workers willing to transfer to growth areas—subject to the approval of the Ministry of Labour. The non-transferability of pension rights continues to be a serious obstacle to the mobility of higher-grade staff. Although the Government has now made the preservation of pension rights at a minimum level on transfer of employment a condition of contracting out of the Graduated Pension Scheme, the Council suggests that the preservation of pension rights on transfer of employment might well be made a condition of approval of a pension scheme by the Inland Revenue.

The Report also contains some suggestions for dealing with the financial hardship of redundancy. The unemployment benefits given under the National Insurance Scheme are much too low in relation to earnings to offer adequate financial protection against redundancy, and the railways are a striking example of the limits in practice to redundancy schemes on an industry-wide basis. They could not deal satisfactorily with a declining industry without assistance from the Government. The Report suggests that there is much to be said for replacing the existing National Insurance Scheme by a comprehensive wage-related contribution and benefit scheme. However, although the high level of benefit in relation to earnings which would then be practicable would substantially reduce the financial hardship of unemployment, such a fundamental re-organization of the existing system could not be achieved quickly. Public opinion would have to be prepared for the change, and the complex financial and administrative problems of a graduate scheme would require examination in detail. Meanwhile, the methods of improving arrangements for dealing with redundancy are being studied by the Ministry of Labour, by the employers, nationalized industries and Trade Unions on the Minister's National Joint Advisory Council. The debates in Parliament on the Beeching Report showed that the Government have the railways particularly in mind in the new arrangements for industrial training, etc. The Beeching Report, as has already been noted, raised the issue of redundancy in two particular sections, and its foresight and planning are manifestly in line with the recommendations which the Acton Society Trust based on a survey of redundancy some five years ago. It was rather on the Government responsibilities in this field that criticism on the debate centred, and here the replies from the Government side suggested that the Government is fully aware of, and responsive to, the recommendations made by the Acton Society Trust relating to the role of the Government in redundancy generally.

Railway costs are predominantly incurred on a time basis and more than 60 per cent of the total is the cost of providing manpower. There are real opportunities for savings by increasing the effectiveness with which men and equipment are used, and apart from closer control of maintenance practices, and the elimination of older types of wagon and coach, rationalization of the workshops offers savings in standing charges estimated at £4 million by 1966. Expert consultants in mechanical handling have been enlisted to study methods of handling and carrying parcels and freight sundries, and the rationalization of specifications for equipment, materials and stores is also expected to offer considerable savings.

These and other measures for increasing efficiency are expected, with the closure of lines and rationalization of traffic, to lead to some considerable reduction in the staff employed on the railways which, it should be noted, has already been reduced from 648,740 in 1948 to 474,538, the largest annual reduction being 25,896 in 1962. These reductions have hitherto been effected with less widespread disturbance than might be expected because there is a high natural wastage of staff and a total replacement rate of about 88,000 a year. It is estimated in the Report that closure of the passenger services as proposed will render some 16,200 staff redundant and a further 10,900 when the lines are later closed completely, while a further 8,600 supervisory clerical and handling staff may become redundant with in the longer term a 4,900 train-operating and maintenance staff.

The average rate of reduction in implementing the proposals of the Report is not expected to be much higher than that in 1962, and the existence of a clear-cut plan will facilitate the strict control of recruitment, which with the high natural wastage will keep the rate of discharge far below the annual reductions in manpower. The pattern of staff displacement and the geographical distribution will not, however, match the pattern of wastage, and

* National Economic Development Council. *Conditions Favourable to Faster Growth*. Pp. v+54. (London: H.M.S.O., 1963.) 4*s*. net. See also *Nature*, **198**, 1123 (1963).

† British Railways Board. *The Reshaping of British Railways*. Part 1: Report. Pp. ii+148. Part 2: Maps. (London: H.M.S.O., 1963.) 20*s*. net. See also *Nature*, **198**, 1233 (1963).

problems are bound to arise from the necessity for extensive redeployment of people. The Railway Board is keenly aware that the reorganization proposed is bound to cause hardship to some people and inconvenience to many others, but it has already shown that it is prepared to ameliorate these difficulties so far as possible. The established arrangements for redundancy have been revised and men who have to move from their appointed post to another in a lower grade will, in future, retain their old rate of pay for up to five years, unless they can be re-instated in their former grade in the meantime. If they have to move home there are substantial payments to meet costs of removal. For those who are discharged or elect to leave rather than move to other work there will be adequate periods of notice and substantial resettlement payments which have been agreed with the Railway Trade Unions. In addition, efforts will be made to help men to find new employment, using existing machinery for placement and retraining and consultation with other employers.

This issue of redundancy was, of course, prominent in the debate in the House of Commons and, for the Government, Mr. Marples, who endorsed the comment of the 1960 White Paper that if the railways were to regain solvency and provide a fair livelihood for their workers, efficiency of operation and the most economic use of manpower were crucial, and Mr. J. Boyd-Carpenter both agreed that it was a major issue. Furthermore, they indicated that the right policy was to ensure so far as humanly possible that those whose lives were affected by the inevitable changes were taken care of and helped. In the Lords debate it was almost the dominant issue, and Lord Robertson was only one of several speakers who warmly praised the Railway Board for its sense of responsibility and the example it had set to other industries. Lord Robertson referred particularly to the retraining scheme started to facilitate movement within the industry and to the appointment of a senior officer to watch over the whole business. Lord Robertson's outstanding speech was concerned essentially with this issue, and he drew from Lord Hailsham the agreement that the Government had a duty to set an example as employer and from the Earl of Dundee the assurance that the Government would do its best to see that men who had to leave the railways were trained for, and placed in, new jobs as soon as possible and without suffering hardship in the meantime.

It is clear that Mr. Marples was fully justified in saying that the extent of redundancy had been greatly exaggerated, and that if the men concerned accepted another job the number of redundancies would be small. Transfer, however, in the railway system may not be a simple matter from the human point of view as was fully recognized in the Parliamentary debates. The Earl of Dundee on the Government side pointed out specifically that the redundancies were not all in one place and that it was physically not possible to fill every vacancy which is caused by natural wastage by moving in a man who had lost his job in another area. Furthermore, the Earl of Dundee expressed the hope that, as Lord Robertson had urged, if it were necessary for an employee to find a job outside the Railway Board altogether, as much care would be taken in helping him as was taken when a soldier was discharged from the Army. He added that the director of resettlement appointed to ensure that all the resources of the railways and the Ministry of Labour were made available to assist men to find other employment and to maintain contact with other employers and with the Trade Unions was Mr. D. Robertson, the Board's present director of establishment and a past president of the Scottish Trades Union Congress.

No unprejudiced reading of these Parliamentary debates or of the Beeching Report itself can leave any doubt as to the sincerity and extent of public concern with the human aspects of this problem of redundancy in the railways or elsewhere for that matter, nor, equally, that anything is to be gained by postponing the issue on the railways. There must, however, be a readiness on all sides to face change and to prepare for it. The Beeching Report at least sets an example in the way in which it is possible to plan for change and to prepare to deal with it in this field of human relations.

SCIENCE IN THE SIXTEENTH CENTURY

Science and the Renaissance

By W. P. D. Wightman. Vol. 1: An Introduction to the Study of the Emergence of the Sciences in the Sixteenth Century. Pp. xv+327+8 plates. Vol. 2: An Annotated Bibliography of the Sixteenth-Century Books relating to the Sciences in the Library of the University of Aberdeen. Pp. xix+293+8 plates. (Aberdeen University Studies Nos. 143 and 144.) (Edinburgh and London: Oliver and Boyd, Ltd., 1962. Published for the University of Aberdeen.) 42*s*. net each volume.

PROF. WIGHTMAN explains that his book originated in a purely bibliographical survey, the first volume being intended as an introduction to the second, "the kernel of the work", a catalogue of early printed books on scientific subjects in Aberdeen University Library. This second volume, which describes 759 separate titles with full annotations, will be of great interest to those engaged in research on the Renaissance antecedents of the seventeenth century Scientific Revolution, in so far as they can be found in the printed literature of the time. In the nature of things, it could not be an all-inclusive bibliography of all the early books on scientific subjects, and many important works are absent from the list. However, much useful information is provided in the material available at Aberdeen, including many works which are both rare and of considerable historical importance. We are, moreover, promised a monograph on the contribution of the library's chief benefactor in those early days, Duncan Liddel, the founding father of Aberdonian science. Since it might throw some light on the vexed subject of the dissemination of new scientific ideas, a study of some of the major libraries of this early period would be a very positive contribution. It is not enough to know that an idea was put forward in a certain book: we should also like to know how widely that book was read.

The first volume will clearly attract a much wider reading public, and Prof. Wightman expresses the hope that it will be found interesting by "scientists anxious to discover the relations of their special disciplines to the others which began to separate out at this time". No doubt he would not claim to have attacked all the problems which complicate our understanding of how modern science began to emerge into the light during this century. Rather, he seems to have intended a bio-bibliographical introduction to them, in the nature of George Sarton's *Six Wings; Science and the Renaissance* could well replace that famous study. He has indeed something of Sarton's amiably conversational manner, drawn from a wide reading, and warm sympathy for his subjects, but he is more concerned to reflect on the real significance of this literature and the concepts involved in it.

Since his starting point was a university library, it is scarcely surprising that this "Introduction" shows a distinctly university bias, which may perhaps be a little misleading in a discussion on "the emergence of the sciences in the sixteenth century". Practical activities of every sort are but briefly treated, and often cast in the shade by the loquacious disputations of the learned. All the same, this bias has one excellent consequence. Medicine was the only scientific faculty at the universities, and the obvious profession to attract those interested in scientific research. Botany, pharmacology, comparative anatomy, even chemistry only gradually began to take autonomous shape, and were still essentially ancillaries to medicine, maturing slowly under her protective cloak. This feature of the science of the Renaissance, and indeed long after, is seldom given its rightful prominence. A general historical study written around medicine and medical controversies makes an interesting change from the usual preference for the more dramatic revolution in astronomy and mechanics.

Prof. Wightman sees the Renaissance as an age of gestation, in preparation for the great advances made during the next century. There were few great discoveries in science, but rather a change in values and outlook, "a re-orientation of observation, a reconstruction of the framework in which the idea of Nature took shape. . . . In what does this re-orientation and reconstruction consist? In nothing less than a technique for the reversal of emphasis from subjectivity to objectivity". This is to be Prof. Wightman's master-key and he applies to each of the sciences in turn this concept of a "transvaluation" within the traditional framework, in which the inherited techniques and data continued in use. At times, one may suspect that he expected more of a transvaluation at the outset of some enquiry into a given science than he was actually to find there. Thus, in his chapters on the development of scientific method through the discussions on medical method, and on the furious controversy over the 'chemical' medicaments advocated by Paracelsus, he begins by implying that a tremendous but silent revolution was going on. By the end of these chapters, one feels a little disappointed. The transvaluation seems to have been so hedged about with qualifications that the men of 1600 end up only a fraction more advanced than the men of 1500. Perhaps, after all, university libraries are places too conservative for a real transvaluation.

A. G. KELLER

INTERNATIONAL HORTICULTURE

Advances in Horticultural Science and Their Applications

Proceedings of the XVth International Horticultural Congress, Nice, 1958. Edited by Jean-Claude Garnaud. Vol. 2: Pp. xii+527. Vol. 3: Pp. ix+578. (London and New York: Pergamon Press, 1962.) 400*s*. net per set of three volumes.

THE papers in these two volumes are grouped under headings which are likely to make a wide appeal to horticulturists. In Volume 2 are papers on fruit growing and flower growing, in Volume 3 papers on ornamental trees and shrubs, Mediterranean and sub-tropical plants, plant pathology and disease control, modern techniques and productivity in horticulture, greenhouses and horticultural climatology. Closer inspection of many of the individual papers, however, suggests that they will interest specialists in the subjects under discussion rather than workers in other branches of horticulture. This does not mean that most of the papers are highly technical in nature. A number of descriptive papers are included, like "Developing a New Arboretum" by W. S. Stewart, "The Situation in Horticultural Research in Hungary" by J. Ormos, and "The Horticultural Herbarium" by C. E. Kobuski, together with several general reports of work in progress at experimental stations. But this more general type of paper does not appear to fit well into the very brief form (usually about four pages), which presumably was imposed by the requirements of the conference. There may be interesting points to glean from the articles, but the shortened form can be indigestible for those attempting to broaden their horticultural knowledge.

The papers concerned with experimental work could scarcely show a greater variation in methods of treatment and presentation. Some of the authors have described a relatively small field of study and have presented data to substantiate their conclusions, others have given results in summarized form from larger experimental programmes. Either of these treatments can convey valuable and interesting information, and examples of the contrasting styles are seen in the papers by J. P. Nitsch and T. Visser. Dr. Visser describes his experiments on effects of environmental and physiological factors on the rooting of tea cuttings and presents his quantitative data in the form of graphs, histograms and tables. Dr. Nitsch summarizes progress made in elucidating the physiological and biochemical mechanisms involved in the photoperiodic regulation of growth in woody plants, drawing on results obtained in a number of experimental projects. In some papers the omission of the more detailed results is less acceptable. For example, in the paper by P. K. Ursulenko on "Biological and Physical Foundations of Fruit Bearing in Fruit Trees", the interesting observation is made that stages in the development of trees are marked by differences in amino-acid complement, but there is little elaboration of this and no discussion of evidence on which it is based. Such treatment may have been desirable in the paper read to the conference but detracts from the value of the published *Proceedings*.

The international nature of the conference is very evident throughout, and a number of the contributions are of interest in that they describe horticultural developments in a particular area or country. In the section on fruit growing, for example, there are papers describing the programmes of two experimental stations concerned with fruit tree research in France, the development of fruit growing in the Ukraine, methods used in combating soil erosion in orchards in North Africa and variety trials with raspberries and strawberries in Poland.

While many of the papers describe work which has been aimed at finding immediate application in horticultural practice others are directed towards a better understanding of the physiology of crop plants. Thus in the section on flower growing there are three papers concerned with problems arising in the growing of gladioli. They deal with temperature and light treatment of corms to hasten flowering and with the irrigation and mineral nutrition of the plants. At the other extreme is the paper by T. C. N. Singh and S. Ponniah "On the Effect of Sound-percussions Transmitted Through the Earth on the Growth and Reproductive Phases of Certain Ornamental Flowering Plants". Further papers on flower crops are concerned with effects of light, temperature and nutrition in controlling growth and development, techniques and problems in breeding, and pests and diseases.

The section on horticultural climatology in Volume 3 is noteworthy in collecting together information which is of general interest to the horticulturist and which otherwise might be scattered through the literature. There are three papers on effects of wind and the use of wind-breaks and eight papers concerned with low-temperature injury to crop plants, with discussions of the prevention of frost damage by the regulation of cold-air streams, the use of water sprinkling, and of heaters and blowers in orchards. Further papers are concerned with the relation between rates of crop development and temperature, the measurement of soil-moisture conditions and the geographic charting of plant climatic adaptability.

It was suggested in the review[1] of the first volume of these *Proceedings* that they should be assessed as a work of reference if their high price was to be justified. On this basis the absence of an index, combined with the somewhat illogical grouping of the papers, can be criticized in that it makes the tracing of a particular subject difficult. Papers on diseases of apples appear both in the section on fruit growing and the section on plant pathology, while papers on electrical soil warming are included under flower growing and under greenhouses. Perhaps more serious is the fact that information in some of the papers was published elsewhere as long ago as 1958, the year of the Congress. I myself noted three papers in the section on flower growing to which this applies.

In spite of the criticisms levelled at these volumes they will represent a worthwhile addition to the horticultural library. From them can be derived a view of horticultural research and thought in many different countries and they are a useful source of factual information on many horticultural topics. Whether the two volumes will prove to be of great interest to biologists, as is suggested on the flap of the dust-cover, is more debatable.

G. P. Harris

[1] Harris, G. P., *Nature*, **194**, 223 (1962).

BIOSYNTHESIS IN BACTERIA

The Bacteria

A Treatise on Structure and Function. Edited by I. C. Gunsalus and Roger Y. Stanier. Vol. 3: Biosynthesis. Pp. xv+718. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 139*s.* 6*d.*

THE fact that this volume is more than 100 pages longer than either of the two previous ones is itself an indication of the enormous interest in and the great fruitfulness of bacterial systems in the elucidation of biosynthetic processes. The bacterial world has become a Utopia for biochemists and this volume will be as much (if not more) welcomed by biochemists as by bacteriologists. There are twelve chapters dealing with every known aspect of synthesis in both 'autotrophs' and 'heterotrophs'. Chapters 1 and 2 survey our knowledge on carbon dioxide fixation, Chapters 3 and 4 on nitrogen assimilation, in 'autotrophs' and 'heterotrophs' in each case. The remaining chapters deal with the synthesis of vitamins and coenzymes, nucleotides, tetrapyrrole, homosaccharides, peptides, cell walls, proteins and nucleic acids, and finally enzymes.

As in Volumes 1 and 2 there are extensive author- and subject-indexes and a wealth of references. More than 2,500 papers are cited (most of these published in the past decade), and numerous headings and sub-headings make reference easy. As a comprehensive survey of the state of knowledge of bacterial biosynthesis this volume cannot be bettered and every effort was made to give an up-to-the-minute coverage at the time of publication. These extracts underline this attitude: "Nitrogen fixation in cell-free extracts has only recently been obtained; information on the requirements and properties of the enzyme systems is now available. Since the data are of recent origin, have not been presented in general form, and reviews covering these enzymatic data are not yet available, this subject will be treated in some detail" (Chapter 3). Chapter 11 (proteins and nucleic acids) ends with three sections entitled "Discussion (1959)", "Discussion (1961)" and "Addendum (1962)". The second of these sections starts, "The period between finishing the script and correcting the galley proofs has seen the publication of over 300 papers relating to the general field which this chapter is supposed to cover and references to some 200 of these have been darned in". The Addendum commences, "The 9 months that have passed since correction of the galley proofs have seen changes and advances that are of fundamental importance to the understanding and interpretation of much that has been written in this chapter". Such is the *tempo* of industry and discovery in the field covered in these chapters.

This volume is also of some interest to geneticists in that the mechanism of heredity must ultimately have a biochemical explanation and there is much which sheds light on the significance of genetic changes, especially in the final chapter. One of the outstanding general impressions obtained by reading this book is the rapidly increasing number of 'vital' processes which may now be performed in cell-free systems, and one gets the feeling that the possibility that man may eventually manufacture living matter from undoubtedly inanimate starters is not so remote after all. Should that day arrive, this volume will have played a part in bringing it nearer.

It is curious how some simple grammatical errors have been overlooked.

L. B. Quesnel

PATHOLOGY IN MAN AND BEASTS

An Introduction to Comparative Pathology

A Consideration of some Reactions of Human and Animal Tissues to Injurious Agents. By G. A. Gresham and A. R. Jennings. Pp. xi+412. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 84*s.*

MORE than at any previous time, effective methods are being sought, at an international level, to improve the quality of domestic animals and to eliminate diseases to which they are susceptible or for which they are intermediate hosts of human afflictions. This trend has led to closer associations between veterinary pathologists and their counterparts in the field of human disease, thus reviving, in some measure, collaboration of the sort that flourished in the late nineteenth century. The joint authorship of this book is encouraging testimony of such co-operation.

Gresham and Jennings have chosen to deal with their subject primarily on an ætiological basis and have presented it in twelve chapters, all roughly of equal length. The authors state that ". . . the examples we have used to demonstrate specific modes of action (of different pathogenic agents) have been taken from well recognized, common naturally occurring disease processes rather than from the sometimes narrow and artificial field of experimental pathology. Where necessary, however, we have not hesitated to describe . . . some purely experimental conditions if by so doing we could better illustrate the topic . . .". It is on the latter basis, presumably, that there are, happily, included in this volume such important aspects of experimental pathology as the general adaptation syndrome, some experimental oncology, diseases attributable to radiations, immunological tolerance and even aspects of auto-immune diseases and aging. The references to these subjects are not quite up to date; however, the very inclusion of these topics is most gratifying. Similar remarks apply to the inclusion of some electron micrographs at least in one chapter and attempts to direct attention to the nature and significance of modern work on the structure and role of DNA in genetically determined diseases.

On the other hand, it is strange to find so little reference to geographic pathology. In a volume on comparative pathology the peculiar world distribution of many diseases is surely appropriate. This applies, perhaps especially, to many viral and parasitic diseases and to particular neoplasms (for example, childhood lymphomata in Africa; primary hepatic carcinoma) so much better known to-day than previously. Little attention has been directed to the significant role of climatic factors and vegetation in promoting or even localizing the

aforementioned and other diseases to certain parts of the world. References are made almost solely to demographic findings in Britain; to-day, when so much effort is being made to develop international studies and control of disease, a broader approach to disease incidence would be of undoubted value.

The limited attention paid to the causes and mechanisms of congenital anomalies is regrettable in view of the growing awareness of their importance. Likewise, more attention could have been directed to the biochemical basis of many pathological processes, if only to illustrate the progress which may result when resources of more than one scientific discipline are directed towards individual diseases. In this regard, too, an early chapter on modern investigative methods applied to pathology would have been most interesting and helpful, and would have eliminated the need for digressions frequently interjected in the text whenever techniques pertaining to particular topics are explained.

The printing and production of the book are excellent. Line drawings might have been useful to clarify certain topics; closer correlation of clinical and other macro- with micro-photographs would assist the reader. Many of the illustrations would have been made more valuable by more detailed legends; also, at least a few symbols to clarify allusions to items in illustrations would undoubtedly assist the uninitiated.

In this book a welcome attempt has been made to compare, correlate and contrast the causes and range of pathological reactions in man and beasts. The scope of such a project is most ambitious, albeit desirable. But when an attempt is made to encompass so vast a field, experts in certain areas are likely to detect flaws in approach and in details. None the less, there is little doubt that this work has much to offer to students of and workers in human, veterinary and experimental pathology.

T. GILLMAN

FERROMAGNETISM

Ferromagnetismus mit einen Beitrag

Quantentheorie und Elektronentheorie des Ferromagnetismus. Von Dr. rer. nat. Eckart Kneller, von Prof. Dr. rer. nat. Alfred Seeger und Dr. rer. nat. Helmut Kronmüller. Pp. xx+792. (Berlin, Göttingen, Heidelberg: Springer-Verlag, 1962.) Dm. 126.

Microwave Ferrites and Ferrimagnetics

By Dr. Benjamin Lax and Kenneth J. Button. (Lincoln Laboratory Publications.) Pp. xv+752. (New York and London: McGraw-Hill Book Co., Ltd., 1962.) £6 8*s*.

IN 1939 there appeared one of the most remarkable books in the history of scientific literature, Becker and Döring's *Ferromagnetismus*. It had been made possible by active and fruitful research along two distinct and often not very closely related lines. Theoretical work by Heisenberg, Bloch, Slater and Stoner had led to a fairly clear understanding of the circumstances in which ferromagnetism might be expected to occur. Experimental studies on single crystals and their interpretation by Akulov and Becker made it seem likely that all the phenomena of technical magnetization could be accounted for by the strain theory of Becker and Kersten. This book represented the high-water mark of the strain theory and with its aid the authors accounted for technical magnetization in all its varied aspects in one comprehensive flourish of the pen.

Ten years later, and despite the intervention of a major war for five of them, Becker and Döring's book was largely and irrevocably out of date. Néel had exposed serious errors of omission in the original formulation of the strain theory that cast several doubts on its validity. New experimental techniques had appeared; microwave experiments led to the discovery of ferromagnetic resonance, neutron diffraction had raised the status of antiferromagnetism from a speculative suggestion to an established fact, and the development of new materials such as ferrites with their potential application at high frequencies meant that the dynamic characteristics of magnetic materials would need to be understood far more precisely than hitherto. Henceforth, to try and do for ferromagnetism what Becker and Döring had done in 1939 would need not merely scholarship but stamina and a strong nerve.

In his preface to Prof. Kneller's new book Prof. Köster describes the difficulty which faced him in finding an author not merely able but willing to undertake this gigantic task. His labours were not in vain for this new work has qualities not to be found in the existing books on the subject. It is magnificently comprehensive; there can scarcely be a reference, let alone a topic, which is not covered. The early sections include excellent accounts of antiferromagnetism and ferrimagnetism, thermodynamics, anisotropy and magnetostriction, often in considerable detail, and although these are treated from an experimental angle the appropriate mathematical details are given where necessary. All these sections are preparatory to the main account of magnetic domain structure and the theory of magnetization processes in static and alternating fields. Nothing is omitted and the treatment is authoritative.

The courses of technical and intrinsic magnetization have always gone their own ways, largely independent of each other. It is therefore no surprise to find a section described as "Quantum and Electron Theory of Ferromagnetism" included as a supplement and being the combined work of two additional authors. This section—a mere thirty pages long—is really too brief to be anything more than a résumé of existing theories.

There are a few small defects. In the first place the book is already somewhat out of date. This stands out most clearly where the properties of the compound Au_2Mn are discussed without reference to its helical magnetic structure. The absence of a section devoted to the magnetic properties of the rare earth metals, although in line with the author's approach, is nevertheless a serious omission in a book with such an all-embracing title. Theoretical treatments are often given in full but without critical assessment and without guidance as to the range of applicability of the final result. Finally, despite an excellent subject index printed both in German and English the absence of a cumulative name index sometimes makes a topic unnecessarily difficult to locate. These are, on the whole, minor blemishes. No comparable book contains anything like as much information between its covers and this should rapidly become the standard work on the subject.

No slight is intended on Prof. Kneller's excellent book by describing Lax and Button's *Microwave Ferrites and Ferrimagnetics* as a *tour de force*. It is prefaced by a fifty-page history of the development of ferrite materials and their microwave applications. Thereafter the subject is developed *ab initio* with no concession to chronology. The early sections on antiferromagnetism and ferrimagnetism are masterly. Ferromagnetic resonance is treated with a wealth of detail which includes spin-waves and magnetostatic modes, relaxation processes, non-linear effects and instabilities. This is by far the most comprehensive review of ferromagnetic resonance phenomena which has yet appeared. The sections on wave propagation in ferrimagnetic materials, measuring techniques and microwave devices take up rather more than half the book, which, as the back cover is approached, becomes increasingly addressed to the microwave engineer.

It is an awesome fact that most of this book deals with developments which have occurred over the past ten years. In its seven hundred pages there is not a single reference to Becker and Döring yet, somehow, one feels that no injustice has been done. It is the story of Siegfried and the Wanderer all over again.

E. W. LEE

Isopodes Terrestres

Deuxième Partie. Par Prof. Albert Vandel. (Faune de France, Vol. 66.) Pp. 417–932. (Paris: Éditions Paul Lechevalier, 1962.) 110 NF.

THE welcome appearance of this volume completes Prof. Vandel's account of the terrestrial isopods of France and forms a valuable addition to the series *Faune de France*.

The first volume, published in 1960, was divided into two sections, a general introduction and a systematic part; Vandel (Volume 1, p. 96), in an outline of the classification, divided the sub-order Oniscoidea into two series, tylienne and ligienne, and the latter into three tribes Diplocheta, Synocheta and Crinocheta; of these, the series tylienne, containing the single family Tylidae and the tribes Diplocheta (Family Ligiidae) and Synocheta (Super-family Trichoniscoidea), are dealt with in Volume 1.

The second volume is almost entirely devoted to a systematic account of the French species belonging to the tribe Crinocheta, so it is unfortunate that in this volume the status of the tribe has been reduced to that of a sub-tribe without any explanation being given; this fact is bound to give rise to some confusion since Vandel (Volume 1, p. 95) refers to the characters on which Legrand (1946) established the Synocheta and Crinocheta and gives no indication that the two are not of an equal systematic level. Crinocheta is divided into two super-families, Atracheata and Pseudotracheata; the former contains six families of which four (Stenoniscidae, Tendosphaeridae, Squamiferidae and Oniscidae) are represented in the fauna of France and the latter seven, five of which (Cylisticidae, Porcellionidae, Armadillidiidea, Eubelidae and Armadillidae) include French species.

As in the first volume, super-families, families, etc., are defined and keys for the identification of genera and species are included wherever applicable. Each species belonging to the fauna of France is dealt with under the following heads: synonyms, morphology, affinities, reproduction, parasites, ecology, and geographical distribution; in many cases the descriptions of species are accompanied by excellent illustrations and in some cases distributional maps are also included.

The volume contains a glossary of relevant scientific terms which should prove of real value to the non-specialist reader; there is also an extensive bibliography and a systematic index, which refers to the contents of both volumes.

E. M. Sheppard

Applied Dynamic Programming

By Richard E. Bellman and Stuart E. Dreyfus. Pp. xxii + 363. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1962.) 52*s*. 6*d*. net.

DYNAMIC programming is one of the characteristic techniques of modern applied mathematics. Basically it is a synthesis, and extension, of variational calculus and statistical decision theory, and its field of application is the multiplicity of problems covered by the terms 'operations research' and 'automatic control'. This very general technique has been made practicable only by the advent of the electronic computer. The principles have been explained by R. E. Bellman and his collaborators in two previous books and numerous papers, but *Applied Dynamic Programming* is the first systematic exposition of the associated computational methods.

The text is a discussion of a wide range of practical problems together with their solutions; they include the warehousing and inventory problem, the transportation and routing problem, space vehicle guidance and feed-back control. Since the straightforward numerical approach is often beyond the capacity of the largest computer a useful discussion of approximation methods is given. The effectiveness of policy space approximations is brought out, and the exploitation of the special features of particular problems is rightly emphasized. It is disappointing to find that one important approximation method is relegated to a short appendix.

The presentation is refreshingly clear and direct and makes the principles accessible to people who do not enjoy struggling with mathematical refinements. Those already familiar with operations research will find this book a valuable addition to the literature on computational methods, while newcomers will find it a superb introduction to the way that problems are formulated and solved.

J. J. Florentin

Modern Physics

An Introduction to Atomic and Nuclear Physics. By Prof. D. E. Caro, Dr. J. A. McDonell and Dr. B. M. Spicer. Pp. x + 222. (London: Edward Arnold (Publishers), Ltd., 1962.) 20*s*. net.

THIS book describes the principal steps in the development of atomic and nuclear physics from the discovery of X-rays and the electron to the identification of the anti-proton—the topics, that is, which are usually labelled 'modern physics', though one feels that the exclusion from this heading of, for example, recent work on solid-state physics is somewhat arbitrary. The authors are at the University of Melbourne and Monash University and they have aimed their book at first-year students there, but they have also tried to make it useful to students in their last year at school. In a 'blurb' to the English edition the publishers suggest its use in sixth forms, remarking that the book covers all the topics in the modern physics section of the syllabus for the General Certificate of Education Advanced-level Examination, though going farther than the immediate requirements of the examination syllabus.

The authors treat the subject historically in order to show not only what a theory is but also how it was developed. In this they are generally successful. The writing is clear, direct, and unusually free from the clichés of scientific exposition. Some of the headings to sections and chapters have the ring of newspaper headlines (of the more sober sort) but are none the worse for that. An unusual feature in books at this level is the inclusion of references to original papers. Although not detailed or taken to great depth, the discussions are by no means superficial. The book should be valuable to its intended readers and to teachers; it has the additional merit that it is cheap by contemporary standards.

K. E. B. Jay

Structural Geology of North America

By Prof. A. J. Eardley. Pp. xv + 743 (figures). Second edition. (New York: Harper and Row, 1962.) 162*s*.; 21.50 dollars.

WHEN the first edition of Prof. Eardley's text-book appeared in 1951, it was widely acclaimed as one of the most outstanding geological works of recent years, giving the only modern treatment of the structural evolution of North America. In the second, extensively revised, edition seven new chapters have been added, one on Precambrian orogenic belts and six on the igneous provinces of the western Cordillera, thus providing a fuller account of the eruptive rocks than has hitherto been available in text-book form. Fifteen coloured maps depicting the palæogeography of the continent, one for each geological period, have been re-drawn to incorporate recent discoveries. Although the work is specifically addressed to advanced undergraduates in geology, its scope is such that it will be more fully used at the post-graduate level; and, by virtue of its comprehensive character and its readability, it will doubtless long continue to be used as a convenient reference book. Minor misprints are rather frequent and the unorthodox page size (11 in. × 8¼ in.) has disadvantages in a volume weighing 4¾ lb.

INTEGRATION OF CELL REACTIONS

By Dr. A. C. R. DEAN and Sir CYRIL HINSHELWOOD, O.M., F.R.S.

Department of Physical Chemistry, University of Oxford

Introduction

THAT living matter reproduces itself by some kind of copying process has been commonly realized for a long time. That this process depends essentially on conformity of new material with existing spacings in molecular patterns was expressed in the various analogies with crystal growth or template action and in the correspondences pointed out by Astbury[1] between various distances revealed in X-ray analysis. That the sequences of monomer units in proteins, nucleic acids and polysaccharides allow an almost infinite variation in possible patterns has long been obvious. All these ideas have in more recent times been succinctly stated in terms of 'information' and 'coding'[2].

Modern X-ray analysis has focused attention on the helical structure of DNA (deoxyribose nucleic acid), on the great importance of base-pairing in the formation of doubly stranded DNA as a major element in replication, and also on the configuration of protein molecules[3–6]. The ultra-centrifuge has revealed the large range of particle sizes and molecular weights characteristic of the macromolecular cell components, and the use of isotopic tracers has revealed the lability of some of these[7,8]. The correspondence between relatively labile low molecular weight RNA (ribose nucleic acid) and the DNA of an invading phage has led to the conception of 'messenger' RNA, an idea which has been supported by the claim that the low-molecular-weight RNA formed during periods of rapid adjustment of cells to new conditions differs in composition from the normal[8,9].

A further elaboration of the conception of information and coding is represented by the 'repressor', a substance which is supposed to prevent an existing gene from manifesting its effect[10]. The action of the repressor itself is counteracted by the appropriate 'inducer', which allows the repressed gene to express itself in the formation of specific enzymes, previously thought to be called into being by a rather more direct and positive action of the inducer. The essential basis of the repressor idea is that certain substrates or products of an enzyme X may inhibit, not so much this enzyme itself (an action which would be entirely analogous to well-known phenomena of heterogeneous catalysis[11]) as the first member of a whole enzyme sequence by which X is ultimately synthesized[10].

All these various lines of approach represent developments in the quantitative side of biology and as such are highly characteristic of the modern trend. Valuable and indeed spectacular as some of them are, they are in many respects unco-ordinated, and do not yet give a truly quantitative treatment of the cell as a working unit. It seems to us that there is still lacking a proper appreciation of what might perhaps be called the 'total integration of cell function', a principle which we feel is unlikely not to be observed by Nature after countless ages of evolution.

Some Difficulties

The way in which some of the present ideas about cell function seem to us to need enlarging and co-ordinating may first be illustrated by a few examples.

(*a*) Transfer RNA of a given type is supposed to combine specifically with a single amino-acid which it presently guides into the correct position in a peptide chain. Since some twenty amino-acids may be involved, since there are only four possible structures for the terminal base in the RNA, and since the influence of side-chains in organic reactions is seldom transmitted very far from the reactive centre, the specificity can scarcely be explained on purely intramolecular structural grounds. Indeed, the assumption is generally made that the amino-acid–RNA combination is mediated by a specific enzyme[12,13]. If it is, then the specificity of the enzyme protein is playing a no less important part than that of the RNA, and it appears arbitrary to rule that one is more fundamental than the other. Moreover, the general control of all the individual steps involved in the complex polymerization processes to form the macromolecular cell components can scarcely be casually dismissed as operated by 'enzyme reactions' as though the mechanism of each enzymatic stage did not itself present in one form or another the same major problems of specificity, and conformity to an existing pattern. Familiarity with enzyme reactions may breed contempt, or at least a tendency to regard the name as providing an explanation of the mechanism and a failure to realize that activating enzymes are, in the words of Zamecnik[12], "unusual catalysts deserving of more attention than they currently receive".

When RNA and DNA are both built up under the influence of specific enzymes, are we really justified in saying that DNA can affect the cytoplasm and not vice versa? Without the cytoplasmic constituents, the right DNA could not be made at all.

(*b*) The idea of repressor action would, it seems to us, benefit from incorporation into a wider setting. At the moment we have the picture of one gene responsible for the formation of an enzyme which is inhibited by a repressor formed under the influence of a second gene. The effect of the repressor can be neutralized by the action of an inducer which the cell may never have encountered before. Natural selection seems to have allowed a good deal of unnecessary activity to escape elimination for a long time. Inhibition of the actual activity of an enzyme, either by a substrate or by a product of its own working, would be perfectly understandable on the analogy of what is frequently observed with inorganic catalysts. Catalytic action depends on some degree of affinity between the catalyst and the substrate or the product, and this fact means that excess of either may block the surface (for example, SO_3 poisons platinum in the reaction of SO_2 with O_2). 'Repression', however, is assumed to affect not the working of the enzyme itself, but that of quite different enzymes preceding it in a synthetic chain. The normal principles of catalysis suggest no reason for this. The difficulty would no longer exist if the actual functioning of the enzyme were in fact impaired and if this impairment reacted back on the synthesis of its own precursors through a cycle of interdependent reactions in the way which will be dealt with later. Thus here, too, the disregard of the integrated function of the cell leaves unsolved a problem which might in the end prove not to be real. Evolution has in all probability ensured that synthesis and function are seldom completely uncoupled.

(*c*) One may well feel some degree of uneasiness about the failure to qualify statements such as the common one that chloramphenicol specifically inhibits protein synthesis. Protein synthesis is a complex affair with many

stages. If protein is not formed, no enzymatic activity or anything dependent on it can continue for long. All that can be said is that the ratio nucleic acid/protein continues to rise for a short time during the unco-ordinated events following the interruption by this drug of normal cell function.

Interplay of Cell Components

Protein cannot be formed without the mediation of RNA. Neither can RNA nor DNA be synthesized without the necessary specific enzymes. Cells cannot function steadily without periodic division, and cell division is not possible without the production of a cell wall. All these constituents are clearly in mutual dependence.

There may well be a sense in which the DNA may be given the most fundamental role. We may say that an imposed mutation affecting DNA soon transmits its consequences to the other constituents, while changes chemically induced in these will soon disappear provided the DNA code remains intact. Even if this is accepted as beyond all doubt, the working of the cell as a machine cannot possibly be understood except in terms of the interplay and coupling of all its parts. Work on isolated cell reactions or components may yield valuable information; but this can never be properly assembled until the laws of integration of cell function are applied.

Although the contrary might sometimes seem to be tacitly implied, the self-replication of DNA in the absence of other cell components and in particular of enzymes has never been observed. The DNA is, moreover, never continuously replicated except in the intact functioning cell. In cell-free systems its synthesis may proceed to a limited extent, but must then stop.

Since the cell cannot continue to function without periodically dividing, the steady formation of wall material is necessary. The structure of the cell wall, with its complex pattern of polysaccharide, muco- and lipo-protein, is highly specific[14], and indeed probably as specific as that of proteins and nucleic acids. It determines essential permeability relations, and is probably the actual seat of certain enzyme activities. Moreover, the enzymes and nucleic acids of the interior cannot be correctly built up unless the cell wall makes the proper provision for the access of the raw materials. Nor can the wall be maintained and expanded without the appropriate activity of the nuclear and cytoplasmic components. Once again we have the picture of total integration.

Something in a way equivalent to a code resides in the secondary structures constituted by the foldings and spatial distributions of the macromolecular components of the cell. Geometrical factors directly affect the diffusion paths of the many labile intermediate metabolites, and thus may determine the ratio of alternative reactions, and may even affect the probability of incorporation of a given type of unit into a growing macromolecular chain. The equilibrium folding of proteins may, as has been suggested[15], be pre-determined by the amino-acid sequence; but equilibrium does not normally prevail in biological systems. In the last resort the primary DNA code may be able to impose its message on everything else, but it would be rash to reject all consideration of the possibility that an established geometry of diffusion paths, controlling the synthesis of enzymes, polysaccharides and nucleic acids alike, can have no influence at all on its own perpetuation, or in any event on the speed of its own modification. Once again it would seem wiser to remember the principle of total integration.

Some Sources of Misconception

The true perspective of cell phenomena is sometimes disturbed by the way in which experiments are planned.

In crossing and recombination tests a given character is often recorded as positive or negative on the result of an observation made at a single fixed time such as 24 or 48 h. The consequence is to impose an artificial discontinuity which the phenomena do not inherently possess. The appearance of sharp distinctions instead of gradations tends to focus attention on structural differences rather than on continuously varying processes and so gives a false picture of the true relationships. The distinction between constitutive and inducible enzymes[16] is, for example, probably much too sharply drawn. The less information there is, the more clearly defined a classification may appear. Suppose, for example, we have three related enzyme activities all quantitatively and continuously variable. Crude criteria of assessment can easily lead to a matrix of possibilities ranging from + + + to − − − with all other combinations of + and − between. This can be interpreted in terms of single and separate genes. Since the activities are related, they may not separate easily, and a further conclusion may be drawn about the contiguity of the genes on a chromosome. The arbitrary nature of this could easily escape attention.

Complaints have been voiced that genetic data not conforming to clear-cut or preconceived patterns are often discarded[17,18]. Indeed, it must be said that the propounders of genetic theories themselves subject one another to a good deal of criticism, which, taken as a whole, suggests the need for a wider view of the entire field.

Lederberg[19] has pointed out that streptomycin gives exceptionally clear-cut results in the distinction between sensitive and resistant types and has directed attention to the fact that in this respect it is 'unusually suitable'. But the distinction he makes is seldom borne in mind by those who quote and base wide generalizations on the results obtained with this drug (which in fact there are good reasons for regarding as quite untypical[20]).

Another experimental need to which insufficient attention may be paid is that of ensuring a proper steady-state relation between the organisms under test and their environment. A strain of bacteria is sometimes maintained on a nutrient agar slope or in broth for long periods, and then in a matter of hours or minutes after transfer to a defined artificial medium it may be tested for growth rate or enzyme activity, or it may be crossed with another strain as far removed as itself from equilibrium with the environment. Such procedures may give very misleading results unless it is borne in mind that at a first transfer to a new medium the growth rate is low, the enzyme activities are abnormal, and the nucleic acid and protein contents out of balance.

Even when thoroughly acclimatized cultures of known history are used, variations in nucleic acids and proteins occur during the growth cycle[21,22] (a completely steady state being reached only in continuous culture[22–27]) so that snap tests taken at a fixed time may be completely unrepresentative.

Even if minutely standardized procedures are laid down, it may still be true that the results are an unspecified function of the experimental conditions and will be difficult to assess unless this function is itself investigated.

The present-day picture tends to be fragmented, static, and in some respects artificially simplified. The cell is often spoken of as though it were an unco-ordinated collection of mechanisms. 'Regulator genes' are spoken of as being switched on and off; but what are regulator genes and repressors if not part of the expression of the total quantitative cell balance ?

The structural code, at whatever level it may be inscribed in the cell, controls the whole set of potentialities. The expression and balance of these are governed by certain general principles which cannot be ignored without resulting confusion.

A number of communications on the kinetics of cell and tissue growth have been published at different times[28–35]; but it may be said on the whole that their influence needs re-asserting.

Integration of Cell Processes

The 'principle of total integration' is capable of mathematical expression. If the rate of formation of a nucleic acid, A_1, depends on an enzyme reaction, we should have in the simplest case $dA_1/dt = k_1P_1$, where P_1 is the concentration of the appropriate enzyme. Conversely if the protein formation is guided by a nucleic acid template, we should have another equation such as $dP_1/dt = k_1^1A_2$. The general principle is unaffected by the consideration that the individual equations are not necessarily of the simple linear form given in the example. Having regard to the active part that the cell wall plays in permeability phenomena as well as being the seat of certain enzyme activities, the polysaccharide, mucopeptide and lipoprotein constituents can all be brought into the scheme.

Now since the cell reproduces itself as a whole, and no part can continuously function without the others, it is reasonable to express the integration principle by closing the set of equations according to a scheme of which the following is the simplest example:

$$dX_1/dt = k_1X_2,\ dX_2/dt = k_2X_3,\ dX_3/dt = k_3X_4 \ldots \ldots\ dX_n/dt = k_nX_1$$

Great elaboration of such schemes, with complicated branchings, is, of course, possible, without change in the basic principle[29].

A second principle, without which no satisfactory theory of cell function is possible at all, is that of the preservation of physico-chemical balance. Inflow of nutrients and outflow of waste products in general can only maintain a proper balance within certain limits of cell size. Hence the necessity for division. Control of division must rest on a third principle, namely, that of some degree of coupling between the act of division and the process of growth. Many factors may be involved in this[36-40], but one simple overall formulation of the condition would be that division occurred when the amount of some key component in the cell reached a critical value[28-30,37]. More elaborate specifications could be laid down without changing the essential need for some such coupling condition. These could bring in reducing potential, size of nuclear particles and so on. The main point is, however, that without some attempt at a formulation no theory can have contact with reality.

Systems of equations based on the foregoing principles have already been examined. They are, it should be emphasized, in no way in conflict with the idea of genetic codes or any other legitimate conceptions of genetics. They lead to some general conclusions which the theory of cell function cannot properly ignore.

The cell emerges as a system which automatically adapts itself to give the optimum growth rate in a given medium. In so doing it changes the proportions of its components, and when it is transferred from one medium to a fresh one it may abandon one pattern of chemical reactions for another. In the process it brings itself into an optimum relation with its environment[29,30].

It is legitimate enough for particular purposes to talk of genes, operons, cistrons, repressors, messenger and transfer RNA, ribosomes, and so on, as parts of a fragmented and often virtually static system; but it is only when they are assembled into a machine that they can possibly make complete sense. When the assembly is ignored, the need will often arise to explain each new fact by a fresh *ad hoc* hypothesis.

It is the actual coupling of the assembly which is responsible for the very important cell property of adaptability to optimum growth, and for the existence of whole continuous ranges of properties rather than discrete differences only. In drug resistance phenomena, for example, although the resistant forms may on occasion show a single degree of response whatever the concentration of drug to which they have been exposed (selection of pre-existent types)[20], they more often display a continuous spectrum of degrees of resistance[30].

We shall not re-capitulate here all the experimental evidence for direct adaptation[30,41-48] nor the objections to the fluctuation test[49] which have been claimed to disprove it. The isolated demonstration (by replica plating) of pre-existent forms resistant to bacteriophage and to relatively low concentrations of streptomycin has, as mentioned here, led to the quite illegitimate assumption that the result is general.

Drabble and Hinshelwood[20] showed that adaptation and mutation occur together in the case of streptomycin resistance, and Kilkenny and Hinshelwood[50] showed that even yeast cells which segregated in a Mendelian manner still underwent direct adaptation (to galactose) after separation.

The examination of the growth equations shows, furthermore, that in a given environment the establishment of a true steady state may be fast or slow[25]. Correspondingly, adaptive changes may be rapidly or slowly reversible. This result corresponds with experimental observations that adaptations are sometimes easily reversible and sometimes persistently held[30], the notion of stable heredity with unicellular organisms being one of degree only. The argument based on stability that adaptive changes are purely selective cannot be consistently upheld. Cells with greatly changed characteristics, relating, for example, to fermentative and respiratory properties and to drug resistance, can be obtained under conditions which preclude any important degree of selection of special types of individual[47,48,51-56]. The importance of enzymatic differences in a directed antigenic transformation is stressed by Austin, Widmayer and Walker[57] and the interplay of microbe and environment in clinical practice is discussed by McDermott[58].

Those who, rightly, lay great stress on the principle of natural selection sometimes fail nevertheless to understand that the selection of an appropriate reaction pattern by self-replicating matter is just as effective and, therefore, may be just as important as the selection of special individuals. A preference could only be justified by some kind of exaltation of structure over function, a distinction which Nature is unlikely to observe.

Unbalanced Growth

A good deal of importance is attached in the literature to observations about the differential synthesis of cell components, for example, uneven replication of DNA at different phases of individual cell cycles. The observations on this seem to have been made entirely with cells not in dynamic equilibrium with their environment. The cells have, for example, been deprived of special growth requirements such as thymine[59], very recently transferred to a new medium[59], or subjected to temperature shock[60]. In these circumstances, the principles we have outlined here would predict very severe disturbances of the co-ordination between the synthesis of different components. To take the most highly simplified example possible, suppose :

$$dX/dt = \alpha Y$$
$$dY/dt = \beta X$$

In dynamic equilibrium X and Y will settle down to average values of X_0 and Y_0 per cell. Suppose we transfer to a medium where the X component (say an enzyme) functions poorly, then the rate of formation of Y is reduced very greatly (possibly to zero), while the formation of X for some time can continue at the original rate. It would be wrong to infer from this that the replication of X necessarily and naturally is a temporal antecedent of that of Y. When continuous growth is allowed again, the proportions will eventually settle down to a balanced ratio. How contingent the whole phenomenon must be is shown by the fact that disturbances in relative synthesis rates characterizing growth of 'synchronous'

cultures has proved to be a function of the way in which the synchronization is effected[27,61,62].

Significance has been attached to the observation that in some synchronized cells mass may increase linearly instead of exponentially with time. Quite apart from the question whether the distinction can be accurately established for the small changes in mass involved, any cell thrown right out of balance can show anomalous rates of increase for any components. With a more carefully planned technique of synchronization (depending only on the selection by filtration of appropriately sized cells and not subjection to chemical or temperature shock) synchronized cells can be obtained in which RNA, DNA and total protein all increase exponentially and maintain a constant ratio over the whole cell cycle[27,61].

Experiments in which the formation of protein or nucleic acid, or the course of specific enzyme activities, is followed for short periods after disturbances such as the addition of drugs are extremely difficult to interpret in the absence of much more complete studies. If a motor-car factory were partially destroyed by a bomb, finished cars might continue to be delivered for a short time, or alternatively individual components might pile up. But conclusions about the sequence of the manufacturing process could be drawn only by the careful co-ordination of various kinds of information.

Conclusion

Although some of the ideas about cell integration put forward in this article have received a good deal of opposition they are supported by much that can be found in the literature.

A very positive attitude is adopted not only by Graham Cannon[63] but also by Uvarov[64], who says: "In spite of a number of cases when environmentally induced variations have been shown to be, at least temporarily, inheritable, there is a reluctance to accept any evidence that might appear to favour the much maligned Lamarckian principle" . . . (and) . . . "this should encourage more entomologists to study their problems without being afraid of infringing some outdated canons of text-book genetics". Ephrussi[65] has remarked: "The second thing we should avoid is basing the distinction of mechanisms on nuclear or cytoplasmic localization. In my opinion this has been a major source of confusion in the past, and it is not going to be so easy to avoid it in the future because we have all been trained to regard the problem of differentiation as a nucleus/cytoplasm dilemma". Danielli[66], commenting on this, refers to: "evidence of either nuclear or cytoplasmic control, or both, in relation to structure and function". Our belief is that the relation needs to be made more explicit.

A common attitude is to admit the possibility of direct adaptive changes in principle but to ignore, dismiss or minimize the significance of the evidence that they occur[67–70]. When this is done the picture of cell function is left very incomplete.

Some authors speak occasionally of specifically induced mutations[71]. They accept the directing influence of the environment on what must, according to the accepted view, be the DNA code. The change in the DNA must involve enzyme action at some stage, so that the distinction between this kind of mutation and an adaptation becomes very indefinite. In this connexion we may refer to the gradual stabilization of induced enzyme adaptation which is observed with bacterial cultures[72,73] and also point out again the arbitrary nature of the distinction between inducible and constitutive enzymes.

Biologists concerned with the differentiation of tissues in complex organisms seem to take a wider view of the potentialities of the cell. Weiss[74], for example, says that: "genic replication, while seemingly the initiating step in the growth process, is followed by chains of events the nature of which is determined by the specific constitution of the (extragenic) rest of the cell". He also says[75]: "In short, the story of the molecular control of cellular activities is bound to remain fragmentary and incomplete unless it is matched by knowledge of what makes a cell the unit that it is, namely, the cellular control of molecular activities". Sonneborn[76] refers to: "successive cycles of differentiation of cytoplasm by nucleus and then of nucleus by cytoplasm". Chantrenne[77] says: "For if the cytoplasm in bacterial systems appears ancillary in regard of the all-mighty genome, embryologists view the cells and embryos as a much more involved system in which the cytoplasm exerts a very strong control on the genome". These authors are concerned with systems much more complex than those in which the kinetic implications can be easily treated but their basic ideas certainly seem to agree with the concept of cell integration.

Perhaps, if all that has been said in this article is conceded, its relevance to the discussion of other modern work may still be questioned. Our contention is that intensive work on individual fragments of a complex machine is not only incomplete but also may be misleading unless attention is paid to the way in which the parts fit together. Coding, information, messengers and carriers are all perfectly valid conceptions: so also are various kinds of enzyme-mediated condensation reaction. But these terms have in themselves little explanatory value, and present-day ideas will only achieve maximum fruitfulness when so combined that they are not left simply to describe bits of a machine without anything to make it work. Sometimes the claim is made that machines can only be understood in terms of their parts. The counterclaim can also be advanced that to understand how the parts fit together the machine must be observed in action. Relatively simple phenomena do reveal the machine in action. Apart from adaptive responses, to which reference has already been made, we have the variation of enzyme contents of cells according to need, enzyme changes compensating for the effects of adverse *p*H, the rise and fall of stores of cell components, and the whole rhythmic variation of cell composition during the growth cycle. One last example may be cited. When cells are multiplying two tendencies may be observed. On the one hand, the DNA content per unit of mass tends to be constant under optimum conditions. On the other hand, the cell tends to divide so as to maintain a constant amount of DNA per cell[21]. When nutrients become deficient a compromise has to be struck: there is an actual increase in the DNA per unit of mass; but a more economical sharing out so that the cells become much smaller and the amount of DNA per cell drops[22]. Facts such as these can only find an explanation in terms of total integration.

It must be emphasized again in conclusion that the ideas we have expressed are in no way in conflict with the conception of the genetic code, nor do they ignore the principle of mutation and selection. Cells in the course of evolution must have profitably exploited the variability of reaction patterns and the consequences of complete integration. It is those who affirm the contrary who fail to give the natural selection principle its due.

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RADIO AND INTERNATIONAL GEOPHYSICAL RESEARCH*

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APART from its direct application to communications and broadcasting including television, and as a vital aid to air and maritime navigation, radio is essential to the practice of telemetry in being the means whereby physical measurements made at a point remote from the observer are recorded and communicated to him. According to the programme of the National Telemetering Conference, we are reviewing the applications of telemetry in such fields as bio-medicine, space electronics and oceanography. In so far as they are concerned with phenomena in the Earth and its surroundings, these subjects fall within the definition of geophysics; and I propose to confine myself to certain aspects of international geophysical research, with which it has been my privilege to be associated for some forty years.

Considering the Earth's atmosphere from the ground upwards, we may conveniently divide it into three regions: (1) the troposphere, or lower atmosphere in which electrical conductivity plays an infrequent and relatively minor part; (2) the ionosphere, that important region of high, but variable, conductivity without which world-wide radio communication would hitherto have been severely limited; (3) the upper, and again poorly conducting region of our atmosphere, which has been one of the subjects of investigation in what we may term the 'space age' using man-made or so-called 'artificial' earth satellites.

* Substance of an address delivered at the U.S. National Telemetering Conference, Albuquerque, New Mexico, on May 21.

International Co-operation

The use of radio exploring and measuring techniques for investigating the Earth and the Universe around it must inevitably be developed on an international basis; and most of what I shall refer to relates to the activities of various scientific organizations whose co-operation is essential to the successful realization of research in these fields.

I am honoured to be the president for the present term of the International Scientific Radio Union (URSI), which has collaborated with its sister bodies, the International Astronomical Union (IAU), the International Geodetic and Geophysical Union (UGGI), the International Union of Pure and Applied Physics (IUPAP), and more recently the International Committee on Space Research (COSPAR), in the exploration by the aid of radio waves of the physics of our Earth, its atmosphere and all the space beyond. I may direct attention to the fact that the URSI will be celebrating the fiftieth anniversary of its formation at the General Assembly to be held in Tokyo

next September. Nevertheless, and in spite of this seeming age, URSI still maintains a position in the forefront of international research, in which radio is so essential for the acquisition and communication of scientific information.

Exploration of the Troposphere

In the British Standard's *Glossary of Terms used in Telecommunication and Electronics* (No. 204, 1960)*, 'radio telemetry' (or radio telemetering) is defined as: "Automatic communication by radio intended to indicate or record a measurable quantity at a distance".

Thus telemetry is essentially the science and practice of making measurements of physical quantities at a point remote from the observer, and then communicating the results by radio to this observer. In accordance with this definition, one of the earliest applications of telemetry was in meteorology, as a means of observing directly conditions in the Earth's atmosphere. A small package, containing a barometer, thermometer and hygrometer together with a radio transmitter and power supply, is carried aloft by a hydrogen-filled balloon. At pre-determined intervals, which can be set by a clock or a wind-vane mechanism, the indications of the three meteorological instruments are used to modulate, at different audio-frequencies, the higher frequency of the radio transmitter. Thus as the balloon ascends, and drifts with the wind, the signals received at the ground are used to record automatically the pressure, temperature and humidity of the air through which the instruments are being carried. In the more advanced techniques developed in the United States, the information from the temperature and humidity elements is combined in such a way that the refractive index of the air is calculated and telemetered to the ground station. Furthermore, by attaching a suitable reflecting 'target' to the balloon, a radar station on the ground is used to plot the horizontal projection of the balloon's position as it is carried along with the wind. From a recording of the pressure, corrected for the temperature as may be necessary, the height of the instruments is deduced; so that a complete knowledge of the vertical structure of the atmosphere is obtained at the ground station, including the two components, direction and speed, of the wind velocity.

The technique of sounding the troposphere, or lower atmosphere up to heights of some 30 km (100,000 ft.), is now a routine operation in many countries; and it provides a major contribution to the meteorological services of the world. Since much of the weather in the United Kingdom and Western Europe comes from a westerly direction, Europeans are very conscious and grateful for the advance information they obtain from ground stations on the American continent, and also from the mutually operated weather-ships in the Atlantic, which make full use of the radio-sounding technique.

Radio transmission and measurement techniques have, however, provided much greater facilities to the meteorologist than that of merely recording the basic parameters of an atmosphere. For example, the physical mechanism by which raindrops form and fall to the ground has been the subject of discussion among meteorologists for a very long time; but in the past decade or so, the centimetre wave radar installation has provided a powerful new tool for the meteorologist. Examination of the echoes scattered back to the observer from raindrops, or from snow and ice particles, has thrown considerable new light on the fundamental physical processes involved, as well as providing advance information on the onset of storms. Associated with this has been the application of radio reception to the investigation and movement of sources of atmospherics, which themselves provide supplementary advance information on weather conditions. Furthermore, during a thunderstorm the actual lightning stroke occurs down a column of ionized air, which as an electrical conductor can give rise to strong radar echoes, even when the actual precipitation of rain is too weak to produce a detectable response. In this way, lightning occurring either from cloud to ground or from cloud to cloud can be detected and followed out to ranges up to 100 km (70 miles) or more; and this again forms a valuable aid to the meteorologist.

I would like now to refer to the subject of 'angels', those mysterious meteorological echoes which have puzzled the investigator for about a quarter of a century. It is at times thought that these echoes are reflexions from areas of sharp change in gradient of the meteorological conditions in the atmosphere, such as the upper or lower boundary of a cumulus cloud or the region of a temperature inversion layer. But it seems to be the experience of many investigators that the gradients required by theory exceed measured or reasonable values by one or two orders of magnitude. However, some of this discrepancy may in the past have been due to the limitations of the measuring technique.

The direct measurement of the refractive index of the air with meteorological type instruments has already been mentioned. In the past decade or so, the technique of micro-wave refractometry has been developed in several countries. Here the natural frequency of a cavity resonator exposed to the atmosphere is compared with that of a sealed and, if necessary, evacuated resonator; and the refractive index determined from the difference in frequencies. The micro-wave refractometer, as the resonator combination is termed, is sufficiently small and light to be carried in a suitable position outside a small aircraft. Various types of this instrument have been developed; and one of these is in the form of an expendable radio refractometer, which can be dropped from a suitable altitude. A self-contained oscillator radiates at the natural frequency of the cavity; and the variations of frequency are recorded at the ground as a direct and continuous measure of the refractive index of the sample of air in the cavity. In this way, a quick and direct record is obtained of the vertical gradient of the refractive index of the air through which the resonator descended.

While much of this research work has been conducted with the object of assisting the meteorologist to gain a better knowledge of the physical characteristics of the atmosphere, it has concurrently been most useful to the radio scientist endeavouring to understand and turn to practical account the transmission of very short waves through this, un-ionized, portion of our atmosphere.

Exploration of the Ionosphere

In the presence of world-wide radio communications on the scale practised to-day it is perhaps not easy for the present generation to realize that it is not much more than forty years ago that the existence of the ionosphere was subject to experimental verification in both Great Britain and the United States. Two methods were used in these early experiments to demonstrate the existence of a reflecting layer of ionized air. The first of these used a frequency sweep technique at the transmitter, and the recording of interference fringes at the receiver where both the ground and sky waves were received. This method has since found another useful application in aircraft altimeters.

The second method consisted in sending out small pulses of radio waves and measuring the time taken for such pulses to travel up to the reflecting region and back again. Knowing the speed with which the waves travel, the distance of the reflecting region could be calculated from the observed time-interval. This method provided the basis for the development of radar about a quarter of a century ago. But even before such applied development started, the pulse-echo technique had found widespread use in the scientific exploration of the ionosphere in various parts of the world.

* British Standard 204, 1960, published by the British Standards Institution, London.

Systematic measurements made by this method, soon indicated that the hypothesis of a single ionized layer was insufficient to explain all the observed facts. The original experiments were made with medium-frequency waves: by using higher frequencies it was found that the waves could penetrate the lower, or E, layer and were reflected from an upper ionized, F, region, which itself was found to be complex, and on occasions it was divided into two parts (F_1 and F_2). Later research has shown that at certain times the short (or high-frequency) waves are reflected from intense clouds of ionization in the first or E layer; and also that very long waves (of low frequency) are reflected from a lower, D, region of smaller conductivity. In this way, we have come to realize that the ionosphere is that portion of the atmosphere situated 50–250 miles (80–400 km) above the Earth, with some highly ionized and so electrically conducting layers at heights of the order of 50 miles (80 km) for the D region, 75 miles (120 km) for the E, 150 miles (250 km) for F_1 and 250 miles (400 km) for the F_2 regions. The actual values of these heights vary markedly, as also do their electrical or reflecting properties, from day to night, and for different parts of the Earth.

World-wide radio communications depend directly on the successive reflexion of radio waves between the Earth and the ionosphere, in order that the circuits may overcome the curvature of the Earth. The design and operation of these circuits therefore involve a knowledge of the conditions in the ionosphere in various positions all around the world: and this, in turn, involves a measure of international co-operation in investigating the characteristics of the ionosphere over different countries. A considerable impetus to this type of international research was given thirty years ago, during the International Polar Year of 1932–33, when a successful exploration of conditions in the ionosphere at high latitude was carried out. The results of this work disclosed the close relationship between magnetic storms and abnormal conditions in the ionosphere leading to difficulties in radio communications, and on occasions to radio black-outs.

In the following quarter of a century, many countries established ionospheric observatories for the regular recording of the characteristics—the height of and the density of ionization of the reflecting regions—in different parts of the world. Under the auspices of the International Scientific Radio Union (URSI), the results of such observations were co-ordinated and used in the preparation of world-wide charts of predicted ionospheric conditions, which were applied by national administrations in the planning of their communication services. By systematically comparing the actual performance of these services with the forecast conditions, the knowledge of the conditions in the upper atmosphere was steadily increased, and the accuracy of the predictions correspondingly improved. It soon became apparent, as was to be expected, that, since the electrification of the ionosphere was dependent on ultra-violet radiation from the Sun, the characteristics of the ionosphere varied with solar activity in accordance with the 11-year cycle already established from astronomical observations. Now, the International Polar Year of 1932–33 was associated with a relatively quiet period of solar activity; and having in mind the rapid rate at which scientific research in radio was advancing, another international co-operative effort was conducted in 1957–58, at which time solar activity would be a maximum. In the event, this turned out to be the most intense period of such solar activity ever recorded since optical observations were first made some 200 years ago. Because of the greater scope of the physical measurements involved, and because the area of investigation was over the world as a whole and not confined to the polar regions, this period was designated the International Geophysical Year, which actually extended from July 1, 1957, to December 31, 1958.

The International Geophysical Year 1957–58

During the foregoing period, scientists of some 66 nations worked at more than 4,000 observatories throughout the world on a co-operative programme concerned with the physical properties of the Earth and its atmosphere, and with the related solar and terrestrial phenomena. In order to ensure the approximate simultaneity of the measurements, a calendar of observation days was drawn up in advance and, in this, provision was made for the declaration of Special World Intervals (SWI) when the state of the Sun indicated the likelihood of a geophysical disturbance which might affect, for example, the ionosphere and the Earth's magnetic field. For this purpose an international network of radio and line communications was used to alert all participating observers and warn them that a SWI might, subject to confirmation, begin 16 h later. During the International Geophysical Year, 44 such alerts were distributed; and of these, 22 culminated in a Special World Interval observing period, during 17 of which, major solar and terrestrial disturbances occurred. In addition, there were 6 severe and 33 mild disturbances for which no previous warning or alert had been issued.

Provision was also made in advance for the setting up of a number of World Data Centres. Two of these, in the United States and the U.S.S.R., collected the observational data relating to all types of measurements at the large number of observatories involved. Two others, in Britain and Japan, confined their collections to radio and associated measurements on the characteristics of the ionosphere, on atmospheric disturbances and on aurora.

The primary aim of the meteorological programme for the International Geophysical Year was to obtain a more nearly complete global picture of the mechanism of the general circulation in the atmosphere. To this end special stations were established in the polar regions, equatorial areas and on oceanic islands to supplement observations made daily throughout the extensive network of permanent meteorological observatories. Measurements made with radio sounding balloons were extended to greater heights up to some 50 miles (30 km) where practicable, thus extending their scope into the lower regions of the ionosphere. More frequent soundings were made on the Special World Days; and considerably increased observational facilities were provided on the Antarctic continent. As a result, the meteorologist has been able, for the first time, to construct charts showing broad circulation patterns at various atmospheric levels above the Antarctic.

Meteorologists also collaborated with radio scientists in investigating the nature and motions of thunderstorms, which make themselves evident by the emission of radio waves from lightning flashes. Among the main objectives of the International Geophysical Year noise programme were measurements of the average power received at radio frequencies from lightning flashes, and a detailed investigation of the waveform of the radiation over a wide range of frequencies. As a result of co-operative work of this type, charts are now available from which an estimate can be made of the level of interference to communications due to atmospheric noise likely to be experienced in any part of the world at any time or season and on any radio frequency. Such information is invaluable to both national and international authorities planning global communications networks, including those used for broadcasting and navigational aids.

One of the most useful results of the extensive and world-wide investigation of the ionosphere during and since the International Geophysical Year is that charts have become available whereby the communicator, as the user of radio communications is sometimes termed, can plan, many months and even years ahead, the frequencies most favourable for his use over various circuits at all times and seasons and in all parts of the world. Such

charts normally indicate the maximum frequencies usable on communication circuits; and they have been based on the assumption that the electron density in the upper, or F_2, region of the ionosphere increases with height according to a parabolic law. The calculation of these ionization densities by manual methods is an arduous operation; but, during the International Geophysical Year, first in the United Kingdom and later in other countries, digital computers have been programmed to calculate these distributions of electron density on a routine basis. As a result, it has been found that these distributions are often far from parabolic in form: and the practical conclusions reached are that, while the maximum usable frequencies obtained from the charts are not seriously affected by the shape of the layer, it would appear to be desirable to use aerial systems which project the radiation at a greater angle of elevation than has hitherto been considered the most appropriate.

The normal methods of sounding the ionosphere take little or no account of the fact that the reflecting layers are not at rest, but appear to be moving with horizontal velocities which vary with the height of reflexion. Special techniques have, however, been developed to measure the horizontal movements, or drifts; and some interesting results were obtained during the International Geophysical Year both in near-tropical regions and in the Antarctic. For example, at Ibadan, in Nigeria, it was found that the drifts of ionization were subject to a diurnal variation, the prevailing directions being to the west by day and to the east by night at all seasons. But these drifts are subject to a long-term change over the solar cycle. Now at Halley Bay, in the Antarctic, new and most interesting results were obtained. Among these was the somewhat unexpected observation that, although the Sun did not rise above the horizon as seen from the ionosphere for many weeks during the winter, the noon critical frequency or density of ionization was greater in midwinter than in midsummer, when the Sun did not set even during the 'night'. It is thus to be concluded that the conditions in the reflecting layers were determined by movements of ionization and not by direct solar influences.

These direct measurements of ionospheric conditions in the Antarctic were supplemented by detailed observations of the Earth's magnetic field and of the incidence of meteors and auroral activity. The systematic reduction of the great mass of these observations has continued over the past few years and several volumes of the results are in course of publication. As a result, our knowledge of the physical conditions both at the ground and in the atmosphere above the Antarctic continent has been considerably enhanced by this international co-operative enterprise.

The general operation of the programme of the International Geophysical Year and the increase in our knowledge already obtained from the results thereof are considered to have been so successful and rewarding that it has been decided to repeat this scientific investigation during the full calendar years 1964–65. During this period the activity of the Sun will pass through its minimum phase in contrast with the very high maximum activity which prevailed during the International Geophysical Year. The forthcoming two-year period (1964–65) is denoted as the International Quiet Sun Year (IQSY).

Rockets and Satellites for Geophysical Research

For some years before the International Geophysical Year, rockets carrying scientific instruments were used for research purposes to investigate the phenomena and characteristics of the upper atmosphere by direct measurement in a manner which ground-based experiments were unable to provide. Radio transmission was used to communicate to the ground station the measurements recorded by the instruments carried in the rocket. The technique may be regarded as an extension of that used in the radio sounding balloon which was developed by the meteorologist many years before. As an indication of the great progress made in this field, it was reported as recently as April 1963 that a *Skylight* rocket sponsored by the British Meteorological Office had reached a height of 145 miles at Woomera in Australia, as part of a programme of direct research into the physical properties of the upper atmosphere.

Reverting to the International Geophysical Year programme, however, the first successful application of artificial Earth satellites carrying scientific instruments for investigating the space around us occurred in October and November 1957, when the U.S.S.R. launched the satellites known popularly as *Sputniks I* and *II*. These were followed in January, 1958, by the American satellite *Explorer I*; and many others of various types and instrumentation have followed in the succeeding five or six years. The immediate success of the early launchings was impressive, and they constituted the most spectacular results arising directly from the operation of the International Geophysical Year. These satellites were equipped with instruments for the measurement of the properties of the atmosphere, the electron density of the ionosphere and the intensity of cosmic and solar radiation in outer space. The output of these instruments is in electrical form, and is either transmitted directly by radio to the ground observing stations, or is recorded on magnetic tape and sent later on receipt of a command signal from a radio station on the Earth. Some of the telemetry systems are relatively simple, but others are more complex and use up to 48 frequency channels. While the early transmissions were on the relatively low frequency of 20 Mc/s, and others on a frequency of 108 Mc/s, it is now customary to use the band 136–137 Mc/s, which has been assigned by the International Telecommunication Union for space research purposes. In one of the typical telemetry systems used, the modulation signals consist of 16 bursts of tone, the information being carried by the tone frequency of 5–12 kc/s, the duration of the bursts and the intervals between them, giving 48 channels in all. The power of transmitters in the early satellites varied from 10 to 100 mW for continuous operation, while for intermittent operation on command, several watts may be available.

Apart from the need to extract the observational information from the satellite, radio transmission is used to track it and supplement the positional information which may be obtained visually under clear sky conditions. Radio interferometers and tracking equipment have been installed in a number of countries to give round-the-world coverage by international co-operation. Additional information provided by the radio signals includes a determination of successive orbit periods, from a knowledge of which information is obtained as to the shape of the Earth, variations of gravity at different altitudes and the drag of the rare atmosphere at the orbit-levels.

While research on cosmic rays at high altitudes was in progress before the International Geophysical Year by means of balloons and rockets, the scope of the work has been considerably extended by the use of satellites carrying instruments for measuring the intensity and the energy distribution of the radiation. Also the discovery by van Allen and his colleagues of the high-intensity radiation belts which surround the Earth is one of the outstanding results of the International Geophysical Year. Later observations have indicated the existence of two belts of high-intensity radiation around the geomagnetic equator: the first is at a height of about 10,000 km, while the second is at approximately 22,000 km. These belts consist of charged particles the density of which recorded on a Geiger counter varies over a range of 10,000 to 1 under different conditions. The examination of these particles trapped in the Earth's magnetic field is providing a major contribution to our knowledge of the characteristics of the outermost portions of our atmo-

sphere, and the influence which the Sun's radiation has on these characteristics.

This subject has now become a very wide and active field of scientific research.

Top-side Exploration of the Ionosphere

So far I have referred to the progress made in the investigation of the characteristics of the ionosphere by radio wave echo-sounding technique conducted from ground stations. In this work it has been known for a long time that there is an upper limit to the frequency of the waves which are reflected from the higher ionized regions; and higher frequency or shorter waves penetrate these regions and travel on into outer space. We have, therefore, in the past been prevented from learning anything about the state of ionization in the atmosphere above the highest, or F_2, region. But the successful launching of artificial Earth satellites and the development of telemetry associated therewith has removed this limitation. By transferring the whole of our ionospheric recording instruments, including both the transmitter and receiver, to a satellite vehicle, we now have the facility of doing the ionospheric sounding from above the F_2 region and, by telemetry, the recorded results of the soundings can be communicated to the scientists in their ground laboratories.

This has been achieved by the collaboration of the U.S. National Aeronautics and Space Administration with the Canadian Defence Research Board and National Research Council. The satellite, termed scientifically 1962 $\beta\alpha 1$ but more colloquially known by the title *Alouette*, was launched on September 29, 1962, by a *Thor Agena B* rocket from the Pacific Missile Range; and it has been an outstanding success.

The *Alouette* satellite contains, in addition to the sounder, apparatus for counting cosmic ray particles, for observing the very-low-frequency radio spectrum and for monitoring the engineering performance. The sounder itself comprises a self-contained transmitter and receiver, both fully transistorized: the frequency is swept over a range of 0·45–11·8 Mc/s at a rate of 1 Mc/s per sec; and with a pulse-width of 100 μsec and repetition frequency of 67 c/s, the peak pulse power is 100 W. In addition, the satellite carries a beacon transmitter, and two telemetry transmitters operating in frequency channels within the 136–137 Mc/s band. The orbital parameters of *Alouette*, as calculated by the National Aeronautics and Space Administration Computation Centre in October 1962, shows that it travels around the Earth in an elliptical orbit with an eccentricity of 0·002 35, a period of 105·4 min and at heights of about 996 and 1,031 km (622 and 644 miles), at perigee and apogee respectively.

The general form of the ionograms obtained from this sounder are similar to those recorded at ionospheric observatories at the ground. At the lowest frequencies the ionized plasma is too dense for penetration; but as the frequency increases, pulses received from below the satellite are observed and show that the waves are reflected upwards from the top-side of the ionosphere, and above the level of maximum ionization of the F_2 region as observed from the ground. A typical top-side ionogram shows that the apparent height of reflexion falls rapidly from the satellite height at 2·0 Mc/s and then, as the frequency increases, the waves penetrate the ionosphere at 8·1 Mc/s, and are afterwards reflected from the ground. Thus the technique provides the means for exploring the ionosphere at all heights from ground-level to more than 1,000 km (more than 625 miles), by combining the *Alouette* records with those normally obtained at some of the ground observatories over which the satellite passes. In addition to an investigation of the preliminary results obtained by the Canadian scientists, two other investigations have been proceeding in the United Kingdom and the United States respectively. In the former, telemetry signals have been recorded from the satellite as it passes over the Radio Research Station, near Slough in England, and over its associated stations in Singapore and the Falkland Islands. In the United States, staff of the Central Radio Propagation Laboratory at Boulder, Colorado, have already examined some of the data recorded during 27 passes of the satellite over the telemetry station of the National Aeronautics and Space Administration at Blossom Point, Maryland. It is already clear that, by international collaboration in the field of geophysical research, the new techniques of radio and telemetry have opened up extensive new vistas in our exploration and understanding of the physical properties of our atmosphere, and the manner in which this is influenced by radiation from the Sun and outer space.

Conclusion

Apart from the application of instrumented satellites to direct scientific research, the recent successful launching into orbit of the *Telstar* and *Relay* satellites has similarly opened up a vast new field of inter-continental communications including the exchange of sound and television programmes, the possibilities of which have already been demonstrated. Truly it may be said that telemetry is a field of electrical or electronic engineering which is destined to play a most important part in bringing the nations of the world into closer and more direct communication, and at the same time provide them with a more detailed knowledge and understanding of the universe around our own planet, the Earth.

THE SHIRLEY INSTITUTE

By Dr. DOUGLAS W. HILL
Director of Research

ON May 21, H.R.H. Princess Margaret, Countess of Snowdon, formally opened the new Chemistry and Finishing Building of the Cotton Silk and Man-made Fibres Research Association at Didsbury, Manchester. Better known as the Shirley Institute, the Association is the second largest of the present fifty-two research associations in Great Britain. It was incorporated in 1919 and moved to its present site on the southern outskirts of Manchester in 1922. The site embraces a total area of 21½ acres and the accommodation now comprises four main groups of buildings providing some 221,000 sq. ft. of laboratories, experimental workrooms, offices, library, etc.

Buildings

The Towers (26,000 sq. ft.): The original, and still central, building is the original residence existing on the estate at the time of its purchase (Fig. 1). It is a large, three-storey, typically Victorian building erected in 1865 and now houses the library and Information Department, in which is maintained what is claimed to be the finest collection in the world of books on textile subjects. It contains about 20,000 books and pamphlets, increasing by about 600 every year; in addition more than 300 technical and scientific journals are taken annually.

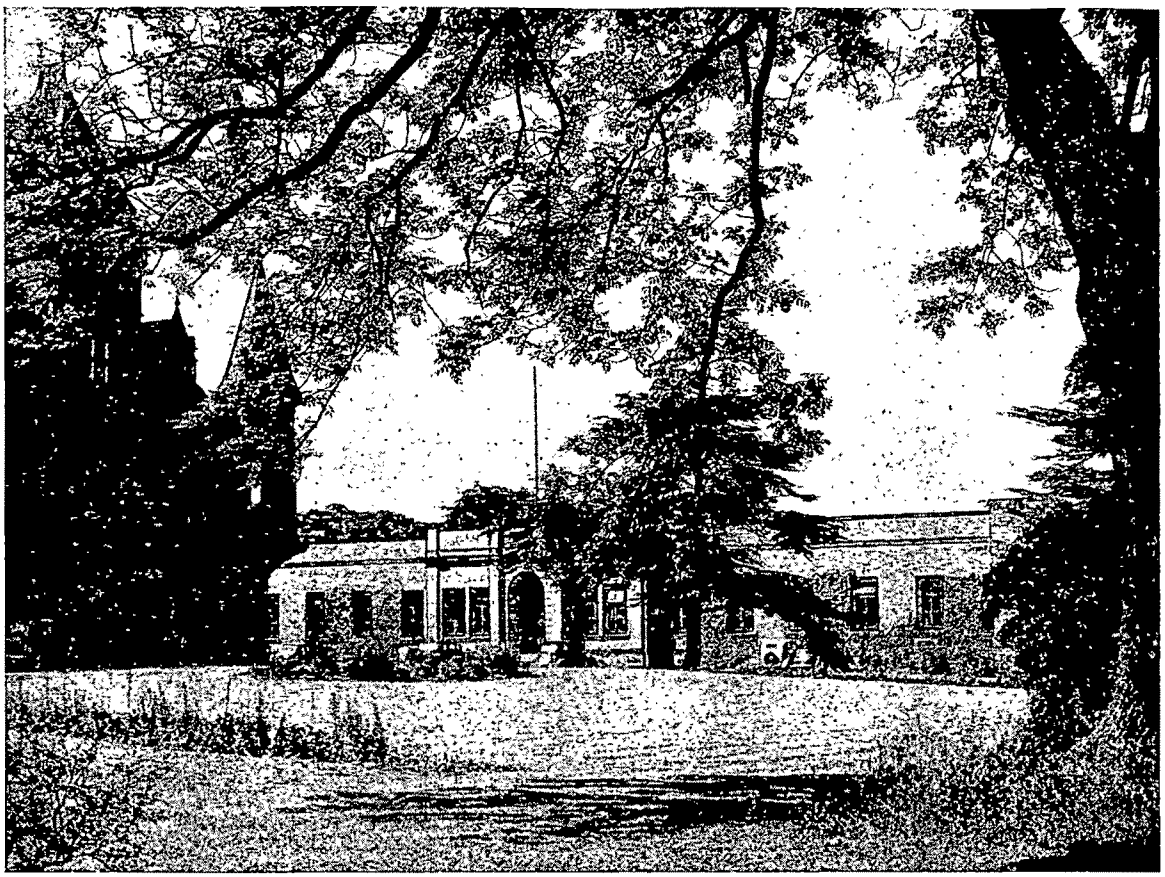


Fig. 1. The Shirley Institute. The Towers and Old Laboratories

Old Laboratories (100,000 sq. ft.) : The earliest part of these buildings, a laboratory block of thirty rooms, was built in 1922 and was formally opened on March 28 of that year by H.M. the late King George VI (then H.R.H. the Duke of York) (Fig. 1). Between 1922 and 1949, six additions were made to this original block, more than one-half in 1936 or afterwards. The buildings have constantly been modernized and re-equipped to bring them in line with present-day standards. They now accommodate the Spinning, Weaving, Finishing, Chemistry, Fibre Structure, and Engineering Departments, and include, in addition to the laboratories, a cardroom, spinning and doubling rooms, a weaving shed and a large engineering shop.

Physics and Administration Building (38,000 sq. ft.): This block, which was erected in 1952, comprises three two-storey wings (Fig. 2). One wing houses the central administration of the organization, together with offices for the Liaison Department. The other two wings are completely air-conditioned for temperature and humidity and provide accommodation for the Physics and Testing Department, and, temporarily, the Silk Department and Drawing Office.

New Chemistry and Finishing Building (57,000 sq. ft.): This building has just been completed (Fig. 3) and is the one recently opened by Her Royal Highness. It has been designed to accommodate staff and machines engaged on research into the finishing of cloth both in the laboratory and in processing rooms simulating works' conditions, and represents the final stage in the amalgamation of the former British Cotton Industry Research Association and the British Rayon Research Association which was effected in April 1961. Of the total area of 57,000 sq. ft., laboratories and offices occupy 32,000 sq. ft. on two floors, and a single-storey machine room somewhat similar to a dyehouse occupies the remaining 25,000 sq. ft. Separate sections at each end of the machine room accommodate pilot size plant for small-scale experimental work. The workroom area is separated from the laboratory block by a sound-absorbing buffer area mainly devoted to storage and service rooms.

The laboratories have been designed to allow for the maximum flexibility in any future desirable re-arrangement. Services to the machine room are generously disposed in underground ducts terminating at regular intervals in grouped assemblies, thus making for easy access and connexion to new machines with a minimum of disturbance to experiments in progress. In the laboratories the services are disposed in a co-ordinated modular arrangement in vertical ducts which, together with a system of hollow tubes resting on the structural floor, enables any service to be run conveniently to any point. The services provided include steam at various pressures, a central vacuum system, two pressures of compressed air, gas, electricity, hot and cold water. A feature of the services is that the whole of the Institute's cold water system is now filtered. The plant and storage tanks for the new system are housed in the basement of the new laboratory. The main heat source for the building is supplied by steam from a central boiler house where new oil-fired boilers have been installed.

The opportunity has been taken to lay out the grounds of the Institute afresh and, of course, to instal new and up-to-date machinery and equipment.

The new building and the associated work have cost about £450,000 while a further £50,000 has been spent on specially purchased equipment.

Chemical and finishing researches have, of course, been in progress at the Institute for many years but the opening of the new building will mean that this work can now be extended and carried out in ideal conditions.

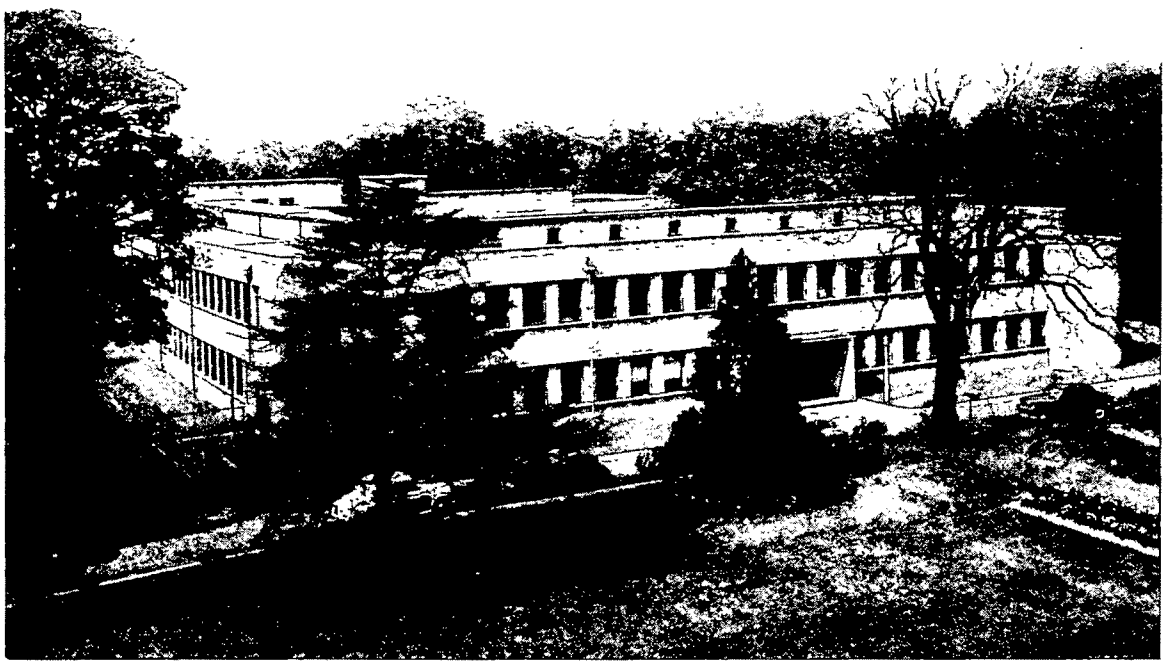


Fig. 2. Physics and Administration Building

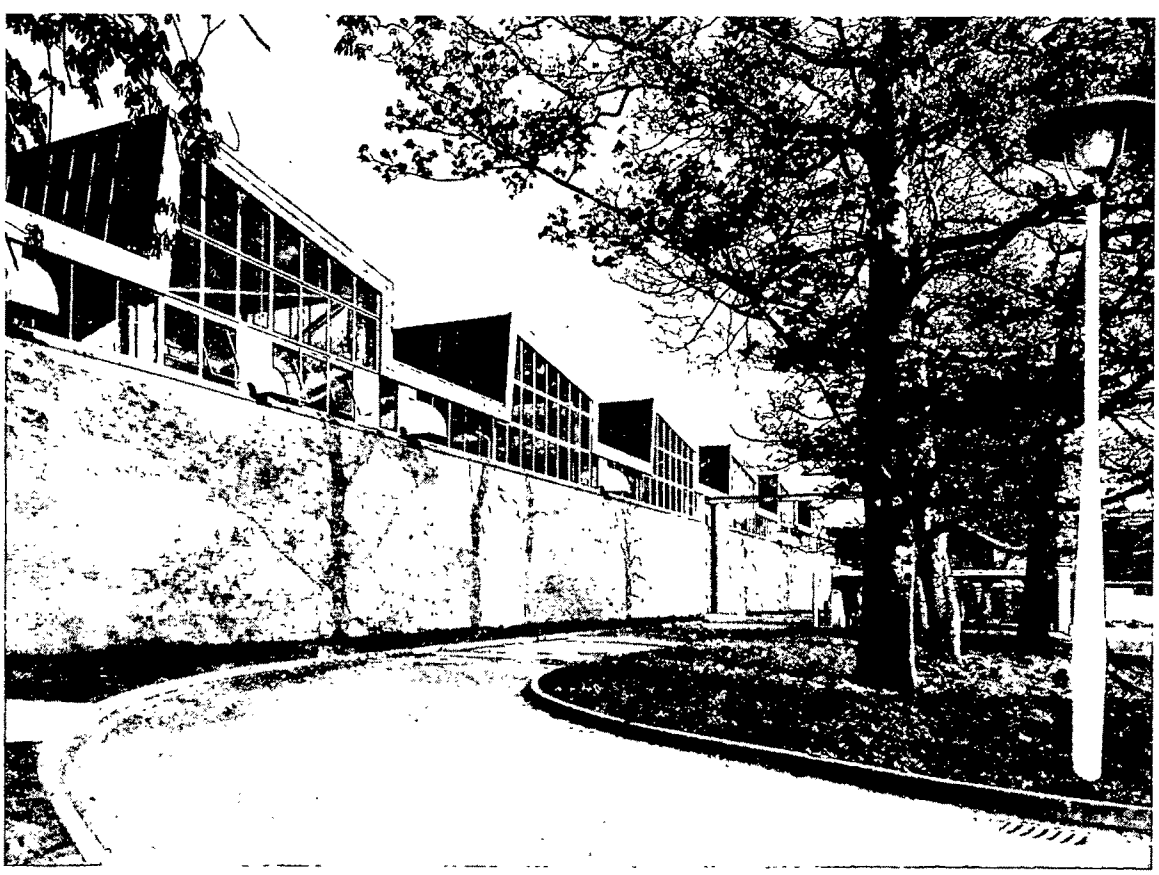


Fig. 3. Western aspect of the new Chemistry and Finishing Building

Work and Objectives of the Institute

As its formal name implies, the Institute is primarily a research organization. The founders expressed its primary aim in the words "Our task is not to make handicraftsmen, but to imbue the whole industry from top to bottom with the spirit of science; not to survey existing fields of knowledge but to break new fields and map out territories yet unknown". It is undoubtedly by adherence to this policy that the Institute has achieved its steady growth to maturity and to world-wide recognition. Starting with an annual expenditure of £4,000 in 1920, it increased this to £51,000 in 1929, to £93,000 in 1939, to £215,000 in 1949, and in the last financial year to almost £650,000. All subsequent experience has amply confirmed the wisdom of the original decision, because fundamental work is the basis of future applied research. In this respect the knowledge provided by fundamental research is the capital invested in the business of providing technological assistance.

In due course, the Institute was able to turn its attention to applied research, and since that time fundamental and applied research have progressed side by side, one feeding progressive ideas to the other and in return receiving fresh basic problems thrown up by their application.

It is difficult, as everyone knows, to draw a sharp line between fundamental and applied research, and equally so between applied research and development. It is, however, not enough to carry out fundamental research, or even to show how it may be applied to existing practices. It is often necessary to develop the new practices to the point of industrial exploitation, which often implies the production of new machines, instruments or equipment. Shortly after the Second World War, therefore, a separate Engineering Department was organized complete with drawing office, instrument section and machine shop. This Department can design and produce, in collaboration with the laboratories, any new prototype machines needed for full exploitation of the research ideas.

With this background of basic research, applied research and development, the Institute is able to assist the industry in a major way to solve its day-to-day problems. The use that the industry makes of this kind of service has increased enormously over the years, both in absolute amount and as a proportion of the resources available. The maintenance of an adequate amount of research as distinct from service work is a problem that faces all research organizations, which are always in danger of becoming the victims of their own research, since each successful investigation creates the need for further service work to ensure its successful application in industry. There is therefore a tendency for service work to increase continuously with disastrous consequences for the amount of research work that can be carried out.

At the present time about 35 per cent of the Institute's resources is utilized in service work to the industry, approximately the same amount in applied research and development, and about 30 per cent in fundamental research. Experience suggests that it would be unwise to permit the fundamental research to fall below this level and that to do so would destroy the character of the organization.

Finally the Institute has grown from a local industrial research organization to a great national and even international institution. Of the twelve thousand or more technical contacts it has each year some three thousand are with organizations not in membership of the Association. These include most of the Government departments, universities and colleges, trade associations, Trade Unions, and industrial concerns outside the textile industry. Many of these arise from the Institute's position as a leading authority on certain fields of science and technology as well as from the need of laymen to have a source of expert advice on textile problems with which they may be confronted.

With the completion of the most recent new building, the course embarked on by the founders may be said to have been completed. The Institute is now well equipped in every field of its activities. It is fitting, therefore, that in opening the new building H.R.H. Princess Margaret should also have formally completed on Tuesday, May 21, what her father H.M. the late King George VI formally initiated on another Tuesday forty-one years earlier.

ABERDEEN MEETING OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

By Dr. R. B. STRATHDEE, O.B.E.
Department of Chemistry, Aberdeen

WHEN the British Association for the Advancement of Science was founded in 1831 Aberdeen had two universities, both degree-granting, King's College, founded in 1494, and Marischal College, founded in 1593. Among the scientists who at that time expressed concern at the unsatisfactory position of science in the country and who were instrumental in forming the Association was John Herschel, who was appointed Rector of King's College in 1842. The Association did not visit Aberdeen until 1859. In the previous year a company had been formed in the city to take over what was then known as the Assembly Rooms, a large building in Union Street erected in 1820, the architect being a distinguished Aberdonian, Archibald Simpson. The new Music Hall Company reconstructed the interior of the building, one of the main changes being the conversion of a commodious dining-room into a hall capable of seating a very large gathering. The first public meeting held in this Music Hall was on the evening of September 14, 1859, when H.R.H. Prince Albert delivered his address, as president of the British Association, to an audience of 2,500, and in the same hall Sir Eric Ashby will also this year address the Association (see *Nature*, 195, 856; 1962). Her Majesty in 1859 graciously entertained to lunch at Balmoral Castle the members of the General Committee and the distinguished foreign visitors present. The British Association visited the city again in 1885 when Sir Lyon Playfair was president and in 1934 when Sir James Jeans occupied the chair. Annual meetings have, on occasion, been used for the announcement of outstanding scientific discoveries, and on the latter occasion the story of vitamin C (ascorbic acid), its isolation, structure and synthesis, was revealed to the chemistry section by Profs. A. Harden, A. Szent-Györgyi, E. L. Hirst and W. N. Haworth.

Aberdeen, the principal town of the maritime county of the same name, has been a Royal Burgh for more than eight centuries and to-day ranks as the third city of Scotland. Throughout the years Aberdeen has sought to develop its natural resources, and the area boasts a rigorous modern agriculture and industry. Situated on the coast between the Rivers Dee and Don, with a splendid hinterland of field and forest, mountain and moor, its attractions as a tourist and holiday centre have long been recognized. The cultural life of the city had its roots in the

ancient church and to-day the city is ideally placed to act as host for the 1963 meeting of the British Association, with its University and its Robert Gordon's Technical College, where the reception centre and administrative offices will be situated. In addition, Aberdeen is the home of many research institutes, namely, the Rowett Institute (for research in animal nutrition), the Macaulay Institute for Soil Research, the Marine Laboratory and the Torry Research Station (Department of Scientific and Industrial Research).

With such potentialities, an interesting and varied programme of excursions, both general and sectional, has been arranged; members will have an opportunity to visit industrial works, the research institutes, places of interest within the city and such well-known areas as Deeside, Donside, Speyside and the north-east coast. The dates of the meeting coincide with the Edinburgh International and the Pitlochry Festivals, and the various Highland Games and Gatherings are also held around this period so that members can include such functions if they wish to extend their visit to the north. Two longer excursions (limited in numbers attending) have been arranged for a period just before and after the meeting; these will be based at Tarradale House, Muir of Ord, Ross-shire, the University Field Centre in the Black Isle, and will be led by geographers from the University.

One of the first of the local committees to function in the arrangements for the Aberdeen meeting was a small committee under Prof. A. C. O'Dell, which was set up by the University to compile and publish a survey, largely scientific, of Aberdeen and district. This small group was later enlarged to become the Publications Committee of the local organization. Authors were chosen for their particular knowledge, and the volume, which will be published at the opening of the meeting under the title *The North-East of Scotland*, will form a valuable contribution to the literature of the area. The sections of the book cover natural history, regional settlement, agriculture, forestry and fisheries, industries, commerce and trade, population, education and research with an account of scientists of the North East. A copy of this publication will be presented to each member attending the Aberdeen meeting, and it is anticipated that a limited number of copies will be available for sale to libraries, etc., after the close of the meeting.

The president of the 1963 meeting is Sir Eric Ashby (see *Nature*, 195, 856 ; 1962), the eminent botanist and Master of Clare College, Cambridge. He will deliver his presidential address on the evening of Wednesday, August 28, on "Investment in Man", a subject which will provide an interesting sequel to Sir John Cockcroft's address last year at Manchester on "Investment in Science". Preceding the address, the Vice-Chancellor, Dr. E. M. Wright, on behalf of the University of Aberdeen, will confer the honorary degree of LL.D. on Sir George V. Allen (see *Nature*, 197, 128; 1963), who this year retires from office as secretary of the Association, on Sir Charles Morris, vice-chancellor of the University of Leeds and president of Section L (Education), and on Prof. J. Monteath Robertson, Gardiner professor of chemistry, University of Glasgow, and president of Section B (Chemistry).

Two evening discourses will be given, one by Prof. C. F. Carter on "Problems of Economic Growth" and the other by Prof. R. V. Jones (subject to be announced later). The presidential addresses of the various sections cover a wide range of topics from "High-Voltage Insulation" (Section A) to "Urban Planning and Location of Industry" (Section X). It is one of the purposes of these annual meetings "to disseminate scientific knowledge among the lay public" and the non-scientist should find much to interest him not merely among the presidential utterances but among the various papers, etc., which make up the sectional programmes. Of much interest to all Scots men and women must be the joint sessions of Sections E (Geography), F (Economics), K (Botany), K* (Forestry) and M (Agriculture), covering two whole days, to discuss "Land Use in the Scottish Uplands". These meetings will be 'chaired' by Lord Lovat, Prof. J. R. Matthews, emeritus professor of botany, University of Aberdeen, Dr. R. F. Hunter of the Hill Farming Research Organization, Edinburgh, and Prof. J. R. Raeburn, Strathcona-Fordyce professor of agriculture, University of Aberdeen. Speakers of many interests will take part including Sir Robert Urquhart, chairman of the Crofters' Commission.

There will be, in various centres, a number of exhibitions: in two of the rooms adjoining the Music Hall there will be a display based on "Forestry and Forest Products" contributed by research institutions and industry, while in the neighbouring ballroom the Ordnance Survey will have on show maps focused on Scotland and on some of the topics under discussion at the meeting. In the Art Gallery there will be on view models and diagrams illustrating the theme "Cities and Universities of the Future". In Scotland to-day very considerable efforts are being made to change the physical appearance of the cities and this exhibition will reveal the lines along which the planners are thinking. Under the auspices of the Institute of Science Technologists there will be at Marischal College an exhibit of teaching aids and apparatus made by the technical staff of various university geology departments. Also in Marischal College (Wilson Room) there will be on display the magnificent St. Ninian's Treasures discovered by Prof. A. C. O'Dell and his party while excavating on that isle in 1958. In the University Library at King's College, there will be available for inspection a number of books, etc., of special and historical interest. "Teaching Machines and Programmed Texts" will be the subject of an exhibition in the College of Education, and in the Chemistry Department of the University will be a display of equipment illustrating the "History of the Microscope". The National Book League is to stage an exhibition of books in the Reception Centre; these will include, in addition to a general section, three subsidiary sections covering forestry, archæology and sociology.

In 1956, Aberdeen Town Council purchased a vacant church—a large Grecian structure, modelled on St. Pancras Church, London—with an imposing portico of four Ionic pillars, above which rises a clock tower. The interior of the building has now been reconstructed to provide on the upper floor an auditorium to seat 390, with dressing-rooms, etc., and on the ground-floor a library and lecture rooms. Now renamed the "Civic Arts and Adult Education Centre", this building will serve as a centre for the arts, drama, music, painting, etc., within the city and it will be used for the first time during the meeting of the British Association. Here the programmes of scientific films will be screened and these will include films from thirteen different countries. There will be two morning sessions for young people, free lunch-time and evening performances for the general public, and afternoon periods for members. In addition there will be a display of audio-visual equipment kindly provided by the Rank Organization.

One of the main functions of the British Association is to enable scientists not merely to meet one another but also to meet the men and women of the city and surrounding districts who are their hosts. To enable this purpose to be achieved, the City and the University are both giving receptions while, in addition, there will be a number of smaller functions and garden parties. It is perhaps invidious to single out any one of these, but one interesting function will be the performance of Haydn's "Creation", given by the Haddo House Choral Society on Sunday evening. A buffet supper will be served and members will have an opportunity to view the house and to walk through the grounds. Haddo House was designed for William, the second Earl of Aberdeen, by William Adam, father of the Adam brothers, in 1731; among its treasures are many fine portraits and some beautiful china. Major

David Gordon, grandson of the present Earl, has with his wife done much to foster a love of music in the north-east. For more than forty years students and graduates of the University have staged a theatre show during their Charities Week. When the British Association visited Aberdeen in 1934 they presented the pageant "Town and Gown" in honour of the visit, and this year, during the first week of the meeting, they are to stage a revue under the title "Element of Surprise".

Following the usual pattern of British Association meetings, a special programme has been arranged for young people. In the High School for Girls there will be a Science Fair, which will be opened on Thursday morning by the President. Here there will be on display exhibitions and experiments devised by the pupils of the secondary schools and colleges of Aberdeen and the neighbouring counties. Of the many items listed among the projects is one, by the Gordon Schools, Huntly, under the intriguing title of "There is more to Transistors than Cliff Richard". The opening ceremony will be followed later in the morning by a reception at which pupils and staff will have an opportunity to meet the President and Officers of the Association. Among the many other items arranged for the young people is a 'Brains Trust' when a panel of scientists will attempt to answer questions sent in by the pupils, and a full programme of lectures including the York Lecture by Sir Raymond Priestley, president of the Royal Geographical Society, on "Antarctic Exploration Yesterday and To-day". Sponsored by Section X there will be on Monday morning a series of talks, under the title "Science in the Sixth Form", given by seven secondary school pupils.

Aberdeen looks forward with interest and with pleasure to its fourth visit from the British Association, which the organizers hope will be even more successful than the three previous meetings.

OBITUARIES

Dr. Theodore von Kármán, For.Mem.R.S.

Theodore von Kármán was born in Budapest on May 11, 1881, son of Prof. Maurice von Kármán, a distinguished pedagogue and philosopher, and Helene Konn. After graduation in 1902 with highest honours as a mechanical engineer from the Budapest Royal Technical University, he spent the next four years in Hungary, teaching at the University and performing theoretical research on machine design problems for Ganz and Company.

The next six years were spent in study in London, Paris and Göttingen. His first direct contact with aeronautics occurred in Paris in 1907 when he saw Henri Farman fly a circular one-kilometre course. He settled for a time at the University of Göttingen to work with L. Prandtl, receiving a Ph.D. degree in 1908. His dissertation on the buckling strength of straight columns was the first of many major contributions. In this communication he set forth the double-modulus theory of column behaviour under loads which stress the column material beyond the elastic limit. Von Kármán continued as a teaching Fellow until 1912. During this period, stimulated by observing the difficulty of his colleague, Hiemenz, in obtaining symmetrical flow of water about a circular cylinder, he developed and published in three communications in 1911 and 1912 his well-known theory of the unsymmetrical vortex arrangement in the wake of a cylinder, commonly known as the Kármán vortex trail.

In 1912 von Kármán accepted an invitation to become professor of aeronautics and mechanics and director of the Aerodynamic Institute in the Technical University of Aachen. The Aachen period was one of rapid growth to eminence on the part of the man and the Institute. His interests turned largely toward fluid mechanics. Some of his most notable contributions dealt with the stability of laminar flow, the theory of turbulence, wing theory, pressure distribution on airship hulls and the Kármán integral relation for solving problems of boundary layer flow and skin friction.

During 1914–18 von Kármán served as director of research of the Austro-Hungarian Aviation Corps, returning to Aachen after the War.

In 1926 he visited the United States at the invitation of Dr. Robert Millikan of the California Institute of Technology and the Daniel Guggenheim Foundation for the Promotion of Aeronautics. Following a trip around the world, he arranged in 1928 to divide his time between Aachen and the California Institute of Technology. In 1930 he settled permanently in California as director of the Guggenheim Aeronautical Laboratory, becoming a U.S. citizen in 1936.

In the nineteen years of his association with the California Institute of Technology, von Kármán published some fifty communications in solid and fluid mechanics, alone and with his students. These included contributions to the theory of the buckling of spherical shells under external pressure, influence of curvature on the buckling characteristics of structures, the Kármán–Moore slender-body theory for bodies of revolution at supersonic speed, compressibility effects, supersonic aerodynamics, statistical theory of turbulence, and many others.

In 1939 von Kármán initiated rocket research at the Guggenheim Aeronautical Laboratory, especially for the development of assisted take-off rockets for airplanes. He directed the jet propulsion project, which later became the Jet Propulsion Laboratory. With a few friends he founded the Aerojet Engineering Corporation, the first American firm specifically engaged in rocket development in the United States.

Von Kármán took leave of absence in 1944 to organize a Scientific Advisory Group for General H. H. Arnold to analyse the use of science in warfare and to forecast the impact of new developments in rockets, jet propulsion, and supersonic flow on future air warfare. He retired from the California Institute of Technology in 1949, devoting much of his time to his duties as chairman of the U.S. Air Force Scientific Advisory Board, which succeeded the Scientific Advisory Group.

In 1951 von Kármán called a meeting of the directors of aeronautical research of the NATO countries, which resulted in the establishment of the NATO Advisory Group for Aeronautical Research and Development (AGARD) with headquarters in Paris. He was chairman of AGARD until his death.

Von Kármán was a leader in the organization of three additional agencies for international co-operation in science, the International Congress of Applied Mechanics, first held at Delft in 1924 following a preliminary meeting at Innsbruck, the International Congress of the Aeronautical Sciences, first held in Madrid in 1958, and the International Academy of Astronautics established within the International Aeronautical Federation.

Von Kármán was a foreign member of the Royal Society of London, a member of the U.S. National Academy of Sciences, Academie de Sciences de l'Institut de France, Academia Nazionale dei Lincei, Royal Academy of Sciences (Madrid), honorary Fellow of the Royal Aeronautical Society and honorary Fellow, honorary member or member of some additional 35 professional societies in 11 countries. He received more than 60 honorary degrees or special awards from institutions in 13 countries, including the U.S. Medal for Merit (1946),

John Fritz Medal (1948), Franklin Gold Medal (1948), Lord Kelvin Gold Medal (1950), Gold Medal, Royal Aeronautical Society (1952), Wright Brothers Memorial Trophy (1954), Daniel Guggenheim Gold Medal (1955), U.S. Medal of Freedom (1956), Ludwig Prandtl Ring Award of the Wissenschaftlische Gesellschaft für Luftfahrt (1956), Goddard Gold Medal (1960), U.S. National Science Medal (1963).

Theodore von Kármán died during a visit to Aachen on May 7, 1963, four days before his eighty-second birthday. His place as a leader in applied mechanics and aeronautical and astronautical science, and in international co-operation in science and engineering, will not soon be filled. HUGH L. DRYDEN

Dr. H. Baines

DR. HARRY BAINES, of Messrs. Kodak, Ltd., died suddenly on June 7.

Dr. Baines was an outstanding figure in the photographic world, where he was extremely popular not only for his wide scientific knowledge, but also for his very happy and genial personality.

Born in 1901 of Yorkshire parentage, Harry Baines spent his youth in Nottingham, where he studied chemistry under the late Prof. F. S. Kipping. He took a University of London external B.Sc. degree with first-class honours in chemistry in 1921 and M.Sc. in 1923, in which year he entered the photographic industry as research chemist to Thomas Illingworth and Co., Ltd. In 1938 he was appointed chief chemist and deputy director of research in Kodak, Ltd., and in 1953 transferred to the head office in Kingsway, London, where he was scientific liaison officer between Kodak, Ltd., and the Press.

He was always keenly interested in raising the standard of photography in Britain, and for the past three years he was responsible for the successful organization of the Kodak scholarships in colour and advanced photography.

His main contributions to photographic science were the devising of a number of new methods of analysis (which formed the basis of a thesis for D.Sc. (London) in 1928), advances in emulsion-making technology and the elucidation of the mechanism of fixation.

He was considerably interested in the work of scientific societies and had been vice-president of the Royal Institute of Chemistry and of the Society of Chemical Industry; but he was more closely connected with the Royal Photographic Society, of which he was twice president. In 1956 he gave the Royal Institution Christmas Lectures. He was the author of *The Science of Photography*, which quickly became a popular standard work of reference.

Among the appointments he held at the time of his death were principal of the London School of Medical Photography, chairman of the Photographic Information Council and member of the Photographic Advisory Committee of the City and Guilds of London Institute.

His leisure time interests included antique furniture and clocks and he was a member of the Antiquarian Horological Society.

Dr. Baines leaves a widow and two daughters.

NEWS and VIEWS

U.S. Presidential Award and Gold Medal : Prof. F. L. Whipple

PROF. FRED L. WHIPPLE, director of the Smithsonian Astrophysical Observatory, Cambridge, Mass., has been given the Presidential Award and Gold Medal for Distinguished Federal Civilian Service. The Award, the highest that can be bestowed on a career Federal employee, is being given to Dr. Whipple for his outstanding achievement in conceiving and developing "an optical satellite tracking system which stood ready to track the first artificial satellite launched and has since provided valuable scientific data". This network of twelve tracking stations, located on six continents, is still the most accurate tracking system in operation anywhere in the world. Dr. Whipple, who is also a professor of astronomy in Harvard University, has made major contributions to investigations of comets and meteors. He was the first person to propose that a 'meteor bumper' be placed about a spaceship to protect space travellers and astronauts from the danger of meteor punctures of a spacecraft. This proposal was made in 1946, eleven years before the first satellite was launched and fifteen years before the first man was placed in orbit.

Light Division at the National Physical Laboratory: Dr. L. A. Sayce, C.B.E.

DR. L. A. SAYCE, who was appointed in 1948 to the position of superintendent of the Light Division at the National Physical Laboratory, retires from this position on June 30 after fifteen years of distinguished service. He took a particular interest in the blending of basic and applied research, and nowhere was this more ably demonstrated than in his personal research on diffraction gratings. During his tenure of office, the Light Division has carried out much basic research on vision, colour and colour rendering, and there have been equally important advances in the more general fields of photometry, radiometry and optics, including the development of new forms of optical masers. The work on diffraction gratings which Dr. Sayce personally supervised has led to the availability of gratings of high efficiency both in the infra-red and X-ray regions, and out of this work an entirely new application of gratings has developed in their use as accurate scales of measurement. This has required a close study of moiré fringe techniques and has resulted in the extensive use of gratings in metrology and engineering. (He will be continuing and extending this aspect of his work for a further period in a special post which has been created for him at the National Engineering Laboratory.) Graduating from King's College, Newcastle upon Tyne, in 1922, Dr. Sayce took his Ph.D. in 1925. He was appointed lecturer in chemistry there in 1927, and his researches thereafter covered a wide range of interest in physical and inorganic chemistry, instrument design and photography. During the Second World War he served in the Ministry of Home Security, and for two years before joining the National Physical Laboratory, in 1948, he was assistant director of physical research at the Admiralty. He was created C.B.E. in 1958. Dr. Sayce is to be succeeded as superintendent of the Light Division by Dr. J. Dyson (see *Nature*, 198, 738; 1963).

Chemistry at Sheffield: Prof. R. D. Haworth, F.R.S.

PROF. R. D. HAWORTH retires from the Firth professorship of chemistry in the University of Sheffield at the end of this academic year. He had his early education in Stockport and then at the University of Manchester, where he came under the lasting influence of Arthur Lapworth. He proceeded with an 1851 Exhibition Scholarship to Oxford, to conduct very fruitful work on the synthesis of the isoquinoline alkaloids. From 1937 until 1939 he was lecturer in chemistry at King's College, Newcastle upon Tyne, where he carried out his extensive

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investigations of those natural phenolic resins which formed the subject of his Tilden Lecture to the Chemical Society in 1942. Since 1939 he has held the Sheffield chair, directing important work, especially on the colouring matter purpurogallin and the chemistry of the tropolone system, and on the hydrolysable tannins. He has served as vice-president of the Chemical Society; he was elected to the Royal Society in 1944 and was awarded its Davy Medal in 1956. During the Second World War, and in the difficult years of expansion which have followed, Prof. Haworth has conducted the affairs of the Department with conspicuous vigour and skill. He collected an efficient and congenial team of academic associates and maintained good external relations to the advantage of the Department. The present impressive laboratories owe much to his inexhaustibly patient and knowledgeable co-operation with the architects. Prof. Haworth, who has been appointed Leverhulme Visiting Professor to India, is to be succeeded to the Firth professorship by Prof. G. Porter (see *Nature*, 198, 737; 1963).

The Imperial College of Science and Technology

Prof. P. M. S. Blackett has asked that he be allowed to relinquish the headship of the Department of Physics at the Imperial College of Science and Technology at the end of the present academic year. The Governing Body, in agreeing to this request, has recorded its great appreciation of his outstanding services in that position during the crucial years of the expansion of the College and the construction of the new physics building. Prof. C. C. Butler has been appointed to succeed Prof. Blackett. Prof. Butler, who has served on the staff of the College for ten years, has been a professor of physics since 1957. Prof. Blackett will remain as a professor of physics and as Pro-Rector of the College.

The Councils for Training of Health Visitors and Social Workers

Sir John Wolfenden has resigned from the position of chairman of the Council for the Training of Health Visitors and the Council for Training in Social Work following his appointment as the new chairman of the University Grants Committee. Sir Charles Morris, who is about to retire from the vice-chancellorship of the University of Leeds, has been appointed to succeed Sir John Wolfenden. The two Councils were set up last October, under the Health Visiting and Social Work (Training) Act, 1962. Under the First Schedule of the Act the Privy Council appoints one person to be chairman of both Councils.

The International Atomic Energy Agency and Trieste Centre for Theoretical Physics

An international centre for theoretical physics is to be established in Trieste in the first half of 1964 under the auspices of the International Atomic Energy Agency. The Centre will be established on a provisional basis; its work will be evaluated so that the Agency, after two years, can decide on the future direction of the Centre's activities. A further evaluation will be made to enable the Board to determine if it is desirable to move the Centre to a developing country after four years. The Italian Government has offered to construct a new building for the Centre and housing facilities for the staff and the fellows; it has further offered a cash contribution of 250,000 dollars per year for five years, staff services and fellowships. The International Atomic Energy Agency will contribute fellowships and professorships to an annual value not exceeding 55,000 dollars for four years and additional annual contributions not to exceed a total of 110,000 dollars in the same period.

The 1964 programme of work and budget for the International Atomic Energy Agency reflects a small increase over 1963. Administrative costs have been trimmed down further through a reduction in staff and decreased costs both for the scientific meetings and the sessions of the Board and the Conference. The Board recommends a regular budget of 7,444,500 dollars as compared with 7,337,500 dollars in 1963. For the operational programme, an expenditure of 2,367,500 dollars is proposed to be financed from voluntary contributions of 2 million dollars, special contributions, miscellaneous income and income from reimbursable services. The 2 million dollar target for voluntary contributions is the same as that of last year. The combined regular and voluntary budgets of 9,812,000 dollars do not include the funds made available to the International Atomic Energy Agency from the United Nations Expanded Programme of Technical Assistance, estimated at a little more than 1 million dollars.

United Kingdom Funds for Nuclear Energy Research

In a written answer in the House of Commons on June 17, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that the United Kingdom was at present called on to pay about 23 per cent of the budget of the European Nuclear Energy Agency, but in the period 1957–62 only £155,000 of the £192,000 provided in the Atomic Energy Estimates was actually required. The contribution for the year ending December 31, 1963, was £30,000, but contributions to the Agency's joint project were separately assessed. It was not possible at this stage to give an estimate of the financial commitment of the United Kingdom to these projects. The commitment of the United Kingdom to the 20-MW *Dragon* project at present under construction at Winfrith was £10,200,000, of which £4,556,000 had been paid, leaving £5,644,000 to be paid over the period 1963–64–1966–67; £2,100,000 was provided in the 1963–64 Estimate. Some additional assistance was given in kind and in certain income tax liabilities of foreign staff attached to the project.

The National Reference Library of Science and Invention

At the report stage of the British Museum Bill in the House of Lords on June 18, when a motion providing for the separation of the National Reference Library of Science and Invention from the British Museum under a separate body of trustees was again pressed to a Division, a further amendment was moved by the Earl of Cranbrook requiring the new Library to provide the staff to operate a National Scientific Abstracting Service. The Earl of Cranbrook expressed the view that it would be essential for an abstracting service to be built around the new Reference Library, though he did not think that a complete abstracting service covering all the sciences was necessary or desirable. Moreover, he referred to a recommendation of the Council for Scientific and Industrial Research in its latest report that the Commonwealth countries and the United States should consider together the whole problem of science abstracting in English-speaking countries. For the Government, the Earl of Dundee said that the whole subject of technological abstracting had been considered by the British Commonwealth Scientific Council and a report was about to be issued. In the Patent Office Library a study was being made at the instance of the British Museum Trustees of the use of the scientific literature in the Patent Office as part of the planning of the new reference library. At the instance of the Advisory Council on Scientific Policy, Prof. B. H. Flowers was undertaking another study on the difficulties experienced by research workers in keeping up to date with new developments. The Earl of Dundee said that the Trustees recognized that it was most desirable that the new Reference Library should play a leading part in considering the adequacy of the coverage of existing abstracting systems, in setting up machinery for co-ordinating them, identifying overlaps and omissions and seeking all possible means of removing them. It was

also recognized that the development of electronic devices for storage and retrieval of information must be a main object of study in the new Library, and research was already proceeding into the possibility of collecting abstracts in punched tape form. Such a method might solve many of the problems of overlapping and delay in abstracts, and he thought the multiple use of the same abstracts should be encouraged rather than repeated abstracting of the same papers. In interpreting the amendment as implying a statutory obligation to provide a comprehensive national scientific abstracting service, which he did not regard as helpful, the Earl of Dundee was strongly supported by the Earl of Crawford, who argued that it would be better to await the outcome of the present enquiries, and regarded the proposal as damaging to the Library and a retrograde step. The amendment was, by leave, withdrawn.

The Commonwealth Development Bill

In moving the second reading of the Commonwealth Development Bill in the House of Commons on June 19, the Under-Secretary of State for Commonwealth Relations, Mr. J. Tilney, said that the Bill amended the Overseas Resources Development Act, 1959, and the Colonial Development and Welfare Act by returning to the sphere of activity of the Colonial Development Corporation—now to be renamed the Commonwealth Development Corporation—all Commonwealth territories within its scope when the Corporation was established in 1948. In accordance with powers conferred under the Bill, the Secretary of State will instruct the Corporation to seek his prior approval before starting operations in an independent country and that approval will only be given with the agreement of the Government concerned. It is not proposed to raise the amount which the Corporation can borrow from the Treasury above the limit of £130 million: net advances outstanding amount to £92 million, and in the past ten years borrowings have averaged less than £10 million per annum. Mr. Tilney said that the Corporation welcomed the policy embodied in the Bill.

On the colonial development and welfare side, the Bill extended the period in which such schemes could be made and Exchequer loans approved from March 31, 1964, to March 31, 1966, and increased from £315 million to £340 million the amount which may be spent on schemes under Section I of the 1959 Act. About £43·5 million remained unspent at March 31, 1963, so that about £68·5 million would be available for the three years 1963–66, and the provision for Exchequer loans was increased from £100 million to £105 million, permitting further issues of £41 million. Mr. Tilney pointed out that expenditure on research and development was now financed by the Department for Technical Co-operation and said that this change did not imply any decrease in research for the benefit of colonial territories. While the Bill was welcomed generally, Opposition speakers criticized the provisions as inadequate, but in replying on the debate for the Government, the Under-Secretary of State for the Colonies, Mr. N. Fisher, put the total of Government aid overseas during the next three years at £180–200 million a year.

Transition from Primary to Secondary Education

In a written answer in the House of Commons on June 17, the Minister of Education, Sir Edward Boyle, stated that when the Central Advisory Council for Education (England) had completed its present task, he proposed to reconstitute it with the following terms of reference: To consider primary education in all its aspects and the transition from primary to secondary education. In due course, he would ask the Central Advisory Council for Education (Wales) to consider the Welsh aspects of this enquiry and he was arranging for some common membership of the two Councils. Lady Plowden had accepted his invitation to become the next chairman of the Council, and the present chairman, Mr. J. Newson, had agreed to serve as deputy chairman of the reconstituted Council.

English Clearly Expressed

Prof. R. Hoggart's inaugural lecture, "Schools of English and Contemporary Society", delivered at the University of Birmingham on February 8, is concerned with emphasizing the practical importance and significance of a love and respect for language—which he regards as a prime duty of a school of English to foster (Birmingham: The University, 1963. 2*s*. 6*d*.). He insists that English has to do with exploring human experience in all its complexity and flux, and what he has to say about the changing meaning of words and the necessity of defining the meaning of words in the context in which they are used is highly pertinent to the scientist and his task of interpretation and exposition, whether it is a matter of exposition to his fellow scientists or to a wider public. Prof. Hoggart gives clear warning of the dangers which attend the careless use of words irrespective of the meaning which is likely to be attached to them by particular audiences or in particular circumstances. His lecture is as apposite in the context of discussions on the content of a general education as it is to the proper functions and value of a school of English.

United States Readings on Development

A Selected List of United States Readings on Development (Pp. xvii+363. Washington, D.C.: Agency for International Development. Obtainable from U.S. Government Printing Office, 1963. 1.25 dollars), prepared for the United Nations Conference on the Application of Science and Technology for the Benefit of the Less Developed Areas by S. M. Katz and F. McGowan, of the University of Pittsburgh, is a companion volume to the twelve volumes of United States papers prepared in connexion with the Conference (see *Nature*, 198, 250; 1963). Its thirteen chapters, containing almost 1,200 references, cover the regional planning experience of the Tennessee Valley Authority and Puerto Rico, production and marketing policies of great industrial enterprises, and the role of universities in concerting research and education in the service of human welfare. There is a short chapter on scientific and development policies, which includes sections on society and science and Government and science, but this is rather too limited in scope to be of service to any but the most elementary student of such subjects, though the chapter lists some references which are not generally known in the United Kingdom.

The Universities Federation for Animal Welfare

The Universities Federation for Animal Welfare has issued an interesting pamphlet setting out briefly some of the immediate interests that are given attention (*Some Things Done, Being Done, and to be Done*. Pp. 6. London: The Universities Federation for Animal Welfare, 1963). Included are: experiments on animals; pain in animals; the gin trap in Britain and overseas; cruel poisons; irresponsible shooting; whaling; correct technique for the electrocution of dogs and cats; tortoise trade; farm animals; anæsthesia; bull-fighting. Some indication of the manner in which the Federation undertakes its work is also given.

The Association of Consulting Scientists

At the annual general meeting of the Association of Consulting Scientists held in London recently the following honorary officers and members of council were elected: *Chairman*, Mr. F. G. Sarel Whitfield (Avebury Research Laboratories Ltd.); *Vice-Chairman*, Dr. H. H. Chambers (Sondes Place Research Laboratories Ltd.); *Honorary Treasurer*, Dr. G. W. Ferguson (Parry and Ferguson); *Honorary Secretary*, Mr. W. H. Stevens (Geochemical Laboratories); *Members of Council*, Dr. G. P. L. Miles, Mr. H. Pritchard, Mr. J. A. Radley (J. A. Radley Labora-

tories Ltd.), Dr. H. J. Stern (H. J. Stern's Laboratory Ltd.) and Dr. A. N. Worden (Huntingdon Research Centre). Dr. M. Barent (Barent and Johnson), the retiring chairman, was appointed liaison officer with the Union Internationale des Laboratoires Independant, to which the Association is affiliated as the United Kingdom and Commonwealth representative association.

Keilin Memorial Fund

The following statement has been received by the Editor of *Nature*: "With a number of our colleagues we have been considering how best to commemorate the scientific achievements of David Keilin. We believe that the establishment of a Keilin Memorial Lecture under the auspices of the Biochemical Society would perpetuate his memory and be of enduring value. It is hoped to hold the lecture every two years, and to realize this aim it will be necessary to establish a fund of £3,000 (8,400 dollars). The Biochemical Society has agreed to administer the fund". The statement is signed by Dr. B. Chance, Dr. M. Dixon, Dr. E. F. Hartree, Dr. R. Hill, Dr. T. Mann, Dr. M. F. Perutz, Dr. E. C. Slater, Dr. Emil L. Smith and Dr. P. Tate. Anyone wishing to contribute to the fund can obtain further information from Dr. E. F. Hartree, Animal Research Station, 307 Huntingdon Road, Cambridge.

U.K. Courses in Nuclear Energy, 1963–64

Under the heading of "U.K. Courses in Nuclear Energy, 1963–65" the May 1963 list of *Nuclear Studies*, compiled by the Science Department of the British Council, was noted on p. 1042 of the June 15 issue of *Nature*. The note refers to *List No.* 7 and not *List No.* 6 as stated. The note also mentioned that "The list has unavoidably appeared too late for application to be made for *any* of the courses for the academic year 1963–64". This is incorrect and should read "The list has unavoidably appeared too late for application to be made for *many* of the courses for the academic year 1963–64". Applications need not be submitted a long time in advance for many of the shorter courses. Furthermore, the *List* does not, in fact, include any courses for 1965, except by implication where courses are run annually.

University News:

Aberdeen

Dr. D. J. F. Bowling has been appointed to a lectureship in botany, and Dr. M. N. Court to a lectureship in soil science.

Durham

The seventeenth annual report of the University of Durham Appointments Board for 1961–62 reports that although significantly larger numbers completed courses in 1962 than in 1961, the demand in several sectors, notably chemical, oil and engineering industry, declined (Pp. 12. Durham: The University, 1963). As yet, the Board sees no signs that scientists or engineers, who would have reasonable prospects of success in, say, the Home Civil Service Administrative or Foreign Service competitions, are being attracted in those directions. Industrial demand for arts graduates also suffered a setback, but the arts graduate with reasonable personal qualities seeking employment outside teaching did not have an especially difficult time. The steady fall between 1958 and 1961 in the number of men joining nationalized industries and public utilities and corporations was reversed in 1962, and public service has increased its share of Durham graduates over the past five years. Reference is also made to the increasing numbers entering university employment, technical colleges and farm institutes, and the increased proportion of men graduates still seeking employment at the end of 1962 is attributed largely to the slack and uncertain state of the economy.

Liverpool

Prof. W. H. F. Barnes, professor of moral philosophy in the University of Edinburgh, has been appointed vice-chancellor in succession to Sir James Mountford, who retires on September 30.

London

The title of professor of chemical engineering has been conferred on Mr. S. B. Watkins in respect of his post at King's College. The title of reader in aeronautical engineering has been conferred on Dr. G. J. Hancock in respect of his post at Queen Mary College.

Announcements

Sir Isaac Wolfson, Bart., has been elected a Fellow of the Royal Society, under the Statute of the Royal Society which provides for the election of persons who either have rendered conspicuous service to the cause of science or are such that their election would be of signal benefit to the Society.

Dr. K. R. Payne has been appointed general manager of L. Light and Co., Ltd., with effect from May 16, in succession to Mr. G. R. Jones, who is leaving the Company.

The second International Pharmacological Meeting will be held in Prague during August 20–23. Further information can be obtained from the Secretariat of the Second International Pharmacological Meeting, Albertov 4, Prague 2.

The thirty-ninth annual Plant Pathology Field Meeting, arranged by the British Mycological Society, will be held at the East Malling Research Station on July 18. Further information can be obtained from Dr. R. C. F. Macer, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge.

The thirteenth general assembly of the International Union of Geodesy and Geophysics is to be held on the campus of the University of California at Berkeley during August 19–31. Further information can be obtained from the Local Arrangements Committee, International Union of Geodesy and Geophysics, University of California, Berkeley, 4, California.

The fifth International Congress of Clinical Chemistry, organized by the American Association of Clinical Chemists and the Canadian Society for Clinical Chemistry, will be held in Detroit, Michigan, during August 19–23. Further information can be obtained from the secretary, Dr. D. G. Remp, Henry Ford Hospital, Detroit 2, Michigan.

The fourth Australian Spectroscopy Conference, organized by the Spectroscopy Committee of the Australian Academy of Science, will be held at the Australian National University, Canberra, during August 20–23. Further information can be obtained from Dr. S. R. Taylor, Department of Geophysics, Australian National University, Canberra.

An international conference on "Lattice Dynamics", organized by the Danish Academy of Technical Sciences, the Danish Physical Society, the International Union of Pure and Applied Physics, NORDITA, the U.S. National Science Foundation and the U.S. Office of Naval Research, will be held at the Technical University of Denmark, Copenhagen, during August 5–9. Further information can be obtained from Dr. S. Lundqvist, Department of Mathematical Physics, Chalmers University of Technology, Göteborg, Sweden.

Erratum. Referring to his communication in *Nature* of May 18, p. 678, Dr. S. M. Silverman writes that the times which he gave on the individual scales in Fig. 2 are incorrect. They should read, from top to bottom (U.T.): 1155, 1055, 1025 and 0955.

FOOD SCIENCE AT THE UNIVERSITY OF LEEDS

By Prof. A. G. WARD, O.B.E.
Department of Food and Leather Science

ON June 18, Mr. Lauchlan Rose, chairman of the Food Manufacturers' Federation, formally opened the extension to the Procter Department of Food and Leather Science of the University of Leeds. The name commemorates Prof. H. R. Procter, who founded, in 1891, the Leather Industries Department, the precursor of the present one. The additions constitute an increase in floor area of almost 50 per cent to a total of more than 18,000 sq. ft. They provide much-needed facilities for the 4-year course leading to the honours B.Sc. in food science, the inaugural year of which is just about to be completed, as well as for postgraduate research in food science.

Apart from offices, the main part of the extension is devoted to the food technology laboratories under the direction of Mr. G. Glew. The purpose of these laboratories is two-fold: first, to enable undergraduate students to handle small-scale equipment in order to obtain an understanding of the operations involved in food processing and, secondly, to provide facilities for research into technological problems of the food industry by undergraduates in their final year, research students, and members of the staff. The facilities of the laboratories include three refrigerated chambers, one controllable down to −29° C, a blast freezer, a small Leybold–Elliott freeze drier, a Kestner laboratory spray drier, a Kestner drum drier, and a Q.V.F. all-glass concentrator with a flavour recovery unit, together with a canning line and a variety of equipment for mixing, milling, baking, boiling and testing.

Attached to the laboratories is a room for bacteriological control work, much of which is carried out in collaboration with Mr. J. Wolf's Bacteriology Section of the Department of Agriculture. This work was illustrated by several exhibits.

Dr. R. Reed's electron-microscopy group with its Siemens Elmiskop II and a full range of ancillary equipment, including two ultramicrotomes, an ultrasonic disintegrator, and a metal shadowing unit, has been re-housed in the extension in a compact suite, and light microscopy, under Dr. F. Olga Flint, is catered for both on the under- and post-graduate levels. A Leitz 'Ortholux' microscope has just been acquired. The Biochemistry Research Laboratory's most prized piece of equipment is a Technicon automatic analyser, at present used for amino-acids, but shortly to be used on carbohydrates as well. A physics research laboratory and a final-year undergraduate laboratory complete the extension.

The previous addition to the Department in 1959 enabled facilities, such as a Spinco model *L* ultra-centrifuge, a Bellingham and Stanley photoelectric spectropolarimeter, and a light-scattering apparatus, to be installed. The present occasion provided the opportunity to add a Perkin–Elmer 237 infra-red spectrophotometer and a 451 gas chromatograph.

In connexion with the opening, the whole department as well as the new extension remained on view for three days, and some two hundred visitors were received. Special food science exhibits illustrated the methods adopted for undergraduate teaching and these included a display by Dr. R. Harper of the Psychology Department, who is responsible in the course for treating problems of perception with special reference to the sensory qualities of foods and food acceptance. A further display, on the history of the Public Analyst's work, was that by the City Analyst, Mr. R. A. Dalley, who lectured on food control aspects.

Present research on view included topics such as the electron microscopy of wheat gluten, the gelling of gelatin and pectin gels, and the isolation of the components of black-currant volatiles.

THE SOUTH-EASTERN UNION OF SCIENTIFIC SOCIETIES

ANNUAL CONGRESS

AT the invitation of the Mayor and Corporation of Tunbridge Wells and the Tunbridge Wells Natural History Society, the South-Eastern Union of Scientific Societies held its sixty-eighth annual congress at Tunbridge Wells during May 9–12 under the presidency of Dr. W. S. Bristowe. The headquarters of the Congress was at the Public Library and Museum; the Young Naturalists' evening was held at the County Grammar Schools for Girls, by permission of the Headmistress.

The delegates were welcomed and entertained by the Mayor, Alderman Donald Savage, who reminded them that the Union was founded at Tunbridge Wells in 1896, only seven years after the town received its Charter. The Corporation takes great pride in its Museum, one of the first to stress the importance of local interest in the display of exhibits.

At the commencement of the Congress, the delegates were conducted on a tour of the town by Mrs. E. Bradley, who, on the route, described the Georgian houses and their past tenants, the origin and development of the Walks, now the Pantiles, and the cures (real and imaginary) obtained by taking vast quantities of the water from the chalybeate springs. Messrs. Boots kindly permitted the examination of the famous bath beneath the floor of their premises. A visit was made to the Church of King Charles the Martyr, where the change in its orientation was discussed.

A Young Naturalists' evening was held, and questions on a wide variety of natural history topics were answered by a team of experts under the chairmanship of Maxwell Knight, prizes being awarded to the girl and boy who submitted the best questions.

After the formal induction to the presidency, Dr. W. S. Bristowe delivered his address on "A Galaxy of Spiders". He commenced by saying how easy it was for a collector in towns to be suspected of misdemeanour. He had been disgraced near Hyde Park Corner where, seeing a prim and proper lady in distress, because of a spider hanging on a thread from her hair, he had removed it, opened his mouth and popped it in. The lady's expression of extreme disgust later became good-humoured. During his visit to Thailand he could not, at first, regard the delicacy of spiders with favour, but, when the feeling of repugnance was overcome, he found they tasted well. Discussing this with a Siamese gentleman, who had attended school in England, the same feeling had occurred when he was asked to eat British green raw vegetables and the decaying carcases of pheasants and hares.

As food, spiders are not poisonous and were used for medicine in early times. He instanced the case of Dr. Muffet's daughter, born 1540, who was treated for all ailments, internal and external, with concoctions of spider. It is possible from this the nursery rhyme of "Little Miss Muffet" evolved.

Throughout the ages human beings have had two reactions to spiders, admiration and fear. A protective mythology throughout the world still causes people to dislike killing spiders, and, in European countries, it is considered lucky to have them alight on one's clothes, a notion originating with the Greeks.

Spiders are of ancient stock, occurring in Carboniferous deposits perhaps 350 million years ago, and, in Asia, some eight species of living 'fossils' survive. Flies appeared 100 million years after spiders, and the first spiders fed on crawling things. These probably evolved wings to enable the avoidance of capture, then spiders spun nets to catch the flying insect; some insects then developed armour (beetles) through which the spider could not penetrate, some became distasteful and hundreds of species of spider would not eat them at all, and what may be described as an evolutionary battle was carried on. The spider population is enormous, millions per acre, and the weight of insects eaten every year is more than the weight of all the people in England. They are good at dispersing themselves, floating in the air on self-woven parachutes; Dr. Bristowe had found four species of spider on a remote arctic island. Although they had nothing corresponding to human intelligence, they had something equally remarkable, the knowledge of how to react.

In studying the mating habits, it is convenient to divide them into three groups: (1) long-sighted; (2) short-sighted; (3) webbed. In the long-sighted group the males are more fragile than the female and decoration has evolved and is displayed to attract the female. The short-sighted males use special grips, sometimes in the jaws, to ensure that once in contact with the female they must not lose her, in case they cannot find her again. The webbed type are rather like the long-sighted and also use a sort of 'Morse code' to attract the female, being able to pass sounds, as vibrations, along the strands of their webs.

The Archæological Section of the Union was addressed by the president, James Money, on "The Iron Age Hill Fort at High Rocks". He spoke of excavations he had carried out, under the auspices of the Sussex Archæological Society, of rock shelters and the fort, mentioning the help science can give to archæology which, in turn, can help science. Charcoal from the rock shelters had been examined by Miss Balfour Browne at the British Museum (Natural History) and the pollen analysis was carried out by Dr. G. W. Dimbleby at the University of Oxford.

The rock shelters were overhanging rocks on the escarpment of the Tunbridge Wells Sand (Lower Cretaceous) and the flint implements found indicated they were occupied during Mesolithic and Neolithic times, ranging approximately from 6000 B.C. to 1800 B.C. There were hearths of sandstone, heavily burnt, and examination of the charcoal confirmed the age arrived at by the artefacts.

At the fortified Iron Age camp, excavation revealed an area of 20 acres protected by double ramparts and ditches, the ramparts made of stone and earth, numerous post-holes showing the defences were strengthened by a timber palisade. A few fragments of pottery were found of the Southern 2*b* and 1*c* types. The outer ramparts were built in the second century B.C. by Wealden inhabitants to repulse the invasions of Belgic people from northern France and Belgium. The inner defences were constructed at the beginning of the first century A.D., and the finds included iron-ware, the upper part of a rotary quern of local sandstone and a fragment of Roman Samian ware. Evidence of agriculture on the site consisted of pollen grains of wheat; a number of hearths, some with layers of charcoal 6 in. thick, were discovered 15 in. below ground-level. Extensive excavation has been carried out at the east entrance, revealing triple fortifications, a paved pathway and an ancient trackway.

The president of the Zoological Section, Prof. H. R. Hewer, chose as the title of his address "Seals". He said he proposed to speak mainly about the grey seals as, during the past ten years, our knowledge of them had greatly increased, for in a book published in 1926, of twenty-six statements regarding them, twenty-two were completely wrong. The methodical examination commenced in 1950 off the coast of west Wales, for the matter had become a political issue through the complaints of depreciation caused by seals from fishermen of north-east England and south Scotland. The matter is now taken seriously, and a responsible officer, J. Smith, of the Nature Conservancy, is stationed in the Orkneys, where the largest colony in the British Isles occurs; as many as 3,000 cubs are born each year. A variety of interests calls for control of a large natural population. When an area is completely protected there is a danger from epidemics and the death-rate increases—the mortality-rate rises to 35 per cent in the first few days of their life. The cubs during the first fortnight of their lives increase in weight from 35 to 90 lb., mostly in blubber, and should a cub leave the breeding ground before reaching this weight it will not survive. Not a single young seal of the hundreds either shot or caught in nets, by people interested in the salmon fisheries, weighed more than 70 lb. In a colony of 1,000 of each sex, a thousand cubs would be born if all the cows became pregnant, and as the cows, on average, live 35 years and the bulls 25 years, controlled preservation is necessary if the colony is to be successfully established. Age is determined by the examination of thin sections, transverse and longitudinal, of the teeth. The weight of the bull must be considerable when it reaches the breeding territory, for it is without food for 6–8 weeks, living on its blubber. The territory of each bull is vigorously guarded against invasion by other bulls, and there are 7–10 cows to each bull; a specimen bull measured 90 in. in length and weighed about a quarter of a ton. Bulls mature in six years and cows in five years; cubs are produced in the following year. Prof. Hewer concluded his address with a commentary on a film he had taken on the Island of Shillay.

Dr. R. Casey, as president of the Geological Section, gave an address on "The Dawn of the Cretaceous Period in Britain". He said that from time to time some new find throws light on an obscure geological problem and permits reassessment of a boundary, such as the one he proposed to discuss, the Jurassic–Cretaceous. Across northern Europe are signs of concerted movements of the sea which indicate a natural starting point of the Cretaceous period. This affects ideas of the geography of Britain in remote times and on the grouping of the strata.

Three separate basins received deposits spanning the Jurassic–Cretaceous boundary: (1) Southern (Kent–Dorset–Bucks.); (2) Spilsby (Lincolnshire–Norfolk); (3) Speeton (Yorkshire). The ammonites of the Spilsby Sandstone show there is no missing interval in the Jurassic marine record in Britain. Genera of ammonites which occur in the Portland of southern England and the Volgian of the U.S.S.R. are also in the lower part of the Spilsby Sandstone. Dr. Casey traced the development through the Portland–Purbeck regression and the incoming Cretaceous tide and said that the Cinder Bed makes a firm datum. He suggested that the advancing Cretaceous sea re-opened the connexion between the Spilsby and Southern basins, flooding the Purbeck swamps and laying the Cinder Bed as the bedrock of the Cretaceous system in southern England; evidence from continental Europe confirmed this view. In conclusion, Dr. Casey said that all lines of evidence lead to the idea that a single advance of the sea initiated the Cretaceous Period in northern Europe, and to overcome nomenclature difficulties he would suggest 'Lulworth Beds' for the sequence

between the Portland Stone and the Cinder Bed, and 'Durlston Beds' for the sequence from the base of the Hastings Beds down to, and including, the Cinder Bed; the former grouped with the Portland Beds, the latter a subordinate and basal division of the Wealden Beds.

Favourable weather enabled the full excursion programme to be carried out: the botanists visited High Rocks and Eridge, the archæologists were conducted to the Rock Shelters and Iron Age camp at High Rocks by J. Money, while the geologists examined beds within the Wealden, in the vicinity of Tunbridge Wells, under the direction of Dr. R. Casey, who dealt especially with the relation between the Wadhurst Clay and the Tunbridge Wells Sandstone.

The excellent arrangements for the Congress were undertaken by the Tunbridge Wells Natural History Society, for whom Mrs. D. Vernede acted as honorary secretary.

F. J. Epps

THE MATHEMATICAL ASSOCIATION

ANNUAL CONFERENCE

THE Mathematical Association's annual conference held at the University of Nottingham in April was outstanding for the high level of interest sustained throughout the sessions. The Association's membership continues to rise steadily and now stands at more than 5,000.

In his report on current affairs. Mr. Combridge spoke of the formation of the Joint Mathematical Council of the United Kingdom which was announced in the Press on April 3. The Council has set up a fact-finding committee to investigate proposals for setting up an Institute of Mathematics.

The Conference on Mathematics in Education and Industry, of which Mr. Combridge is chairman, had held a meeting on March 23 and had adopted the report of its working party. This report was being abbreviated into a pamphlet which he hoped would be circulated to members of the Mathematical Association this summer. The Conference gave its blessing to a scheme put forward by Mr. B. T. Bellis of Highgate School to form a group of schools which would form links with industry which might assist, in particular, with the teaching of mathematics in the later years of school. Mr. Combridge emphasized the need for strengthening the links between schools and industry, and between schools and training colleges and departments of education.

The Universe

In his presidential address, Prof. V. C. A. Ferraro reviewed the advances made in our knowledge of the universe since Galileo first directed his newly made telescope to our nearest neighbours in space. Galileo's discovery of the satellites of Jupiter demolished the last remaining vestiges of the Ptolemaic system. Then followed the rise of descriptive astronomy associated with the remarkable researches of Sir William Herschel, who stressed the need to build large telescopes for a better understanding of the architecture of the universe. He also suggested, with remarkable insight, that the problem of the Milky Way might be resolved by supposing that the stars extended much farther into space along the plane than in the direction of the poles, so as to appear as a great mass of stars along its edge.

The nineteenth century saw the rise of astrophysics and the use of the spectroscope in unravelling the physical conditions of the stars. The classification of stars according to their spectral classes attained its most important form in the so-called Hertzsprung–Russell diagram in which the luminosities of the stars are plotted against the structural classes. The points are found to lie in a narrow belt running diagonally from the brightest stars to the faint red ones, now known as the mean sequence. This diagram has proved invaluable in the study of stellar evolution.

Our knowledge of the structure and size of the universe could only be obtained by the use of large telescopes and principally by the 100-in. telescope at Mount Wilson and the 200-in. telescope at Mount Palomar. They reveal that the Milky Way consists of hundreds of billions of stars arranged in a flattened disk so large that it would take light travelling at 186,000 miles a second 100,000 years to cross the disk. Herschel had long ago suspected that the nebulosities seen in the Milky Way might be systems of stars similar to our own, but lying outside it. His conjecture was amply confirmed by Hubble from his observations at Mount Wilson. Our nearest nebula, Andromeda, is two million light years away from us, while the farthest nebula so far photographed with the 200-in. telescope at Mount Palomar is about two thousand million light years away. Radio telescopes have probed even farther into space to distances of the order five thousand million light years. Within this great expanse there are probably thousands of millions of these galaxies the distribution of which appears to be uniform in space.

Early in 1930 an observation by an electrical engineer, Jansky, marked the beginning of the study of the new science of radio astronomy. He detected some radiation in the decimetre wave-length which he was convinced came from outer space since the signals he received were not observed at the same time on consecutive days but were observed 4 min earlier each day, corresponding to the lapse of a sidereal day instead of a solar day. Jansky's discovery did not occasion much enthusiasm at the time and it was left to an amateur, Reber, to confirm his conclusions. Later still it was recognized that radio signals could be picked up from regions of space farther away from us than would be possible by observations from optical telescopes. Some of the radiation is now known to originate in the interstellar gas, and a brilliant prediction by the Dutch astronomer van der Hulst that this was radiation of 21-cm wave-length emitted by hydrogen clouds in interstellar space was confirmed by his Dutch colleagues. By this discovery it was recognized that our galaxy has a spiral structure very much like that of the great Andromeda nebula.

Prof. Ferraro then referred to the first-fruits of space research and in particular the discovery of the high-energy particles trapped in the Earth's magnetic field and known as the van Allen radiation belt. This consists of two belts, the inner one, at about 2,000 km from the Earth, being quite stable and generally believed to be produced by neutron decay, while the outer belt, extending from three to five Earth radii or more from the Earth, consists for the most part of high-energy electrons. This is rather variable, especially during magnetic storms.

Recent flights by space probes have also shown the absence of a magnetic field on the Moon and Venus and the high temperature on this planet. In the foreseeable future it seems unlikely, however, that space probes could travel much beyond the confines of the Solar System and that, so far as could be seen, space exploration by probes and satellites was a limited venture. Further knowledge of the vast universe around us must still be derived from observation by conventional optical and radio telescopes.

Prof. Ferraro then briefly referred to cosmological theories which had been proposed and noted that there were, at the present day, two rival theories in the field, the evolutionary theory and the steady-state theory.

In the evolutionary theory the universe evolved from a single act of creation in which the whole of the universe was contained in a primeval atom. Because this high concentration of matter was highly unstable the atom exploded with great violence. After a certain period the matter settled down sufficiently for gravitational force to introduce some degree of stability. Thereafter the galaxies became subject to a repulsion represented by the cosmical constant, so that the galaxies began to recede from one another with speeds proportional to their distance apart in accordance with the observed Doppler shift. In the steady-state theory, although galaxies recede beyond the observable horizons, other galaxies will be formed to take their place so that the universe always presents the same aspect. To maintain this steady-state, the advocates of the steady-state theory have had to invoke that matter, in the form of protons, is continually being created at the rate of about one proton per cubic mile of space per year.

Attempts to discriminate between the two theories by observation of numbers of radio sources in space have so far not proved very conclusive. Prof. Ferraro pointed out that in spite of its many attractive features the steady-state theory, in the form proposed by Bondi and Gold, violates the principles of energy and momentum. There is so far no reason to doubt that these principles are valid universally, and to obviate this feature of the theory Hoyle has proposed a variant in which an as yet undiscovered field of force is invoked. Hoyle's theory has the merit of retaining these conservation principles.

Mathematics in Technical Education

A discussion on mathematics in technical education was opened by Mr. D. G. Toose, who outlined the structure of technical education. The mathematics courses aim at showing the students the theoretical structure of their technology and include basic courses in mathematics.

In the past few years there has been a demand for mathematicians as technologists in their own right. They need to be competent mathematicians with an ability to convert a physical situation into a mathematical statement. At present the training of an industrial mathematician may be by a course at a university or at a college of advanced technology. Higher National Certificate and Diploma schemes exist, but at present there is no mathematical institute to award a professional qualification comparable with those available to engineers.

Dr. Kerr spoke of the Diploma in Technology in mathematics. The colleges take great care in placing students in industry for the industrial part of their training whose success depends on co-operation between the college and the firm concerned. The problems set in college have to be somewhat artificial so that they can be solved in two or three hours, but from the beginning of their time in industry the students see how problems really occur; this gives them considerable motivation for the academic work on return to college.

The academic courses have honours degree standing. A general course is followed by specialization later. Numerical analysis, statistics and computing are usually introduced early in the courses. Geometry and modern algebra tend, as a result, to get less time than is usual in university mathematics courses. Dr. Kerr was able to report the average attainment at entry as rising.

Continuing the discussion on mathematics in technical education, Mr. M. Bridger pointed out that there are full-time degree courses available at a large number of colleges of technology. Young people are becoming increasingly aware of the opportunities for programmers, numerical analysts and systems analysts. Some dozen colleges have installed computers and some half-dozen are completing their installations in the near future. An adequate number of mathematicians with industrial experience have taken up teaching posts so that a reasonable number of colleges of technology are in a position to offer courses in industrial mathematics. The trend in these and other courses is away from part-time study.

New Developments in the Teaching of Mathematics

Opening a discussion on new developments in the teaching of mathematics, Mr. A. P. Rollett referred to work being done by schoolmasters in different parts of Britain and went on to speak of the work of the School Mathematics Project of which Prof. B. Thwaites is director. It is clear that he has been influenced a little by activities in the United States. There is no objection to this, provided it is clearly realized that conditions are vastly different and that the real difficulty in the United States is a shortage of suitably qualified teachers. In Britain there are still enough of sufficient calibre to be able to discuss with university teachers what topics are desirable and feasible in the schools. With regard to the reforms advocated by recent publications by the Organization for European Co-operation and Development, Mr. Rollett quoted Prof. R. L. Goodstein's review in the February 1962 issue of *The Mathematical Gazette*, and he went on to contrast the classroom atmosphere in the United States, France and England. In Britain traditions exist, and the immediate need is more recording and publication of teachers' impressions and pupils' reactions to new work done in the schools of Britain.

Dr. A. G. Howson, of the University of Southampton, explained that the School Mathematics Project has concentrated on one of a number of present-day problems: How can new mathematical topics be introduced into the syllabuses and syllabuses be designed that are more relevant to present conditions? It is hoped to encourage more pupils to pursue the study of mathematics further and it is also hoped to bridge the gulf which at the moment separates university from school mathematics. One of the major changes made by the School Mathematics Project has been in the increase in the algebraic content of the school course. The aim has been to convey something of the nature of the concepts involved rather than to impart a definite body of knowledge. Euclidean space is studied by means of geometrical transformations and the teaching of geometry by rote is discouraged. Another important part of the syllabus is the introduction to probability and statistics in which it is hoped to show how data can be collected and displayed, and what conclusions can be logically drawn from them. Throughout, the emphasis has been shifted towards mathematical ideas and away from the acquisition of techniques.

Mr. C. Hope told the conference of the origins of the Midland Mathematics Experiment. He outlined some of the aims of the syllabus: the pupils must be able to solve problems which have arisen in the past; the problems must arise out of the experience of the children; and any topics which are included should have the widest possible applications in science, mathematics, industry, commerce, etc.

The syllabus of the Midland Mathematics Experiment includes the algebras of sets and vectors, vector geometry and matrices, calculus, stage *A* probability, and arithmetic applied to real living and the solution of problems. Mr. Hope spoke of the advantage it would be both to examining bodies and to schools if there could be formed a joint sub-committee which would examine proposed syllabuses so that experiment should not be hindered by the fact that neighbouring schools are often examined by different boards. He suggested that a series of courses should be held at regional centres to consider what can be done with ordinary children, and to stimulate teachers to experiment in their own schools.

Among the other lectures, Mr. G. A. V. Leaf gave an absorbing and refreshing account of some of the practical problems of the hosiery industry, Prof. H. Bondi gave an absorbing lecture in which he introduced special relativity from an unusual point of view, and Prof. D. B. Scott gave a vivid introduction to some of the ideas of topology.

Elections

At the annual general meeting, the election of Mr. J. B. Morgan as president for 1963 was announced. Mr. R. E. Green was elected honorary treasurer and the honorary secretaries, Mr. F. W. Kellaway and Miss R. K. Tobias, were re-elected. Dr. E. A. Maxwell was elected as editor in place of Prof. R. L. Goodstein, who remains honorary librarian. Mr. B. J. F. Dorrington and Dr. E. Kerr were re-elected, and Mr. J. K. Backhouse, Miss E. M. Holman and Mr. C. Steele elected as honorary assistant secretaries. This represents an increase of two assistant secretaries, which reflects the growing amount of work being undertaken by the Association; the annual subscription was raised to 30*s.* in order to finance the Association's commitments. Mr. N. De Q. Dodds was re-elected as honorary assistant treasurer. J. K. BACKHOUSE

THE DANISH ATOMIC ENERGY COMMISSION

THE annual report of the activities of the Danish Atomic Energy Commission for the period April 1, 1961–March 31, 1962*, consists of six chapters dealing respectively with the construction at the Risø Research Establishment; the recent work of the Establishment; international co-operation in the peaceful use of nuclear energy; geological surveys in Greenland and other activities; the technical and administrative organization of the Commission; and the financial accounts of the Commission. The text of Act 170 of May 16, 1962, on nuclear installations (atomic plants), the list of publications and of papers written by members of the staff during the year under review, and a map of the Risø Research Establishment, form annexes to the report.

Eight meetings were held by the Commission, and thirty-six by the Executive Committee during the year. There was no alteration in the membership of the Commission and all members were re-appointed for the period of three years commencing February 1, 1962, with the exception of Profs. J. Bøggild and H. H. Jensen, whose terms of office did not expire until February 1, 1963. The late Prof. Niels Bohr was chairman and Dr. H. P. Christenson vice-chairman of the Commission, with Mr. H. H. Koch, permanent under-secretary of State, chairman of the executive committee. The staff on March 31, 1962, numbered 723, of which 495 were employed in the technical and scientific divisions. The workshop staff consisted of 41 people, including seven apprentices, under the direction of a superintendent, and the library staff of 14 people, headed by the librarian.

The Commission's research activities, which were considerably extended during 1959–60 when the large reactor *DR*3 was completed, have continued to develop and have led in particular to the accomplishment of several joint research projects in co-operation with atomic energy establishments in various countries and with European joint enterprises. Irradiation experiments for the *Dragon* project have been carried out on certain steel samples in the research reactor *DR*2, and work has continued on graphite and uranium irradiation experiments in co-operation with the United Kingdom Atomic Energy Authority.

In the physics department of the Establishment, which houses the meteorological station, the reactor *DR*1, and the linear accelerator, a number of practical courses were held for students from the Technical University of Denmark, for students from the Universities of Århus and Copenhagen, for engineers employed in industry and for operators attached to the reactor *DR*2. The linear accelerator was used for experimental irradiation for both industry and the department. In addition, the sterilization by irradiation of medical equipment, including blood transfusion sets, female catheters, artificial lungs and disposable hypodermic syringes, was undertaken for individual firms. The meteorological station continued its investigation of the meteorological conditions in the lower layers of the atmosphere and the data collected were processed statistically. The neutron crystal spectrometer attached to the reactor *DR*2 was used to measure the total cross-section of organic coolants. A start has been made on the construction of a triple-axis crystal spectrometer for solid-state physical examinations. A low-temperature laboratory is being built and measurements made on superconductive materials. Other work in solid-state physics includes the study of the transport properties of pure metals.

The electronics department was largely concerned with the development and assembly of instruments, but in addition a study was made of tunnel diodes and their use in nuclear instruments, especially discriminators. During the summers of 1961 and 1962 the airborne scintillometer developed by the electronics department was tried out with promising results by the Greenland Geological Survey. The reactor engineering department was occupied with the helium heat transmission experiments *HTTC* for the *Dragon* project, and the organic heat loop was completed. Activities in connexion with the deuterium moderated, organic cooled, reactor (*DOR*) project, included the construction of fuel elements, the hot-cell facility and apparatus for experiments with some of the components for the primary cooling circuit. The reactor physics section, which consists of a theoretical reactor physics group, an experimental reactor physics group and a computer group, concentrated its attention on calculations and measurements in relation to the *DOR* project. A major effort was made to prepare computer codes for use in *DOR* calculations, and, during the year under review, codes were prepared for the calculation of the thermal utilization factor, resonance escape probability, fast-fission factor, and Fermi age. The main tool of the computer group is to be the digital computer *GIER*, which was produced by the Danish Institute of Computing Machinery and which was installed at the Risø Establishment during February 1962. The fast memory of *GIER* contains 1,024 words, each of 42 binary digits, and its magnetic drum memory contains 12,000 words. The addition time is 50 μsec.

The results of the attempt by the chemistry department to obtain, by examination on a laboratory scale, suitable processes for a continuous extraction method for the utilization of the uranium and thorium deposits in Greenland were encouraging. In addition, calculations of the investment and operation costs of an extraction plant showed that the economic use of the deposits in the future is a possibility, and some 200 tons of ore were therefore mined and brought to Risø during 1962 for further experimentation. The radiolysis and pyrolysis of terphenyls, of importance in connexion with the *DOR* project, were studied and the decay schemes for arsenic-78, metastable indium-116 and lanthanum-140 were investigated. The radiochemistry of proteins, the possibility of separation of uranium isotopes in a Ranque–Hilsch vortex tube, and the use of electron spin resonance are some of the other activities of the department mentioned in the report.

During the period under review, more than 6,000 people visited Risø and were shown around the Establishment on conducted tours. The scientific staff of the Commission have co-operated in the teaching at institutes of higher education and several physics courses have been held for primary school teachers and technical college teachers.

* Report on the Activities of the Danish Atomic Energy Commission for the Period from 1 April, 1961, to 31 March, 1962. Pp. 84. (Copenhagen: Danish Atomic Energy Commission, 1962.)

In collaboration with the University Extension Committee of the Technical University of Denmark a series of lectures entitled "Atomic Energy—its Implications and Utilization" have been given in Hjørring and Elsinore. The Library issued 24,014 items on loan during the year, and 2,045 books, periodicals and reports were borrowed from other libraries. On March 31, 1962, the total holding of the Library consisted of 95,000 bibliographical units, of which 30,000 were on microcards.

S. Weintroub

RADIATION HAZARDS IN PERSPECTIVE

THE Expert Committee on Radiation of the World Health Organization consists of a group of international experts under the chairmanship of Lord Adrian. The Committee, together with representatives of several other organizations, met in Geneva during October 24–30, 1961, to discuss the relative somatic and genetic effects of ionizing radiation and of other agents and substances in our environment which have toxic, carcinogenic or mutagenic properties. The report of that meeting has now been published*.

Prominence has been given in recent years to the hazards of ionizing radiation and intensive studies have been made of radiation injuries and elaborate protection measures devised. Emphasis, however, should also be given to the beneficial aspects of many procedures involving radiation exposure, for it is well known that the benefits of the appropriate medical use of radiation in diagnosis and therapy far outweigh the hazards. With adequate protective measures, radioactive materials and nuclear energy can be peacefully and usefully applied in industrial and technological fields.

The report warns that preoccupation with the problems of radiation hazards may obscure the fact that there are many other toxic agents in the environment, some very widespread, which could prove a greater menace to public health. Man-made hazards to health must be reviewed against the background of known or unknown causes of disease, co-extensive with the whole field of medicine. In less-developed areas, man-made hazards account for only a small proportion of illness, but in highly developed areas they are responsible for a significant fraction of illness and death. The statistics given in the report show that in many countries accidents rank third as a cause of death and constitute the leading cause of death in the younger and more productive age groups. Most of the reported injuries caused by chemical toxins have occurred in industry, but increasing attention is being directed to the dangers of air pollution, pesticide residues in food, and food additives. A wide variety of chemical compounds are now known to be carcinogenic and others have been shown experimentally to be capable of genetic changes.

Turning to self-imposed hazards, the report states that it appears quite obvious that cigarette smoking or something closely associated with it is largely responsible for the high incidence of bronchiogenic carcinoma in certain countries. Some anomalies in the geographical distribution of lung cancer strongly suggest that other agents are partly responsible. The most substantial evidence appears to be the higher incidence in urban than in rural smokers. The medical use of radiation for therapy of non-malignant conditions and of diagnosis, where, in the early days of radiological practice, excessive doses may have been given, has occasionally been shown to lead to malignant disease, and there is still need for care and research in this field. The genetic effects of radiation, chemicals and temperature are discussed in some detail. The total incidence of hereditary diseases and defects in man may be estimated at about 6 per cent of all live births. The discovery recently that a surprisingly high frequency of defects in man are associated with anomalies of chromosome number or structural aberrations of the chromosomes suggest, since such types of abnormalities can be produced by radiation, that radiation hazard is more extensive than suspected before. The role of mutation in maintaining the hereditary burden in man is still under debate and an answer is likely to be arrived at very slowly. Mutations in all animals and plants occur naturally, and although the causes of these spontaneous mutations are not well understood, it is estimated that only a small fraction could be due to the natural background radiation to which all living things are exposed. There is no *a priori* reason for believing that ionizing radiation is the only, or even the main, environmental factor in the production of mutations. Some hundreds of chemical agents are known to be mutagenic, but none has been investigated in such detail as radiation. The report calls for more research to assess the genetic hazards of mutagenic chemicals and temperature.

In their concluding remarks, the Committee recommends that studies be undertaken to establish criteria for safe exposure-levels to toxic agents, both for individuals (as in the case of occupational exposure) and for populations (as in the case of pollution of water, food and air). The suggestions that certain chemical agents may have deleterious somatic and genetic effects similar to those produced by ionizing radiations should be investigated, and the international standards of statistical reporting of effects, damage, disability and death because of toxic materials should be improved. In addition, epidemiological and genetic studies of the effects of toxic materials should be made and intensive research stimulated on the mechanism of action of these materials including their carcinogenic and genetic effects.

* World Health Organization. Technical Report Series. No. 248. *Radiation Hazards in Perspective: Third Report of the Expert Committee on Radiation.* Pp. 37. (Geneva: World Health Organization. London: H.M.S.O., 1962.) 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars.

TOBERMORITE GEL IN CONCRETE

TOBERMORITE is now known to be the chief cementing compound in hardened Portland cement and its structure largely determines the properties of concrete. When Portland cement is mixed with a limited amount of water, a plastic, mud-like material called 'cement paste' results; it sets in a matter of hours, becoming reasonably firm, then continues to harden for a period of months, if not years. The hardened paste is the matrix into which the fine and coarse crushed rock or natural sand and gravel enter, thus forming the artificial rock called concrete. Stephen Brunauer, of the Portland Cement Association, Illinois, discussed the role of tobermorite gel in concrete at a lecture to the Institute of Civil Engineers in London in July 1962 and a transcript appeared in *Structural Concrete* (1, 7; January/February, 1963); this has since been reprinted for the Cement and Concrete Association, London.

Brunauer's thesis is briefly as follows. Portland cement is a complex mixture of eight or more compounds of which the two most important are known to be tricalcium silicate (Ca_3SiO_5) and β-dicalcium silicate (β-Ca_2SiO_4), these two silicates constituting about 75 per cent of an

average Portland cement by weight. Reaction of either or both these silicates with water produces similar calcium silicate hydrates but with different amounts of calcium hydroxide. The calcium silicate hydrate is similar to the natural mineral known as tobermorite and is now designated as tobermorite gel, the most important constituent of hardened Portland cement paste, hence of concrete. "Tobermorite gel plays a vital role in determining the rheological or flow properties of fresh cement paste, which properties in turn determine the consistency and workability of fresh concrete. It plays a dominant role in the setting and hardening of the paste and in determining the strength and dimensional stability of hardened paste and concrete. For these reasons I have called tobermorite gel the 'heart of concrete'."

Examination of hardened tricalcium silicate paste after complete hydration suggests the following stoichiometry of the reaction: $2(3CaO.SiO_2) + 6H_2O = 3CaO.2SiO_2.3H_2O + 3Ca(OH)_2$. Dicalcium silicate reacts with water much more slowly than tricalcium silicate and the examination of a mature, four-year-old dicalcium silicate paste can be represented by the equation: $2(2CaO.SiO_2) + 4H_2O = 3{\cdot}3CaO.2SiO_2.3{\cdot}3H_2O + 0{\cdot}7Ca(OH)_2$. Thus the lime/silica ratio in tricalcium silicate is 3, in dicalcium silicate it is 2. Tobermorite gel obtained from tricalcium silicate does not have the same composition exactly as that obtained from the dicalcium silicate, but otherwise the two gels are similar, having the same X-ray diffraction pattern, and when examined with the electron microscope the gel particles look the same. There is, however, in the specific surface area of each a marked difference. It is the nature of the surface and the extent of that surface which combine to determine the cementing power of tobermorite gel. H. B. MILNER

TOTAL TRAPPING OF CHROMATOGRAPHIC EFFLUENTS IN ARGON CARRIER GAS

By DR. P. A. T. SWOBODA

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THE quantitative collection of fractions in preparative gas chromatography is difficult because of the tendency of the sample to condense into fog particles which are then swept by the carrier gas through the collecting trap[1]. This difficulty is avoided if a method of trapping is used in which there is total condensation of the carrier gas together with the sample, followed by low-temperature evaporation of the condensed gas without loss of the frozen sample. Liquid nitrogen (b.p., −196°) is used as the coolant to condense argon (b.p., −186°). Since on liquefaction there is about a thousand-fold diminution of volume considerable amounts of carrier gas may be collected in a small simple trap.

If a closed vessel cooled in liquid nitrogen is connected to the exit of the chromatograph the condensed argon has such a low vapour pressure (*c.* 200 mm mercury at −196°) that a partial vacuum is applied to the detector and column outlet with a consequent disturbance of the operating parameters of the separation. Usually the exit of the chromatograph is at atmospheric pressure and this is achieved by venting the trap to the atmosphere. The reduced vapour pressure of argon on condensation then results in a back-flow of air into the trap, where it is also condensed. With the apparatus shown in Fig. 1 continuous back-flow rates of air between 200 and 400 ml./min were observed with argon in-flows ranging from 11 to 310 ml./min. To prevent contamination by atmospheric pollutants and to reduce the extra volume of liquid collected, it is preferable to allow pure nitrogen gas to be sucked back. This is most easily achieved by connecting the outlet of the trap to a length of glass tubing the end of which dips under the surface of liquid nitrogen. Back-flow rates of between 75 and 200 ml./min of nitrogen gas were observed with argon in-flows ranging from 11 to 600 ml./min. There must, of course, be an upper limit to the rate of flow of argon which can be completely condensed in a particular design of trap and this is solely determined by the rate of heat transfer to the coolant. That this is high is indicated by the fact that in every experiment where initially argon was passed through the 'Drechsel' tube trap at room temperature, the reversal of flow at the outlet of the trap occurred within 5–10 sec of immersion in liquid nitrogen.

Quantitative recovery of the frozen sample on evaporation of the liquid argon–nitrogen mixture is achieved if the input of heat is not so excessive as to cause explosive vaporization, and the ultimate temperature attained is not so high that the sample has a considerable vapour pressure. The simplest procedure is to transfer quickly the trap, with its inlet now closed and the outlet vented to the atmosphere, to a 150 × 25 mm test-tube half-filled with liquid nitrogen. After standing for a few minutes the liquefied gases have evaporated and the trap will eventually attain room temperature. To limit this final temperature rise the test-tube may be immersed in a suitable cryogenic mixture (for example, melting pentane at −131°). For extremely volatile samples it is preferable

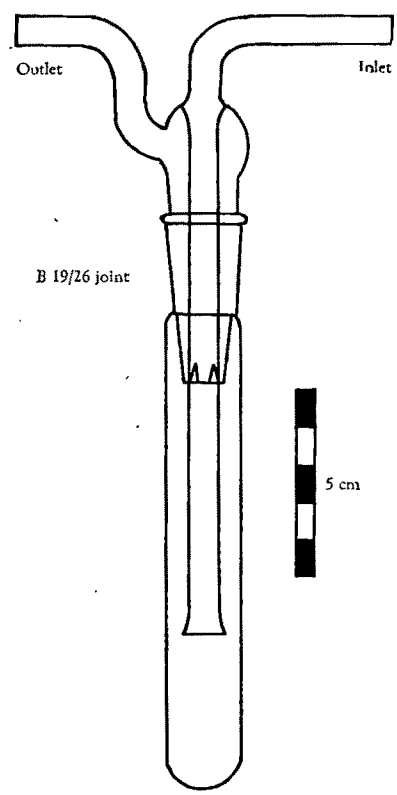


Fig. 1. 'Drechsel' tube used for total trapping. Not shown are the retaining spring and the silicone rubber tubing connexions sealed by Mohr's spring clips

to use liquid oxygen (b.p., −183°). That no loss of sample has occurred may most conveniently be confirmed by connecting a second trap with its outlet closed and re-condensing the total effluent gases, repeating the evaporation and assaying the residue.

Recoveries of 100±3 per cent, usually quantitative within the precision of assay, were obtained with 0·6–26 mg samples of acetic acid, anisole, benzene, hexanal, phenol and propanal. While no loss of acetic acid or of phenol into the second trap was observed when using the pre-cooled test-tube procedure of controlling evaporation, benzene required the use of the melting pentane cryostat. The effectiveness of limiting the rise in temperature is best illustrated by the finding that less than 5 per cent of the sample in the first trap was transferred to the second trap for hydrogen sulphide when using the cryostat, and for ethane when using liquid oxygen.

The design of trap shown in Fig. 1, which consists of a Quickfit 'Drechsel' head *MF*28/2 with shortened stem inserted in an *MF*24/2/6 test-tube, was chosen because it may conveniently be rinsed with small volumes of solvent and yet has sufficient capacity to contain considerable amounts of liquefied gas without development of hydrostatic pressure gradients. Moreover, during the evaporation step the effluent gas does not pass through the inlet tube and therefore cannot cause loss of the sample which may have partly condensed there.

The ease with which quantitative recoveries are obtained by this procedure of total trapping suggests its application not only to preparative gas chromatography but also analytically in combination with independent chemical or physical assay procedures. The trap can be directly connected to an analytical argon chromatograph using the collection adapter previously described[2]. The trap design is in no way critical and the principle of total trapping may equally well be used with other carrier gases such as oxygen, carbon dioxide or steam. Even air at atmospheric pressure can be totally condensed by liquid nitrogen; the 'Drechsel' tube trap with its outlet closed will liquefy more than 200 ml./min (vapour pressure about 640 mm mercury at −196°).

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IMPORTANCE OF MIXING SEQUENCE WHEN USING SET-RETARDING AGENTS WITH PORTLAND CEMENT

By G. M. BRUERE

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INITIAL experiments in an investigation of the actions of set-retarding agents in Portland cement pastes, showed that the sequence of adding these agents to pastes had large effects on setting times. These effects could account for some of the anomalous behaviour of set-retarders in concrete practice.

The set-retarding effects of various amounts of calcium ligno-sulphonate and citric acid were studied in cement pastes made with water/cement ratios of 0·35 by weight, and mixed with a propeller-type mixer at 1,000 r.p.m. Pastes were made from two commercial cements, the calculated mineral compound compositions of which are shown in Table 1. The initial and final setting times of the pastes were determined at 20° ± 1° C with a Vicat needle according to A.S.T.M. method *C*191. Pastes were mixed by the following methods: (1) Water containing a dissolved retarding agent was added to dry cement and the paste was mixed for 6 min. (2) Dry cement was pre-mixed with 90 per cent of the mixing water for 4 min, then the remaining 10 per cent, containing the retarding agent, was added and the paste was mixed for a further 2 min.

Table 1. Calculated Percentage Mineral Compositions of Cements

Mineral	Cement No. 1 Ordinary Portland cement	Cement No. 2 Low-heat Portland cement
Tricalcium silicate	51	31
Dicalcium silicate	25	43
Tricalcium aluminate	10	4
Tetracalcium aluminoferrite	7	16
Total alkali*	1·30	0·15

* Sodium oxide + 0·658 potassium oxide.

The data in Figs. 1 and 2 show that when both cements were pre-mixed with water before adding calcium ligno-sulphonate the setting times were considerably longer than when calcium ligno-sulphonate was added in the mixing water to the dry cements. In addition, differences between setting times produced by the different mixing sequences were much greater in pastes made from cement No. 1, which had a high tricalcium aluminate content, than in pastes made from the low-heat-type cement No. 2, which had a low tricalcium aluminate content. Similar differences between setting times caused by the different mixing sequences were obtained when citric acid was used as a set-retarder. However, at all concentrations, citric acid was a more powerful retarder than calcium ligno-sulphonate.

The mixing sequence effect on setting times is possibly due to competition between the gypsum in cement and

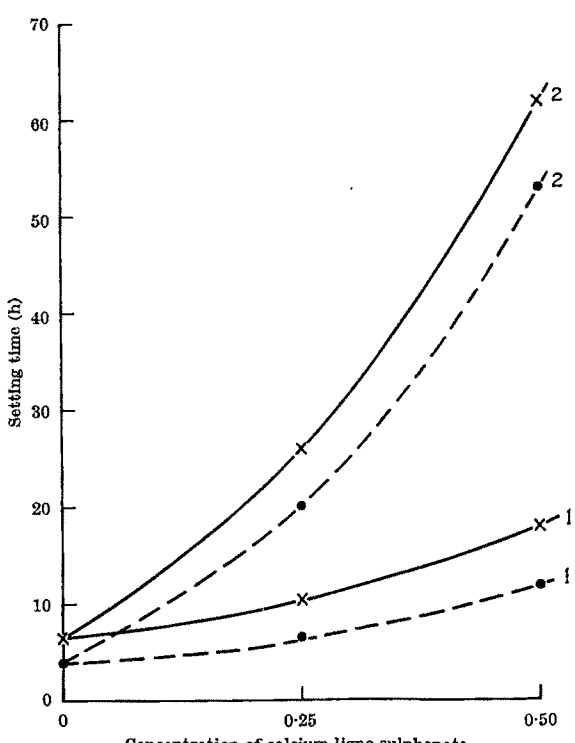


Fig. 1

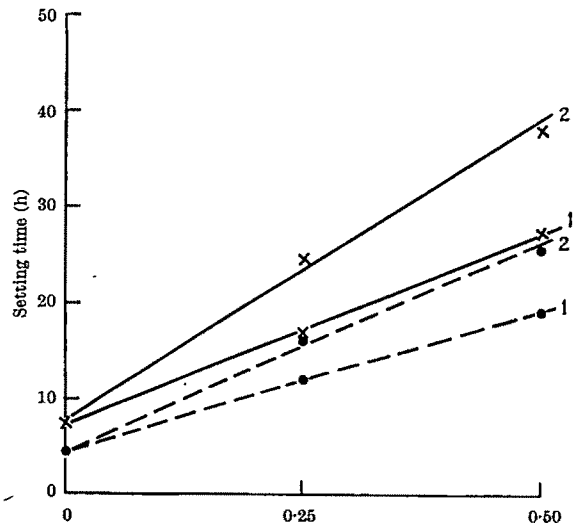


Fig. 2

Figs. 1 and 2. Setting times of Portland cement pastes containing varying concentrations of calcium ligno-sulphonate. Temperature of pastes = 20 ± 1° C, water/cement ratio = 0·35 by weight. ×, final setting time; ●, initial setting time; 1 denotes that calcium ligno-sulphonate was added in the mix water to dry cement; 2 denotes that pastes were pre-mixed with water for 4 min. before adding calcium ligno-sulphonate. The data in Fig. 1 were obtained with cement No. 1 and the data in Fig. 2 with cement No. 2

the organic retarder to combine with or adsorb on the tricalcium aluminate surfaces. Measurements[1] of adsorption isotherms of calcium ligno-sulphonate and salicylic acid on cement and pure cement mineral surfaces showed that tricalcium aluminate adsorbed both these organic materials much more strongly than either dicalcium or tricalcium silicate. Gypsum, which is interground with cement clinker to prevent the rapid hydration reaction of tricalcium aluminate and consequent flash setting of cement, performs this function by rapidly combining with the tricalcium aluminate surface forming calcium sulphoaluminate. When a retarder, such as calcium ligno-sulphonate or citric acid, is added directly in the mixing water to dry cement, it should adsorb on the tricalcium aluminate before any appreciable quantity of gypsum dissolves. This adsorption removes a large amount of retarder from solution and leaves only a small

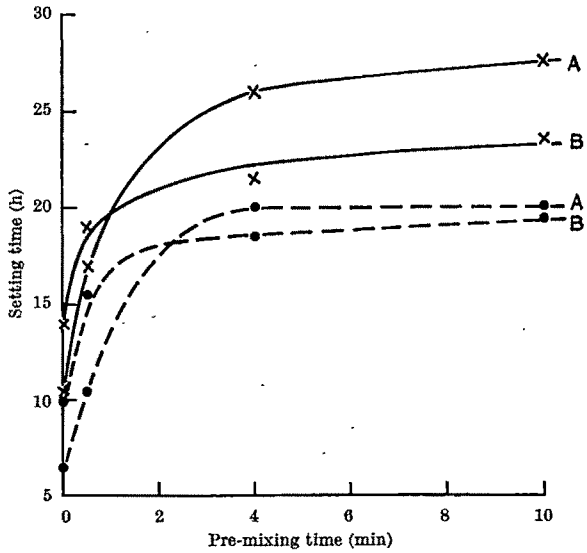


Fig. 3. Variations in setting times of Portland cement pastes with varying periods of pre-mixing before adding set-retarding agents. Cement No. 1, temperature of pastes = 20 ± 1° C, water/cement ratio = 0·35 by weight; ×, final setting time; ●, initial setting time; *A*, 0·25 per cent of calcium ligno-sulphonate by weight of cement, *B*, 0·10 per cent of citric acid by weight of cement

amount to adsorb on the silicate minerals and regard the silicate hydration reactions. In contrast to this behaviour, when cement is pre-mixed with water for a few minutes, gypsum has ample time to dissolve and coat the tricalcium aluminate. Consequently, when the retarder is added to the pre-mixed paste, the tricalcium aluminate is unable to adsorb it and a large amount of retarder is available to retard the silicate hydration reactions.

Fig. 3 shows how setting times of pastes varied with different pre-mixing periods before a retarder was added. The data demonstrate that although pre-mixing periods of 4 min were necessary to get maximum extensions of setting times, pre-mixing periods as short as 30 sec extended setting times by as much as 70 per cent.

In practice, control and prediction of setting behaviour of set-retarded concrete should be improved by specifying the time and sequence of adding water and retarding agent to cement during concrete mixing operations.

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A MOLECULAR APPROACH TO TERATOGENESIS: EFFECT OF VITAMIN A ON INFLUENZA VIRUS *IN OVO*

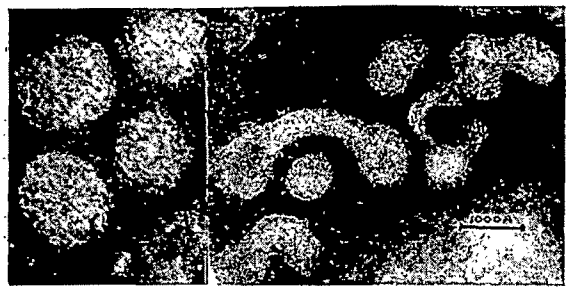
By Dr. H. A. BLOUGH

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IN vitro studies have demonstrated that excess vitamin A may decrease the stability of lipoprotein membranes[1–3]. In a recent investigation, it was shown that vitamin A alcohol drastically altered the morphology of influenza and Newcastle disease viruses *in ovo*[4]. Since the influenza viral envelope represents a large sampling of cell membrane, the negative contrast technique[5] provides a rapid and unique method for detecting alterations in surface structure. The purpose of the work recorded here is to compare the effects of various vitamin A preparations on the integrity and shape of the viral envelope and to attempt to correlate these changes with the known teratogenic action of vitamin A (refs. 6 and 7).

The classical *PR*8 strain of influenza *A* virus was used throughout the experiments. Ten dozen 11-day-old eggs were inoculated with 100 EID_{50} of virus and incubated at 35° C. 6 h after infection, the eggs were divided into four groups and inoculated allantoically with either 150 μg, 50 μg or 5 μg of vitamin A alcohol. The remaining eggs served as viral controls. Allantoic fluids were gathered after 48 h, clarified, and infectivity and hæmagglutination assays[8] were carried out within 24 h. This experiment was repeated on consecutive weeks with all-*trans* vitamin A aldehyde, vitamin A acetate and vitamin A acid.

Virus preparations were partially purified by adsorption to and elution from human group O erythrocytes. Vitamin-treated virus was sedimented at 7,000*g* for 30 min; untreated viral controls were sedimented at 44,000*g* for 1 h. Resultant pellets were resuspended in 0·1 ml. of 1 per cent ammonium acetate, mixed with twice their



Figs. 1 and 2. 1 (left), 'Normal' influenza virus (*PR8*) (× *c.* 124,000); 2 (right), pleomorphic *PR8* virus (vitamin A acetate, 5 μg) (× *c.* 86,000)

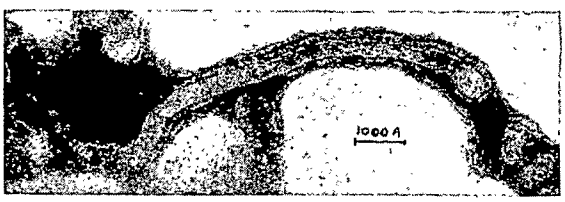


Fig. 3. A filamentous form of *PR8* (vitamin A acetate, 5 μg) (× *c.* 68,000)



Fig. 4. A row of beaded forms of *PR8* (vitamin A acid, 150 μg) (× *c.* 94,000)

volume of 2 per cent potassium phosphotungstate (*p*H 7·0) and sprayed on to carbon-coated grids[5]. These were examined in a Siemens Elmiskop electron microscope. Particle counts, with a minimum of 500 particles, were made at random. Particles were classified as either spherical, filamentous or 'aberrant'—this latter group included all particles > 2000 Å in diameter and others the morphology of which defied description. All experiments were repeated with samples of *PR8* from the World Influenza Centre (courtesy of Dr. H. Pereira).

Untreated viral particles (Fig. 1) were similar to those described by Horne *et al.*[9]. In contrast to these, all vitamin A preparations produced marked pleomorphism (Fig. 2). Filamentous forms (Fig. 3) were most prevalent, constituting approximately 33 per cent of all specimens examined. Occasional rows of beaded particles were observed separately (Fig. 4) or arising from or within filamentous forms. Terminal bodies were occasionally seen. When virus was grown in the presence of 150 μg of (all) vitamin analogues, 'aberrant' forms (Figs. 5 and 6) accounted for 10–12 per cent of the particles. These latter particles possessed external projections and measured up to 5000 Å in diameter (or along their longest axis); with lower concentrations of vitamin, the number of these forms decreased to 5 per cent. The acetate, aldehyde, acid and alcohol were equally effective in the production of filaments and aberrant forms. However, the possibility of vitamin A acetate being hydrolysed *in ovo* to a more active form (for example, the alcohol) could not be excluded.

Table 1. Effect of Various Vitamin A Analogues on the Infectivity and Hæmagglutination Titres of Influenza Virus Strain *PR8*

Vitamin A preparation (150 μg)	EID_{50} (log 10)	*HAU** (log 10)	*ID/HA*
Nil	8·45	2·81	5·64
	8·71	2·81	5·90
	8·50	2·71	5·79
Alcohol	8·45	2·71	5·74
	8·20	2·51	5·69
Acetate	8·50	2·51	5·99
	8·43	2·51	5·92
	8·70	3·01	5·69
Aldehyde†	8·82	2·81	6·01
	8·71	2·81	5·90
Acid‡	8·67	2·51	6·16
	8·14	2·51	5·63
	7·95	2·41	5·54

* Reciprocal of viral dilutions at titration end-point.
† Gift of Dr. G. H. Clement and Hoffmann-La Roche, Basle, Switzerland.
‡ Gift of Dr. J. Lucy, Strangeways Laboratory, Cambridge.

Some partially disrupted particles were seen with all analogues tested. In others, large portions of the viral envelope were dissolved out, revealing a tightly bound internal helix (Fig. 5). Most striking was the effect of vitamin A acid; multiple areas of the envelope were rendered electron-opaque (Fig. 6). In contrast to the effect of saponin on the Rous sarcoma virus *in vitro*[10], hexagonal arrays were not observed. Infectivity and hæmagglutination titres (Table 1) showed little change from the controls, thus suggesting that the nucleoprotein helix was unchanged.



Figs. 5 and 6. 5 (left), Large 'aberrant' particles showing dissolution of viral envelope revealing internal helix (vitamin A acetate, 5 μg) (× *c.* 68,000); 6 (right), partial dissolution of viral envelope (vitamin A acid, 150 μg) (× *c.* 98,000)

The importance of the cell surface in virus-induced congenital malformations[11], and to the localization of defects in an *in vitro* chick embryo system using antimycin-*A* (ref. 12), have been noted. Animal studies qualitating the embryopathic effect of vitamin A have shown that the neural tube, lens primordia and oral cavity are most frequently involved[6,7]. Since these latter structures are surface ectodermal ones, it is suggested that vitamin A exerts its teratogenic effect by a direct physicochemical attack on the cell membrane.

Vitamin A can act on the cellular surface in two ways. First, alterations may occur in the packing of the bimolecular lipide leaflet[13]; a linear orientation of cholesterol-phospholipide molecules would account for the production of viral filaments[4]. Secondly, because of the high degree of unsaturation of vitamin A, a sudden increase in Van der Waals' forces between lipide molecules would cause a 'separating out' of cholesterol from the leaflet[14]; this would account for defects in the envelope. Since cholesterol probably exists as an inclusion compound[15] it is unlikely that these changes are reversible *in vivo*, as has been suggested recently[16]. It would appear that the greater toxicity of the acid on the membrane is due to its larger head group (—COOH) and/or its potential ease of ionization in an *in ovo* system. Furthermore, recent experiments[17,18] have shown that the oxidation of vitamin A aldehyde to the acid is irreversible; thus suggesting that vitamin A acid may be the effective form of the vitamin.

The possibility of utilizing a viral system to elucidate alterations in membrane structure during morphogenesis may provide yet another tool for the rapid screening of potential teratogenic agents.

This investigation was supported in part by a fellowship (*EPD*-11, 468) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

I thank Prof. J. D. Boyd for reviewing this manuscript.

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EFFECT OF 3,4-DEHYDRO-DL-PROLINE ON GROWTH AND PROTEIN SYNTHESIS

By DR. L. FOWDEN, S. NEALE and H. TRISTRAM

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AZETIDINE-2-CARBOXYLIC acid, the lower homologue of proline, acts as a potent proline analogue producing very marked growth inhibition of seedlings[1] and of *Escherichia coli*[2]. Growth inhibition was attributed to the production of abnormal protein molecules in which an extensive and stoichiometric replacement of proline residues by azetidine-2-carboxylic acid had occurred. Pipecolic acid, the higher proline homologue, showed no growth inhibitory effect and was not incorporated into cell protein.

Recently, 3,4-dehydro-DL-proline was shown to produce strong inhibition of growth of several micro-organisms[3]. This inhibition could be reversed by an exogenous supply of proline. No attempt was made to show whether the compound was incorporated into microbial protein. Other biological effects of 3,4-dehydroproline have been demonstrated (cited in ref. 4). The experiments described here show that 3,4-dehydro-DL-proline is incorporated into both higher plant and bacterial protein, replacing an approximately equimolar amount of proline. The effect of this incorporation on the rate of bacterial protein and enzyme synthesis was also determined.

Mung bean seeds were supplied with various amounts of 3,4-dehydro-DL-proline dissolved in water imbibed during 18 h at 26°. The seedlings were then transferred to moistened vermiculite and grown for further periods in the dark[1]. Radicles were detached from batches of collected seedlings (usually 50), and their combined fresh weights used as the index of growth. Proline was supplied together with dehydroproline in the water of imbibition in experiments designed to investigate the reversal of analogue inhibition.

*E. coli C*4 (ref. 5) was grown in a glucose/mineral salts medium[6] in shake-cultures at 37°. This strain forms an inducible β-galactosidase and carries a mutation rendering the formation of alkaline phosphatase insensitive to repression by orthophosphate. In experiments in which induced β-galactosidase formation was measured glucose was replaced by 0·5 per cent maltose and 10^{-3} M methyl-β-D-thiogalactoside added as inducer. Growth was determined by optical density measurement. 3,4-Dehydro-DL-proline was added to exponentially growing cultures (when cell density reached 35 μg dry weight/ml.) at concentrations indicated in the text. Incorporation of amino-acids labelled with carbon-14 into the trichloroacetic acid (TCA)-insoluble fraction and alkaline phosphatase and β-galactosidase levels in *E. coli* were determined as described by Neale and Tristram[8].

In order to extract protein, radicles were macerated in 1 per cent sodium borate solution (*p*H *c.* 9), centrifuged at 25,000*g* (15 min) and a protein fraction precipitated from the supernatant by adjustment to *p*H 4·5 with acetic acid, followed by warming at 60° for 10 min. This protein fraction was twice extracted with 5 per cent TCA (15 min at 90°), twice with 75 per cent ethanol (15 min at 80°), and then successively with ethanol, acetone and ether. Protein of *E. coli* cells was obtained by the procedure described by Roberts *et al.*[7]. The insoluble fraction finally obtained consists of most of the cell protein, together with cell wall material, and is referred to as the 'principal protein fraction'.

Protein fractions were hydrolysed with 6 N hydrochloric acid at 100° for 24 h. Paper chromatography was used to establish the presence of dehydroproline in the hydrolysates. A specific method for identifying the analogue has been developed. Chromatograms were first treated with 0·2 per cent isatin in acetone to reveal the proline spots (dehydroproline gave no colour at this stage); they were then oversprayed with Ehrlich's reagent when the blue spots of proline were rapidly decolorized and dehydroproline spots became an intense pink colour. Less than 0·5 μg dehydroproline could be detected on one-dimensional chromatograms run in butan-1-ol–acetic acid–water (90 : 10 : 29, by vol.) when the analogue moved slightly slower than proline. For analysis, portions of hydrolysates were chromatographed together with a series of known amounts of proline and dehydroproline; an estimate of the proline and dehydroproline content of the hydrolysate could be made after colour development as here.

Incorporation of dehydroproline into seedling protein. Dehydroproline produced a marked growth inhibition of mung bean seedlings although quantitatively it was less effective than azetidine-2-carboxylic acid at similar concentrations (Fig. 1). The growth inhibition caused by providing 4 mg dehydro-DL-proline to 50 seeds was reversed when 8 mg (or more) L-proline were supplied simultaneously. The possibility that dehydroproline became incorporated into protein was examined using radicles detached from seedlings grown for total periods of 24, 48 and 72 h. Dehydroproline could be detected in protein isolated from each type of radicle. However, the number of proline residues replaced in the protein by the analogue decreased as the age of the radicle increased and represented 12–15, 6–8, and 3–5 per cent of the total prolines for 1-, 2-, and 3-day radicles respectively. The growth rate of the radicles in the presence of L-azetidine-2-carboxylic acid or dehydroproline is recorded in Table 1. L-Azetidine-2-carboxylic acid supplied at a level of 3 mg/50 seeds allowed slight initial growth but soon proved lethal. In contrast, after an early initial inhibition had been overcome, it is probable that growth in the presence

Table 1. Rate of Growth of Single Radicles of *Phaseolus aureus* (Mung Bean) Radicles in the Presence of L-Azetidine-2-Carboxylic Acid and 3,4-Dehydro-DL-Proline

Analogue	mg/50 seeds	Radicle fresh weights Day 1 g	Day 1 $\log_{10}$g	Day 2 g	Day 2 $\log_{10}$g	Day 3 g	Day 3 $\log_{10}$g
None	—	0·68	−0·17	1·46	0·16	2·95	0·47
L-azetidine-2-carboxylic acid	3	0·21	−0·68	0·22	−0·66	0·23	−0·64
3,4-dehydro-DL-proline	6	0·31	−0·51	0·58	−0·24	1·14	0·06

of the same amount of the L-form of dehydroproline became logarithmic, as in control seeds.

Effect of dehydroproline on growth and protein synthesis in E. coli. When added to exponentially growing cultures of *E. coli* dehydroproline (20 or 50 μg/ml.) caused severe inhibition of growth. After a short initial period, during which increase in optical density occurred at a rate only slightly lower than that of a control culture, growth became 'linear'. The linear increase continued at a constant rate for about 3 h and then at a slightly diminished rate for at least a further 3 h. Exposure to 5 μg dehydroproline/ml. still resulted in 'linear' growth but after about 2·5 h the culture partially escaped from inhibition, growth becoming exponential, though at a somewhat lower rate than that found in control cultures (Fig. 2). Microscopic examination of stained smears revealed that the average length of cells exposed to dehydroproline was 2–3 times that of control cells.

Dehydroproline (25 μg/ml.) was added to cultures growing exponentially in 15 ml. basal medium supplemented with L-[U-^{14}C]-arginine (3 μmoles; 0·8 μc./μmole). At suitable intervals incorporation of radioactivity into TCA-insoluble material and increases in optical density were measured. Growth and incorporation of radioactivity were also followed in appropriate control flasks without analogue. The results are presented in Fig. 3 and demonstrate that the differential rate of incorporation of arginine into the TCA-insoluble fraction is unaffected by growth in the presence of dehydroproline. Similar results were obtained when basal medium was supplemented with L-[U-^{14}C]-phenylalanine or L-[2-ring-^{14}C]-histidine.

Dehydroproline (3 mg) was added to an exponentially growing culture of *E. coli* (final volume 120 ml.). During the subsequent growth period the optical density of the culture increased linearly from 0·075 to 0·200 in 5·5 h. At the end of the growth period the culture was rapidly cooled and the 'principal protein fraction' isolated as already described. A similar fraction was isolated from a control culture. The 'principal protein fraction' from control cells contained 20–25 μg proline/mg, while that from treated cells contained 8–9 μg proline and 12–14 μg dehydroproline/mg. The analogue accounted for about 60 per cent of the total imino-acid residues present in protein and therefore must have almost completely replaced proline in those protein molecules synthesized after addition of analogue.

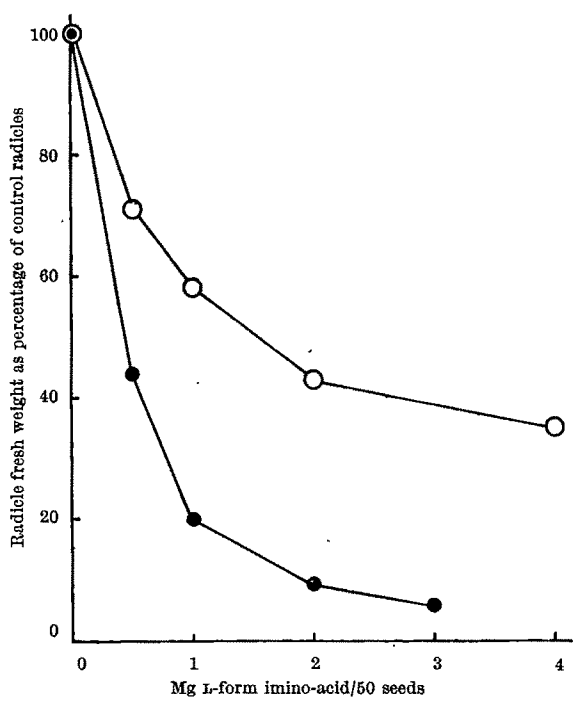


Fig. 1. Fresh weight yield of mung bean radicles after 3 days' growth in the presence of L-azetidine-2-carboxylic acid (●) and 3,4-dehydro-DL-proline (○)

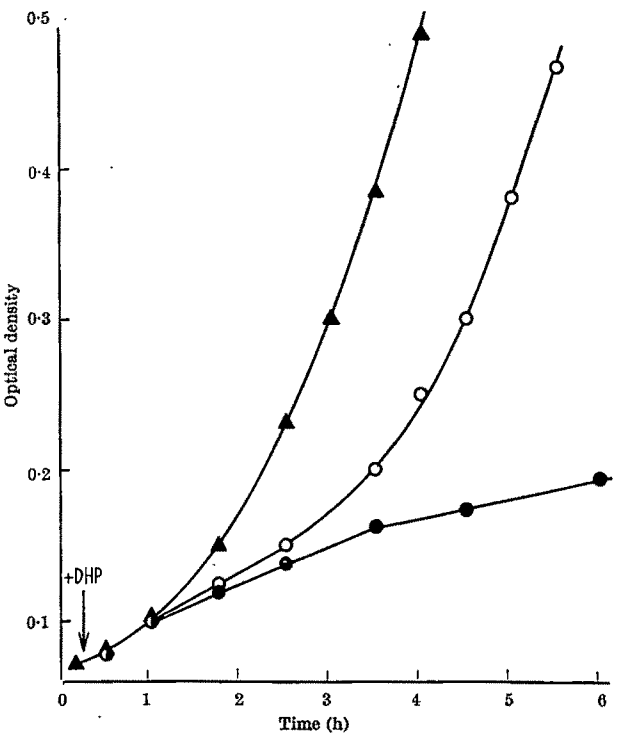


Fig. 2. Growth of *E. coli* in the presence of varying concentrations of 3,4-dehydro-DL-proline. Analogue was added to exponentially growing cultures at the point indicated (+ DHP). Control (▲); plus analogue, 5 μg/ml. (○); 20 μg/ml. or 50 μg/ml. (●)

Synthesis of alkaline phosphatase and β-galactosidase in E. coli. The production of alkaline phosphatase and induced β-galactosidase was compared in normal cells and cells growing in the presence of dehydroproline. The differential rate of synthesis of both enzymes in control cultures remained constant throughout the experiment (Fig. 4*a* and *b*). The presence of dehydroproline (20 or 50 μg/ml.) completely prevented the appearance of β-galactosidase activity. Dehydroproline (5 μg/ml.) also initially prevented β-galactosidase synthesis, but enzyme activity developed later, the differential rate of synthesis being identical with that in control cultures (Fig. 4*a*). The production of active β-galactosidase coincided with the increase in growth rate already noted (Fig. 2).

The differential rate of synthesis of alkaline phosphatase was also affected by the presence of dehydroproline (25 μg/ml.). Initially it remained comparable with that observed in the control culture, but later the differential rate of synthesis of active enzyme decreased. Although the change in rate was not great it was reproducible in a series of experiments (Fig. 4*b*).

Discussion

The initial level of dehydroproline incorporation, although restricting early growth of mung bean seedlings by the production of biologically ineffective protein molecules, apparently did not prevent limited hydrolysis of reserve seed protein. Therefore the amount of free

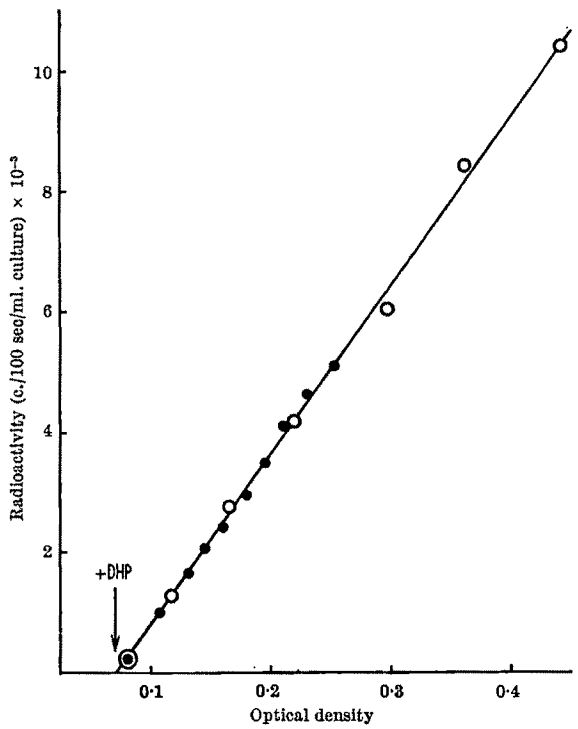


Fig. 3. Incorporation of radioactive arginine into the TCA-insoluble fraction of *E. coli*. 3,4-Dehydro-DL-proline (25 μg/ml.), where required, was added to exponentially growing cultures, as indicated by the arrow (+DHP). Flasks contained 15 ml. basal medium +L-[U-^{14}C]-arginine (3 μmoles; 0·8 μc./μmole). Control (○); plus dehydroproline (●)

proline in the seedlings would increase gradually and eventually would be sufficient to exclude effectively any further incorporation of the analogue into proteins of growing radicles. This stage may be reached at about 48 h, after which the increase in the value of $\log_{10}$ (radicle fresh weight) was about the same for the control and dehydroproline-treated seedlings.

The growth response of *E. coli* to the presence of dehydroproline resembled closely that caused by other amino-acid analogues known to be incorporated into protein (for review, see ref. 9). During the first 45–60 min exposure to analogue growth inhibition was slight, suggesting that the cells at first produced sufficient proline to compete with analogue for incorporation into protein, but that the levels of available proline afterwards decreased, so allowing greater incorporation of dehydroproline, accompanied by 'linear' growth. This behaviour is consistent with the view that dehydroproline repressed enzymes involved in proline synthesis. That amino-acid analogues can 'mimic' naturally occurring amino-acids in operating repression mechanisms leading to cessation of formation of biosynthetic enzymes has been demonstrated in other systems[10,11]. The possible repression of proline biosynthetic enzymes has not been investigated in detail, but a proline-dependent *E. coli* mutant accumulated Δ^1-pyrroline-5-carboxylic acid, an intermediate in the conversion of glutamate to proline, when grown in proline-limiting medium but not when grown in proline-rich medium[12].

This observation could be interpreted as repression of proline biosynthetic enzymes by proline, and although Strecker[12] demonstrated inhibition of Δ^1-pyrroline-5-carboxylic acid formation as a result of end-product inhibition by proline the possibility of partial repression of proline biosynthetic enzymes was not excluded.

Using the value reported by Roberts *et al.*[7] for the weight of proline/g dry weight *E. coli* cells it was calculated that the recovery from growth inhibition observed in cultures exposed to 5 μg dehydroproline/ml. (Fig. 2) was not due to exhaustion of analogue as a result of complete incorporation into protein. Escape from growth inhibition in the presence of low concentrations of dehydroproline could be explained by metabolic conversion of the analogue to a non-toxic compound, though there is no evidence for such a conversion. Alternatively, if dehydroproline repressed proline biosynthetic enzymes, the fall in dehydroproline concentration due to incorporation of some of the analogue into protein could lead to dehydroproline levels no longer effective in repression. Proline biosynthesis would then recommence, leading to proline concentrations sufficient to compete with the analogue and prevent its further incorporation into protein.

Incorporation of either arginine, phenylalanine or histidine occurred at the same differential rate in both control and dehydroproline-treated *E. coli* cells, indicating the continued synthesis of protein in the presence of the analogue. A high level of replacement of proline by dehydroproline residues in protein was attained and was accompanied by failure to produce active β-galactosidase. Earlier workers[13,14] have concluded that such observations are consistent with the view that enzymatically inactive proteins may be formed as a result of replacement of naturally occurring amino-acids by appropriate structural analogues. However, it has been demonstrated[15] that β-galactosidase formation by a 'regulator-constitutive' (*i*⁻) *E. coli* strain could be reduced by growth under conditions leading to accumulation of metabolic intermediates, and it has been suggested[9] that this mechanism may operate under conditions of low growth rates, as observed during exposure to amino-acid analogues. The possibility that formation of a β-galactosidase-like protein occurs in the presence of dehydroproline is under investigation. In *E. coli C*4 the synthesis of active induced β-galactosidase was prevented even when growth was little affected. It has been suggested[16] that induction involves the antagonism

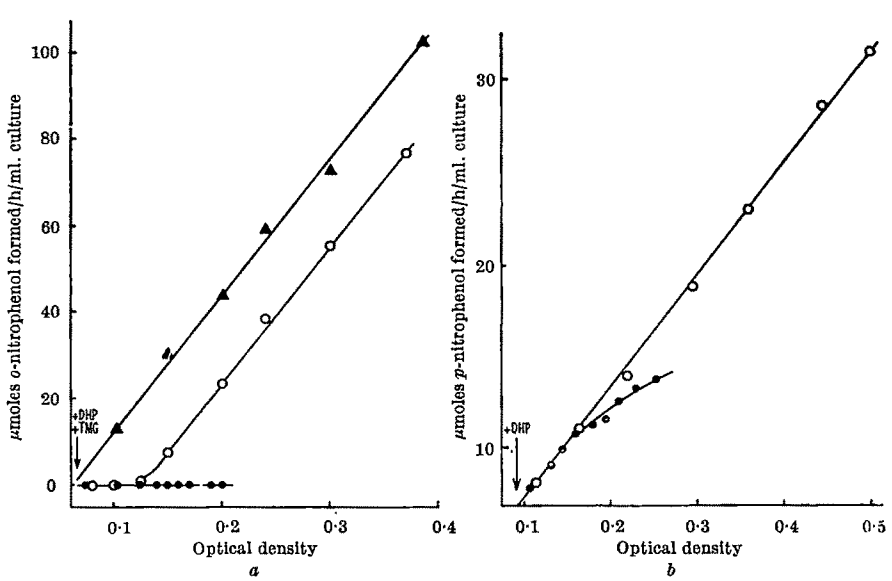


Fig. 4. Differential rate of synthesis of (*a*) β-galactosidase and (*b*) alkaline phosphatase in the presence of 3,4-dehydro-DL-proline. Arrows indicate the point of addition of analogue (+DHP) and, in (*a*), 10^{-2} M methyl-β-D-thiogalactoside (+TMG). (*a*) β-Galactosidase: control (▲); plus dehydroproline, 5 μg/ml. (○); 20 μg/ml. or 50 μg/ml. (●); (*b*) alkaline phosphatase: control (○); plus dehydroproline (25 μg/ml.) (●)

by the inducer, of a repressor formed by the relevant regulator gene. If, as appears likely, the repressor is protein[17], minor modification resulting from incorporation of very small amounts of dehydroproline might render it incapable of combination with the inducer. This explanation is unlikely since formation of active β-galactosidase is also stopped abruptly by addition of dehydroproline (25 μg/ml.) to exponentially growing cultures of 'regulator-constitutive' (i^-) and 'operator-constitutive' (o^c) strains of *E. coli*[18]. In both these strains, since production of β-galactosidase is not dependent on the presence of a functional repressor, even if the repressor were affected by dehydroproline, synthesis of the enzyme might be expected to continue at least during the period when growth was scarcely affected (cf. alkaline phosphatase).

Synthesis of alkaline phosphatase differed from that of β-galactosidase in that the differential rate of formation was at first identical with that of control cells, but later decreased. Since, at identical analogue-levels, the differential rates of incorporation of several labelled amino-acids remained constant, it seems likely that the fall in the differential rate of formation of active alkaline phosphatase coincided with the production of enzymatically impaired protein due to the incorporation of progressively increasing amounts of dehydroproline towards the end of the experiment.

We thank Dr. B. Witkop of the National Institutes of Health, Bethesda, Maryland, for a gift of dehydroproline, and Dr. A. Garen of the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, for a culture of the bacterial strain used. One of us (S. N,) thanks the University of London for the award of a Keddey Fletcher–Warr studentship.

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ACTION SPECTRUM OF MITOTIC FERROUS COMPLEX

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"UNKNOWN mitotic ferrous complex" was my provisional name for an endogenous iron compound which I deduced must exist in root tips, and which seems to be an indispensable participant in the mitotic process[1]. My chain of reasoning was as follows. Cell divisions actually in progress in pea root tips can be arrested by oxygen lack or cyanide poisoning[2]. It would be natural to assume that the arrest occurred because of the stoppage of respiration through cytochrome oxidase. However, quantitative measurements of the rates of mitosis and respiration as functions of oxygen tension showed that a far higher degree of anaerobiosis was required to arrest mitosis than sufficed to stop respiration[3,4]. Hence not cytochrome oxidase but some other component of the cell must be responsible for the stoppage of mitosis. Now the fact that both oxygen lack and cyanide inhibit mitosis suggests that a heavy metal is involved. If the heavy metal should happen to be iron, then it would be expected that carbon monoxide would also be capable of arresting mitosis (in the dark), and that the inhibition would be relieved by illumination[5,6,21]. Both these predictions were fulfilled by experiment[1], which provides very strong evidence that an iron compound is indeed responsible for the arrest of mitosis. Further tests showed that the inhibition of mitosis by hydrogen cyanide was also reversed by light[1] (experiment based on ref. 7), which indicates that the iron compound remains always in the divalent state. Hence the responsible component was referred to as the 'mitotic ferrous complex' (MFC).

It will be recalled that this chain of reasoning lies closely parallel to that of Warburg[6] in his celebrated series of investigations leading to the recognition of the *Atmungsferment*, now known as cytochrome oxidase. That being so, it was considered desirable to pursue the analogy one step further, and try the efficacy of light of different wave-lengths in reversing the inhibition of mitosis by carbon monoxide. This procedure enabled Warburg *et al.*[8] to plot the relative photochemical absorption spectrum ('action spectrum') of the respiratory pigment, which was not identified or isolated until many years later. In the hope that history might repeat itself, I was encouraged to embark on what is technically rather an exacting series of experiments. However, in order to find out which parts of the spectrum would later repay the most detailed investigation, the whole visible spectrum was first surveyed in a single preliminary experiment. The results of this experiment showed so clearly that the MFC is a ferroporphyrin, that it was felt to be of interest to report the results at this stage.

Under selected conditions, the rate of mitosis may be calculated from the mitotic index (M.I.) observed at a known time after excising the root tips from the seedlings[4]. The largest difference in M.I. between darkness and (white) light was observed[1] in 10 per cent carbon monoxide, 20 per cent oxygen and 70 per cent nitrogen, so this gas mixture was chosen for the present purpose. The experimental cell was constructed from a Beckman silica cuvette of 1-cm light path (Fig. 1). The 2-mm long root tips (*Pisum sativum*, var. Alaska, 2-day seedlings) were placed on a 3 cm × 1 cm glass slide in pairs, with their cut surfaces in contact, so as to form a continuous double file of 20 pairs, covering a length of 20 mm. A spectrum 20 mm wide from red edge to violet edge would be projected on to the root tips, so that each pair of tips would be exposed to radiation of different wave-length, and between them they would cover the whole visible spectrum. The invisible extremes of the spectrum were masked out, and two sets of 3 tips were placed near the ends of the slide, to serve as controls in 10 per cent carbon monoxide in darkness. The tips were moistened with about 0·03 ml. of water, and the slide was inserted in the cuvette. A cup containing 0·05 ml. N potassium hydroxide was included to absorb carbon dioxide, and the cuvette was closed by a square gum-rubber stopper pierced with two 20-gauge hypodermic needles. The moist gas mixture (150 ml.) was flushed through the cell at the start of the experiment, and a further 50 ml. was passed each hour thereafter to replace the oxygen consumed by respiration.

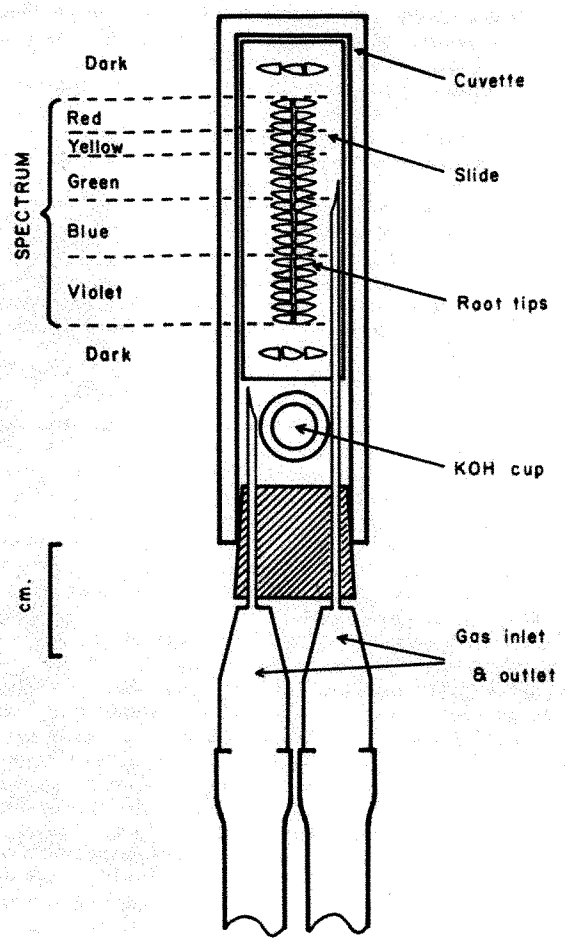


Fig. 1. Experimental cell for illuminating root tips with light of different wave-lengths, under controlled atmosphere

The cuvette was placed in a Beckman cell holder laid flat on a piece of black velvet, and clamped in position.

I thank Prof. J. E. Gullberg of this Department for kindly setting up the optical and electrical equipment necessary for projecting the spectrum, determining the wave-length and measuring the light intensity. The light source was an upright, 21-W straight filament incandescent lamp. The light passed horizontally through an aspheric lens and a large triangular flint-glass prism laid flat, being finally reflected vertically downwards in focus on to the root tips by a surface aluminized mirror. The whole apparatus was set up in a darkroom at 23° C. At the end of the experiment the exact positions of the root tips relative to the red end of the spectrum were found by placing the glass slide on a 1 cm wide strip of mm ruled co-ordinate paper. This paper was later placed in the cuvette *in situ*, and the distribution of wave-length along the cuvette was plotted by inserting in turn in the light path a series of 33 narrow-band interference filters of known maximum transmission wave-length, and noting the position of the transmission band on the co-ordinate paper. The distribution of light intensity through the spectrum was determined with a windowless Moll-type linear thermopile (1 mm slit width) which was moved by millimetre increments through the spectrum at a position equivalent to that of the root tips. The galvanometer deflexions were converted to absolute units (μcal/cm²/sec) by calibration with a radiation standard lamp.

A time of 65 min elapsed between excising the root tips and getting them in place in the cuvette. They were exposed to 10 per cent carbon monoxide in the spectrum for 230 min; and a further 20 min had passed by the time the root tips had been removed (in pairs) and fixed in separate vials of acetic alcohol. The M.I. was counted for each pair of tips, and converted by means of a calibration curve derived from previous experiments[3,4] into amount of mitotic progress. The mitotic progress made by root tips while exposed to 10 per cent carbon monoxide was expressed as a percentage of the mitotic progress made by root tips exposed to air for the same period. This percentage represents the 'relative rate of mitosis', that is, the average rate of progress of cells through the visible phases of mitosis[4].

The results are shown in Fig. 2. The upper broken line represents the rate of mitosis in air (100 per cent), and the lower broken line shows the effect of 10 per cent carbon monoxide in darkness, which decreased the relative rate of mitosis to 58 per cent of normal. The upper curve demonstrates clearly that only certain regions of the spectrum were effective in preventing the inhibition of mitosis by carbon monoxide. Light in the blue-violet and in the yellow-orange regions completely removed the inhibitory effect of carbon monoxide. In fact, a slight stimulation of the rate of mitosis was revealed. The most effective wave-lengths were 436–466 mμ and 578–612 mμ. (There was also possibly a minor peak in the blue-green at 510–525 mμ, but the height of this peak was close to the counting error in determining the M.I.) The lower curve shows the steep fall in light intensity from the red towards the violet, on account of the nature of grey-body radiation from the filament, and the greater dispersion by the prism in the violet. It is notable that although the blue light had only about one-tenth the intensity of the yellow, yet it was just as effective in reversing the inhibition by carbon monoxide.

Insufficient data are yet available to convert these results into a complete photochemical absorption spectrum according to Warburg's procedure[5]. Nevertheless, the form of this simple action spectrum (Fig. 2) allows some important conclusions to be reached. The spectrum is definitely that of a (carboxy-) ferroporphyrin, with its

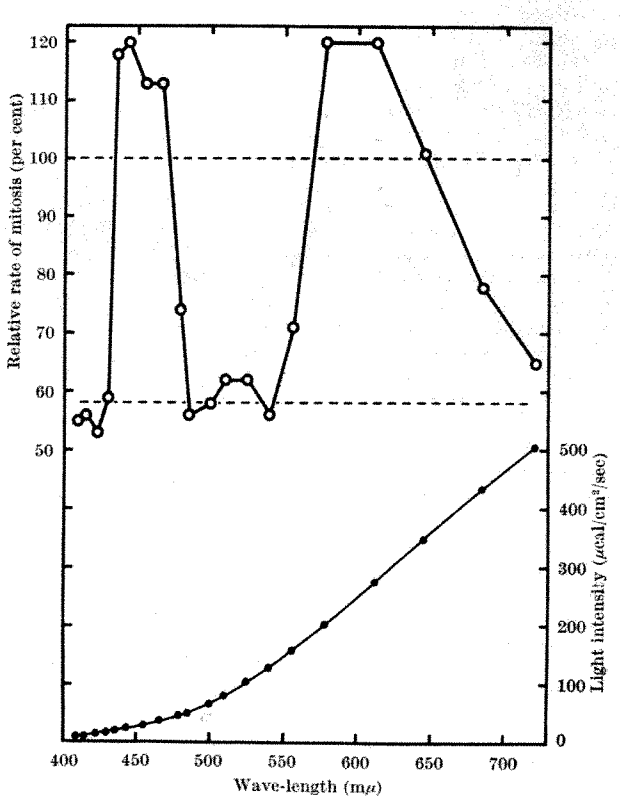


Fig. 2. Action spectrum of the mitotic ferrous complex (MFC, or mitohæm), in 10 per cent carbon monoxide. The upper curve shows the relative rates of mitosis at each wave-length, and the lower curve the corresponding light intensities

Soret or γ-band in the violet and its α-band in the yellow. The roughly 10 times stronger absorption in the violet compared with the yellow is in keeping with this conclusion. One may even follow Warburg's argument[9] a little further; the γ-band is at too long a wave-length for it to be a red hæmin, whereas the α-band is at too short a wave-length for it to be a green hæmin. In fact the positions of the absorption bands suggest that the MFC belongs to the class of red/green hæmins, or phæohæmins. The phæohæmins include Warburg's *Atmungsferment* or cytochrome oxidase, the invertebrate respiratory carrier chloroeruorin, and the disputed 'Pasteur enzyme' of Stern and Melnick[10].

From quantitative measurements of the relative affinities of MFC for oxygen, carbon monoxide and hydrogen cyanide, I had earlier concluded that the MFC must be very closely analogous to *Gastrophilus* hæmoglobin and to the reduced form of cytochrome oxidase[1]. The absorption spectra of *Gastrophilus* hæmoglobin[11] suggest, however, that it is a red hæmin rather than a phæohæmin. The relative affinity of chlorocruorin[12] for carbon monoxide and oxygen is completely different from that of the MFC. Hence, among the known naturally-occurring ferrohæmoproteins, only cytochrome oxidase (cytochrome a_3) and the Pasteur enzyme remain as close relatives of the MFC. Functionally the MFC is distinct from both, because it is involved with mitosis, whereas cytochrome a_3 is concerned with respiration and the Pasteur enzyme perhaps controls glycolysis. In its detailed properties MFC is distinct from cytochrome a_3 because with oxygen the latter undergoes autoxidation to the ferric state (at least in the presence of cytochrome *c*, ref. 13), whereas the MFC becomes oxygenated rather than oxidized. Also cytochrome oxidase differs from the MFC in the relationship of the partition constant to the CO/O_2 ratio, for the partition constant for cytochrome a_3 depends on the simple ratio (CO/O_2), whereas that for the MFC requires the fourth root thereof $(CO/O_2)^{0.25}$. The Pasteur enzyme has not been characterized in enough detail for it to be possible to decide which of the two, cytochrome a_3 or MFC, it resembles the most.

The function and properties of the MFC seem to be sufficiently distinct and definite to qualify it for a separate name. From its demonstrated involvement in *mito*sis, and its characterization as a ferro*hæmo*protein, I suggest that the MFC be called *mitohæm*.

The discovery of mitohæm, and the consideration that cytochrome a_3, Pasteur enzyme and mitohæm may all co-exist in dividing cells, seem to call for some general comments on their interrelationships. Their chemical properties, particularly their susceptibilities to inhibitors, are so similar that it is experimentally rather difficult to distinguish between them and their respective functions. There are in fact long histories of controversy, both as to whether the Pasteur effect is due to respiratory inhibition[14], and as to whether the arrest of mitosis is caused by stoppage of respiration[1,15]. There is evidence that the order of susceptibility of the three hæmoproteins to the same inhibitor may vary from one tissue to another; for example, the Pasteur enzyme is more sensitive than respiration to oxygen lack in retina, chorion and sarcoma, and less sensitive in nucleated blood cells[16]. Such variability may help to account for the recent finding of Epel[17] that mitotic arrest in sea urchin eggs by oxygen-lack, carbon monoxide or hydrogen cyanide is actually caused by the fall in adenosine triphosphate (ATP) content due to respiratory inhibition, whereas carbon monoxide failed to exhibit any selective effect on MFC; and my opposite finding[18] that the arrest of mitosis in pea roots by these inhibitors is quite independent of ATP-level, but is brought about through the MFC.

I have shown that carbon monoxide can lead to stimulations of both mitosis and respiration, provided its inhibitory effects on the iron-containing systems are overcome by strong light, and I suggested that some other heavy metal, not iron, must be involved in mediating the stimulation[1]. The known occurrence of copper, of indefinite function, in cytochrome oxidase[19], suggests the possibility that it may act as a regulator of the activity of the cytochrome oxidase. If copper should also be associated with the closely related mitohæm, it could explain the stimulating effect of carbon monoxide in the light on mitosis.

There is recent evidence from microspectrophotometric investigations that a hæm pigment is present in the chromosomal regions of mature erythrocyte nuclei in the newt, and it is suggested that it may play some part in maintaining the condensed state of the chromosomes[20]. A possible concordance between these results and mitohæm suggests itself.

It must be emphasized that mitohæm represents an important, vulnerable participant in the mitotic cycle. Following its chemical identification, it should prove possible to devise selective co-ordinating agents which will combine with and inactivate the ferrous atom of mitohæm, without affecting the Pasteur enzyme or cytochrome oxidase. For example, carbon monoxide itself demonstrates the possibility[1,21], and ethylisocyanide may be a further step in the right direction. Ethylisocyanide selectively inhibits the Pasteur effect without affecting respiration[22], and it is also a very potent inhibitor of mitosis[23]. This last observation prompts the thought that some other known mitotic inhibitors may actually bring about their effect by inactivating mitohæm.

Considering the close relationship among cytochrome oxidase, Pasteur enzyme and mitohæm it seems appropriate to mention Warburg's belief that cancer cells are those which have reverted from respiration to fermentation[24]; in other words, that their Pasteur effect is out of control. Remembering that cancer cells are almost by definition those in which mitosis is out of control, it may be suggested that the cancerous condition is associated with a reciprocal imbalance among the normal activities of cytochrome a_3, Pasteur enzyme and mitohæm. In any event, it is clear that the complex relationships among these three phæohæmoproteins will amply repay the most detailed investigation.

I have already thanked Prof. J. E. Gullberg for his help. I also thank Prof. D. Mazia for the hospitality of his Laboratory. The work was carried out while I was a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

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ACETYLCHOLINESTERASE ACTIVITY OF CAPILLARY BLOOD VESSELS IN THE CENTRAL NERVOUS SYSTEM OF THE RABBIT

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DURING an investigation into the localization of acetylcholinesterase in the rabbit's central nervous system (to be published) it was noted that capillaries giving a positive reaction to acetylcholinesterase histochemical staining were present in some areas of the brain.

After being killed by intravenous 'Nembutal', normal rabbits were perfused via the aorta with saline followed by neutral formalin for 3 h, after which the brain and medulla were removed and placed in cold buffered formalin overnight (4° C). Frozen sections 40μ thick were then treated by Shute and Lewis's[1] modification of the Koelle[2] technique using acetylthiocholine iodide as substrate.

To confirm that the esterase present in capillaries was acetylcholinesterase, butyrylthiocholine iodide, which is hydrolysed by cholinesterase but not by acetylcholinesterase, was used as a substrate on a number of sections. Also in another series of controls using acetylthiocholine iodide as the substrate the specific acetylcholinesterase

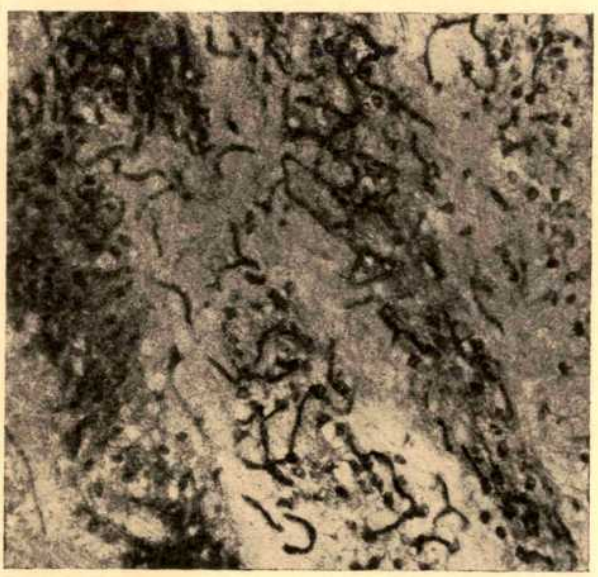


Fig. 1. Medulla. Olive: acetylcholinesterase-positive capillaries (× 75)

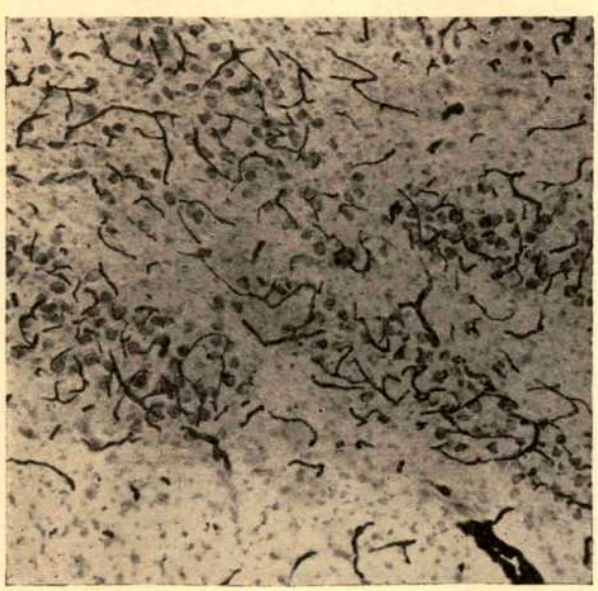


Fig. 2. Medulla. Olive: indian ink-filled capillaries (× 75)

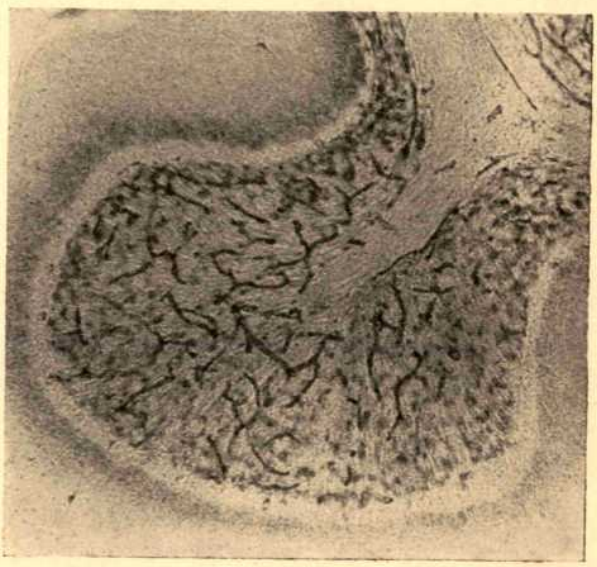


Fig. 3. Cerebellum. Acetylcholinesterase-positive capillaries in granular layer only (× *c.* 66)

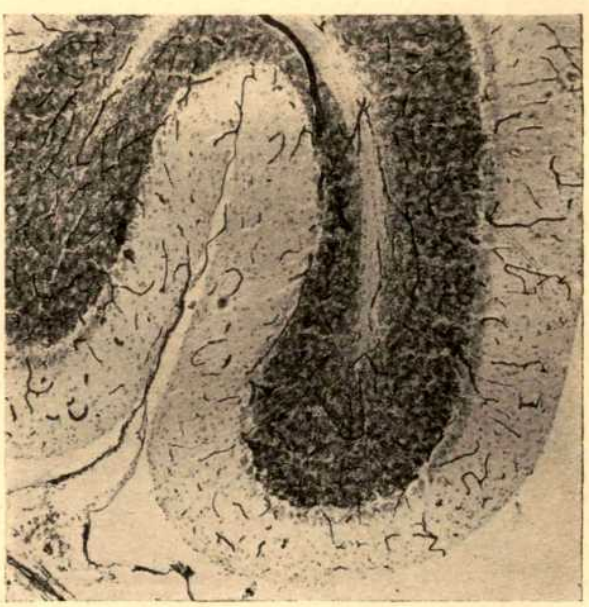


Fig. 4. Cerebellum. Indian ink-filled capillaries present in all layers (× 60)

inhibitor 62 *C*47 was added. These two control series gave consistently negative results.

As an indication that the structures concerned actually were capillary blood vessels, the general picture presented was compared with that provided by indian ink perfusion.

In all about 25 rabbits were used for the enzyme preparations and two for the indian ink perfusion.

Although the indian ink preparations showed that small capillaries were abundant throughout the central nervous system with an increase in density in grey matter relative to white, the acetylcholinesterase-positive capillaries were limited in their distribution and appeared to be present in the following areas only:

(*A*) *Medulla.* (1) Olive (Figs. 1 and 2); (2) a small area of the nucleus of the spinal tract of the V nerve; (3) vestibular nucleus.

In addition weakly positive acetylcholinesterase-positive capillaries were often found lateral to the X nerve nucleus and in the reticular formation, particularly around the lateral nucleus.

(*B*) *Cerebellum* (Figs. 3 and 4). Acetylcholinesterase-positive capillaries were abundantly present in the granular layer yet absent in the molecular and Purkinje cell layers. Indian ink preparations showed that capillaries were present almost equally in all layers with perhaps a slight preponderance in the granular layer.

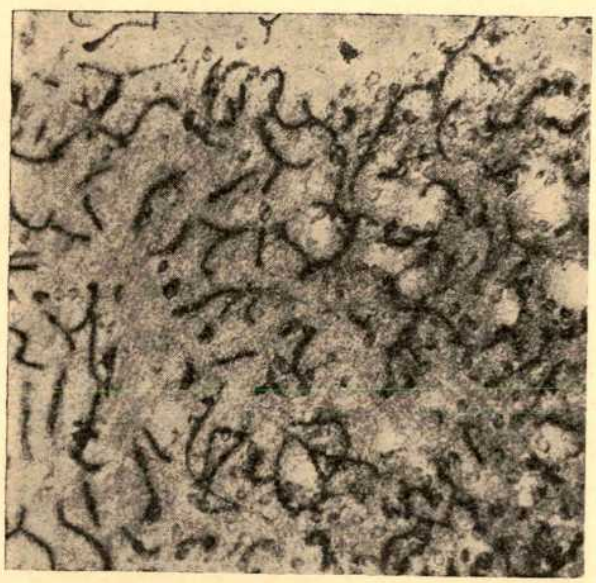


Fig. 5. Thalamus. Acetylcholinesterase-positive capillaries (× 75)

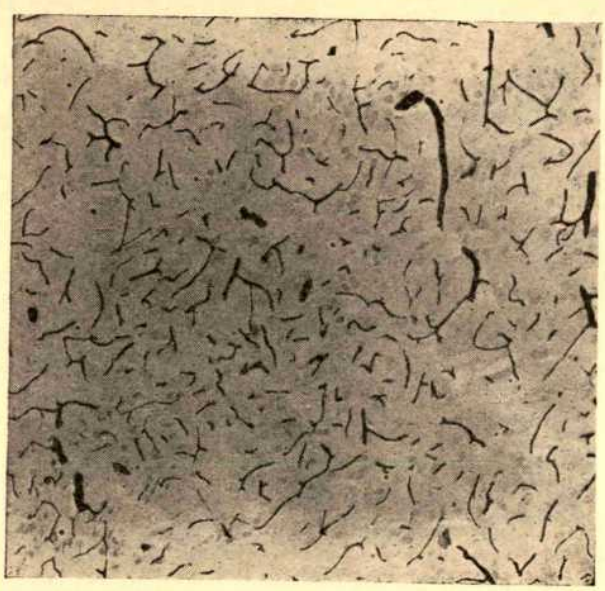


Fig. 6. Thalamus. Indian ink-filled capillaries (× 75)

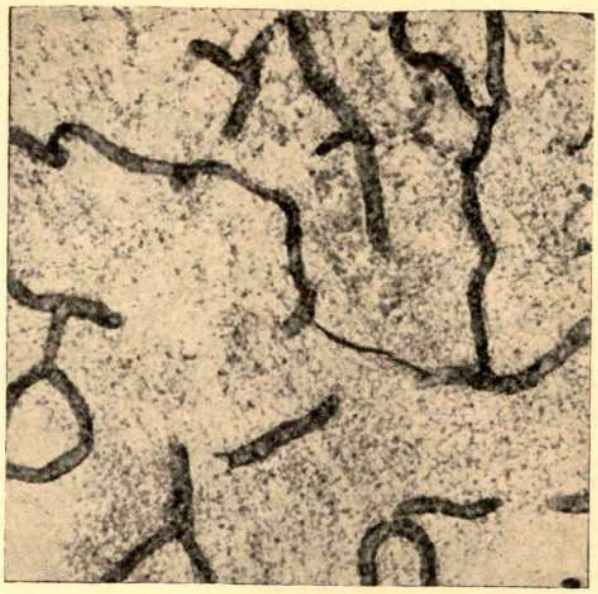


Fig. 7. Thalamus. Acetylcholinesterase-positive capillaries and threads (× *c.* 200)

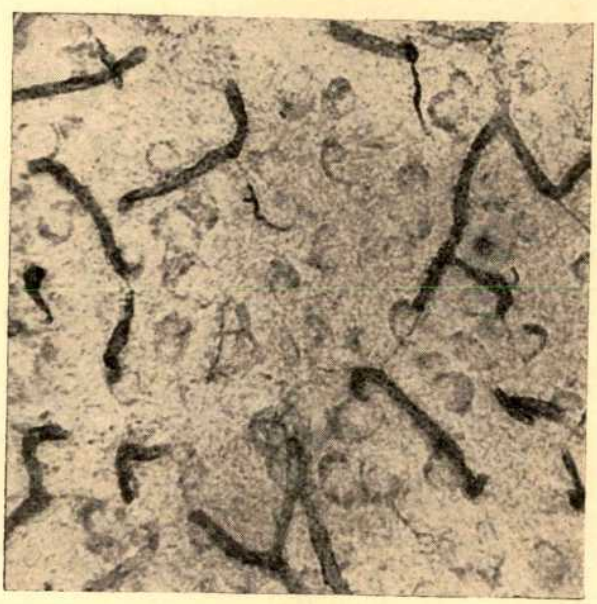


Fig. 8. Thalamus. Acetylcholinesterase-positive capillaries and threads (× 200)

(*C*) *Forebrain.* (Figs. 5, 6, 7 and 8.) Acetylcholinesterase-positive capillaries were more abundant in the region of the thalamus than in any other area of the brain investigated while none was present in the region of the hypothalamus. Indian ink preparations showed that capillaries in the thalamus were denser than those in the region of the hypothalamus bordering the third ventricle. No acetylcholinesterase-positive capillaries were observed in the cerebral cortex or corpus striatum although indian ink-filled capillaries were abundant.

Figs. 7 and 8 are high-power views of acetylcholinesterase-positive capillaries in the thalamus. They have a cylindrical appearance and are hollow on cross-section, indicating that the enzyme activity resides in the wall of the vessel and not within the lumen: although apparently endothelial, the exact site of activity has not been determined. Close inspection shows the presence

of isolated acetylcholinesterase-positive strands which link two or three capillaries together and terminate in their walls. The nature of these strands has not been investigated.

Discussion. Capillaries in most parts of the body do not give a histochemical reaction for cholinesterase but D'agostino and Rossatti[3] have shown acetylcholinesterase to be present in the walls of blood capillaries supplying the germinal centres of lymphatic follicles in mammals and man, and Cavanagh and Holland[4] describe acetylcholinesterase-positive capillaries in the spinal cord and brain stem of chicken. In addition, Koelle[2], Palmer and Ellerker[5] have described cholinesterase in the capillary walls of rats and sheep brains.

Gerebtzoff[6] believes that where cholinesterase localization in capillary walls occurs it is due to a common diffusion artefact. This is difficult to understand in the case of the present investigation because the acetylcholinesterase-positive capillaries are always present only in localized areas and by no means to those areas showing histochemically most tissue acetylcholinesterase, for example, corpus striatum.

Wolff[7] pointed out that the grey matter of the central nervous system is much more richly supplied with capillaries than is the white matter: this investigation has shown that acetylcholinesterase-positive capillaries are confined to parts of the grey matter and particularly to those parts where the capillary pattern is densest although the converse is not true—the cerebral cortex being devoid of acetylcholinesterase-positive capillaries.

The blood supply to the brain, despite the large number of publications, is still imperfectly understood. The pial vessels are known to be supplied with nerves and to react to adrenaline and acetylcholine, but little has been reported on the intrinsic capillaries. The findings of this investigation may perhaps be relevant to the innervation of these capillaries but no final conclusion can be drawn.

I thank H. F. Colgrave for the preparation of the specimens.

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IMMUNOLOGICAL REACTIVITY OF BURSALESS CHICKENS IN GRAFT VERSUS HOST REACTIONS

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THE bursa of Fabricius has recently been shown to play a fundamental part in the development of immunological competence in the chicken[1–3]. The original reports[1,2] showed that surgical removal of the bursa at one day of age or up to 2 weeks of age greatly impaired the subsequent capacity of chickens to produce circulating antibodies to protein and bacterial antigens. This suppressive effect of bursectomy was found to be even more marked if the development of the bursa was prevented in embryonic life by the injection of testosterone or some of its derivatives[2–4]. In investigations on the immunological competence of chicks reared from hormone-treated eggs, it was found that if the thymus in the bursaless birds were intact, then the capacity to reject foreign skin grafts was unaltered[3,5]. It was concluded from this work and from other investigations[6] that, in the chicken, the bursa of Fabricius is concerned with the development of antibody-forming capacity, and the thymus with the development of cells potentially capable of homograft immunity, that is, that there are at least two distinct populations of immunologically competent cells in the chicken.

Several reports by Papermaster *et al.*[7–9] have inferred, however, that chicks reared from embryos treated with 19-nortestosterone showed a deficient homograft response, as assessed by their ability to overcome immunological attack by adult homologous spleen cells, the tentative conclusion being that the lymphoid development and functions of the avian thymus and bursa are similar and are both suppressed by hormone. This is difficult to reconcile with our previous findings which show that the development of cellular homograft immunity is thymus-dependent and unrelated to the bursa of Fabricius. The aim of this present work was therefore to determine the basis of the impaired capacity of young bursaless chicks to reject homologous spleen cells.

All birds used in these experiments were outbred White Leghorn–Australorp hybrids. Bursaless chickens were obtained from eggs injected on the twelfth day of incubation with 4 mg of a corn-oil solution of testosterone proprionate (B.D.H.). Polyvalent tolerance was induced in 15-day-old embryos by the method of Hilgard *et al.*[10]. The recipient birds of one or both of these groups together with untreated controls were injected intraperitoneally with a washed spleen cell suspension prepared from adult birds. Two ages of recipients were used, those between 6–18 h post hatch were given 100 m spleen cells, and those 12-day-old post hatch were given 400 m spleen cells.

All birds were killed 6 days later and spleen and body-weights recorded. The graft versus host (*GVH*) index is the ratio of the mean spleen weight to mean body-weight, and the chimeric index[7] the ratio of the *GVH* index of the cell injected group to control group.

The results obtained with one-day recipients given spleen cells from normal birds are shown in Table 1. There is no statistically significant difference between the chimeric indices of any group. The presence of macroscopically visible lesions was observed equally in all groups. This type of assay does not seem to detect any homograft immunity in normal one-day recipients, as the chimeric index of the normals is not lower than that of the polyvalent tolerant chickens, and there is certainly no evidence that the bursaless chickens were more susceptible to immunological attack by the homologous spleen cells as was suggested by Papermaster *et al.*[7].

These results (Table 1) thus fail to confirm those of the American workers and we can only suppose that there is some difference in the birds used or in the way they were handled. With the 12-day recipients, however, this was not the case. Table 2 shows the results obtained, in which

Table 1. GRAFT VERSUS HOST REACTION IN 1-DAY CHICKENS

Group	Mean body-weight (g)	Mean spleen-weight (mg)	*GVH* index	Control *GVH* index	Chimeric index
Normal	43·4	63·3	1·53(33)	0·86(22)	1·78
Bursaless	40·7	28·6	0·70(31)	0·46(20)	1·55
Polyvalent tolerant	35·5	46·6	1·31(15)	0·90(14)	1·46

GVH index is the ratio of spleen-weight to body-weight, control *GVH* index being the value for an uninjected similar group of chickens. Values in brackets in fourth and fifth column show the respective numbers of chickens per group. Homologous spleen cells were from normal adult donor fowls.

it is evident that there is an impairment of the ability of these bursaless chickens to counteract the attack of the homologous spleen cells. Thymic sections from these birds showed them to be completely normal in this regard, so impairment of homograft immunity due to failure of thymic development is apparently not involved.

Table 3 demonstrates that spleen cells from two four-month-old bursaless birds were fully competent in initiating a graft versus host reaction in 1-day normal recipients.

As both the rejection of homologous spleen cells and the initiation of a graft versus host reaction by spleen cells are in all probability directed towards the same antigens, we must attempt to reconcile the results of Tables 2 and 3. In view of the latter results (Table 3) and our previous observations[5] showing that bursaless fowls have full competence to reject skin grafts, it is felt that the heightened chimeric index of the 12-day bursaless recipients cannot be ascribed to a failure of the population of cells associated with the cellular response to transplantation antigens.

Table 2. Graft versus Host Reaction in 12-day Chickens

Group	Mean body-weight (g)	Mean spleen-weight (mg)	*GVH* index	Control *GVH* index	Chimeric index
Normal	104·0	225	2·13(12)	1·45(15)	1·48
Bursaless	86·7	102·3	1·20(13)	0·52(12)	2·31

See legend to Table 1.

Table 3. Graft versus Host Reaction in 1-day Normal Recipients induced by Spleen Cells from Bursaless Birds

Mean body-weight (g)	Mean spleen-weight (mg)	*GVH* index	Control *GVH* index	Chimeric index
43·0	63·0	1·41	0·82	1·72

cf. with Table 1, in which chimeric index for normal recipients given normal adult homologous spleen cells was 1·78.

There are at least two other alternative explanations. (1) The spleen of bursaless chickens is relatively poor in lymphoid elements and there may simply be more potential space available for the proliferation of reacting host and donor elements. (2) If the difference is more directly immunological in nature it is likely that the failure to suppress the immunological attack is associated with the failure of antibody production. There are growing indications from mammalian systems that circulating antibody may play a part in the complex process of homograft rejection. We are specially interested in the possible significance of the *B* group hæmagglutinins in the chicken since their relation to histocompatibility reactions was shown by Schierman[11]. Search for the relevant cytotoxic antibodies is being undertaken in this laboratory and in due course we hope to be in a position to determine experimentally whether these known or possible antibodies related to histocompatibility antigens are under the control of thymus, bursa, or both organs.

Whatever the eventual decision, we see no reason to modify our opinion that the thymus and not the bursa is concerned with cellular histocompatibility reactions. Papermaster and Good have confirmed our earlier finding that lymphoid cells of bursaless chickens are fully capable of provoking graft versus host reactions[9], so that it is difficult to understand their contention that the functions of bursa and thymus in the chicken are similar. All the available evidence is consistent with the view that the thymus and bursa are concerned with the development of two basically different populations of immunologically competent cells, the precise nature of which is yet to be completely determined. This work was supported by a research grant from the N.H.M.R.C., Canberra, which is gratefully acknowledged.

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INHIBITION OF RIBONUCLEIC ACID-POLYMERASE REACTIONS BY ACTINOMYCIN AND PROFLAVINE

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ACTINOMYCIN inhibits DNA-directed RNA* synthesis of intact cells[1] and enzyme preparations[2–4], and this inhibition is dependent on the ability of the antibiotic to bind to the DNA primer[3–6]. Several effects of actinomycin on RNA-polymerase activity appeared inconsistent with the view that the antibiotic acted simply by obstructing polynucleotide chain growth. One unexplained phenomenon concerns the different susceptibility to actinomycin of DNA and RNA synthesis[4]. The most puzzling observation relates to homopolymer formation which, in the case of DNA-directed poly A and poly U formation, is very resistant to actinomycin[4,6]. Since heteropolymer synthesis is very sensitive to the antibiotic, it might be expected that the presence of actinomycin would shift the enzymatic reaction toward the formation of homopolymers. However, this does not occur, suggesting that in the presence of actinomycin the enzyme might be fixed on portions of the DNA incapable of priming homopolymer formation. We have, therefore, investigated the possibility that actinomycin may be involved in a reaction which binds RNA-polymerase to DNA. The results reported here demonstrate that this is not the case. In addition, we have attempted to establish whether biologically inactive actinomycin derivatives might compete with the parent compound and prevent the inhibition by the latter of RNA-polymerase; this possibility has also been ruled out. Finally, we have investigated the action of proflavine on RNA synthesis primed by synthetic DNA's in order to differentiate the effect of this compound from that of actinomycin.

The details of enzyme isolation, and assay, and the preparation of radioactive nucleoside triphosphates have been described previously[6]. All experiments were performed at an enzyme concentration corresponding to the linear portion of the velocity/enzyme concentration curve.

The possibility that enzyme binding to DNA might be a factor in the action of actinomycin was tested in the following manner: The synthesis of polynucleotides by RNA-polymerase is known to be insensitive to actinomycin when the primers do not bind the antibiotic, for example, when dAT or polyribonucleotides are used as primers[6–8]. If inhibition of RNA polymerase by actinomycin were related to binding of enzyme to DNA, the presence in a reaction mixture of a DNA species (for

Table 1

Exp. No.	Nucleotides	DNA	Actinomycin (μg/ml.)	UM^{32}P incorporated (mμmoles)
1	UT^{32}P, ATP	dAT	—	2·07
	,, ,,	dAT	50	2·07
	,, ,,	dAT + C.T.	—	1·01
	,, ,,	dAT + C.T.	50	1·26
2	UT^{32}P, ATP	dAT	100	1·57
	,, ,,	dAT + C.T.	100	0·89
	,, ,,	dAT + C.T.	—	0·63
	,, ,,	C.T.	—	0·04
	,, ,,	C.T.	100	0·01
3	UT^{32}P, ATP CTP, GTP	C.T.	—	0·78
	,, ,,	crab dAT	—	2·27
	,, ,,	C.T. + crab dAT	—	2·06
	,, ,,	C.T.	40	0·03
	,, ,,	crab dAT	40	1·85
	,, ,,	C.T. + crab dAT	40	1·82

RNA synthesis was measured according to Chamberlin and Berg (ref. 10) for 30 min at 37°. In Exps. 1 and 2, calf thymus DNA (highly polymerized, obtained from Sigma Chemical Co., St. Louis, Missouri) was present at 48 mμmoles, and in Exp. 3 at 6 mμmoles per 0·25 ml. reaction volume. Synthetic and crab dAT were added, where indicated, at 0·01 O.D. units (260 mμ) per 0·25 ml. Enzyme preparation equivalent to 2 μg protein was used in Exps. 1 and 2, and 3 μg protein/0·25 ml. in Exp. 3. The specific activity of UT^{32}P was 4×10^6 c.p.m./μmole. In an experiment conducted in parallel with Exp. 3, the RNA product was subjected to alkaline hydrolysis (0·3 N potassium hydroxide, 18 h, 37°) and the radioactivity of the resulting 2′ (3′) nucleoside monophosphates analysed following paper electrophoresis (0·05 M citrate buffer, *p*H 3·5); the results showed that when both calf thymus DNA and crab dAT were present as indicated, the priming due to calf thymus DNA accounted for 18 per cent of the total RNA produced in the absence of actinomycin; in the presence of actinomycin the product resulted entirely from priming due to crab dAT.

example, calf thymus DNA) known to complex the antibiotic should result in the immobilization of at least some of the available enzyme. Under conditions in which enzyme concentration is rate limiting the addition of calf thymus DNA to dAT or poly A-primed reactions should render the latter partially susceptible to actinomycin. The results in Table 1 demonstrate that this is not the case. The presence of a large excess of calf thymus DNA in the dAT-primed reaction mixtures markedly lowers the overall reaction rate; dAT is the most efficient DNA primer for RNA synthesis (a reflexion of its high AT content) and calf thymus DNA presumably competes with the dAT for the enzyme. The addition of actinomycin does not further lower the reaction rate, as would be expected if the antibiotic facilitated enzyme binding to and consequent immobilization on the calf thymus DNA. On the contrary, the antibiotic reproducibly stimulates the reaction conducted with UT^{32}P and ATP alone, and does not significantly affect the synthesis in the presence of four NTP. These results are compatible with the displacement, by actinomycin, of a fraction of the enzyme bound to calf thymus DNA, and appear to rule out antibiotic-dependent enzyme binding to DNA. Corroborative evidence is presented in Table 2. In this experiment the actinomycin-sensitive primer was *Micrococcus lysodeikticus* DNA, and poly A was the actinomycin-resistant primer. Alkaline hydrolysis of the product of the mixed reaction (tube 3), followed by analysis of the radioactivity of the resulting 2′ (3′) nucleoside monophosphates, revealed that 26 per cent (0·20 mμmoles) of the incorporation of UM^{32}P was due to *M. lysodeikticus* DNA priming, the remainder (0·57 mμmoles) being due to poly-A. As

Table 2

μg/0·25 ml. Poly A	DNA mμmoles/0·25 ml. *M. lysodeikticus*	Nucleotide	Actinomycin (μg/ml.)	UM^{32}P incorporated (mμmoles)
5	—	UT^{32}P, ATP, CTP, GTP	—	0·83
5	—	UT^{32}P, ATP, CTP, GTP	40	0·80
5	48	UT^{32}P, ATP, CTP, GTP	—	0·77
5	48	UT^{32}P, ATP, CTP, GTP	40	0·66
—	48	UT^{32}P, ATP, CTP, GTP	—	0·37
—	48	UT^{32}P, ATP, CTP, GTP	40	0·01

Assay conditions as in Table 1, but performed at 25°, 30 min, in final volume of 0·25 ml. containing 4 μg enzyme protein. Analysis of the nature of the radioactive product revealed that with both poly A and *Micrococcus lysodeikticus* DNA present, the priming due to poly A accounted for 74 per cent of the RNA synthesized in the absence of actinomycin.

Table 3

	AMP-^{14}C incorporated (mμmoles)
Control	1·32
+ *N*-β-aminoethylactinomycin C_2-25 μg/ml.	1·24
+ *N*-*p*-aminophenylactinomycin C_2-25 μg/ml.	1·35
+ Actinomycin C_1, 0·5 μg/ml.	0·54
+ Actinomycin C_1 (0·5 μg/ml.) + *N*-β-aminoethylactinomycin C_2-25 μg/ml.	0·47
+ Actinomycin C_1 (0·5 μg/ml.) + *N*-*p*-aminophenylactinomycin C_2-25 μg/ml.	0·52

Assay conducted as in Table 1, 30 min, 37°, in 0·25 ml. containing 6 μg enzyme protein ATP-8-^{14}C (sp. act. 6·8 μc./μmole, obtained from Schwarz Bioresearch, Mt. Vernon, New York), was the radioactive precursor. Calf thymus DNA was present at 30 mμmoles deoxynucleotide/0·25 ml.

Table 4

		GMP incorporated (mμmoles)	Inhibition (per cent)
Control		0·704	
+ Proflavine	30 mμmoles/ml.	0·362	49

Experiment performed as in Table 1, 30 min, 37°, in final volume of 0·25 ml. containing 1·5 μg enzyme protein. The radioactive precursor was GTP-8-^{14}C (spec. act. 8·28 μc./μmole), obtained from Schwarz Bioresearch, Mt. Vernon, New York. dGC was present at 7·4 mμmoles/0·25 ml.

already seen with calf thymus DNA and dAT, the addition of actinomycin to the mixture stimulates modestly the poly-A primed reaction. This finding would be expected if actinomycin prevented the attachment of some enzyme molecules to *M. lysodeikticus* DNA, thereby making them available for poly A-primed RNA formation. A similar observation has been recorded by Weiss[8] using a system of mixed primers in which calf thymus DNA was the actinomycin-sensitive and polycytidylic acid the actinomycin-insensitive primer for RNA synthesis.

Lack of competitive relationship between actinomycin and inactive derivatives. The results in Table 3 show that two biologically inactive derivatives[5] of actinomycin were incapable of relieving the inhibition of RNA polymerase caused by a much smaller concentration of an active compound. It is noteworthy that one of the inactive compounds, *N*-β-aminoethyl actinomycin, interacts with DNA, but in a manner which does not affect the functional activity of DNA *in vivo* or *in vitro*[5].

Effect of proflavine on RNA synthesis primed by dAT and dGC. Previous work has shown that proflavine inhibits DNA-directed RNA synthesis of enzyme prepara-

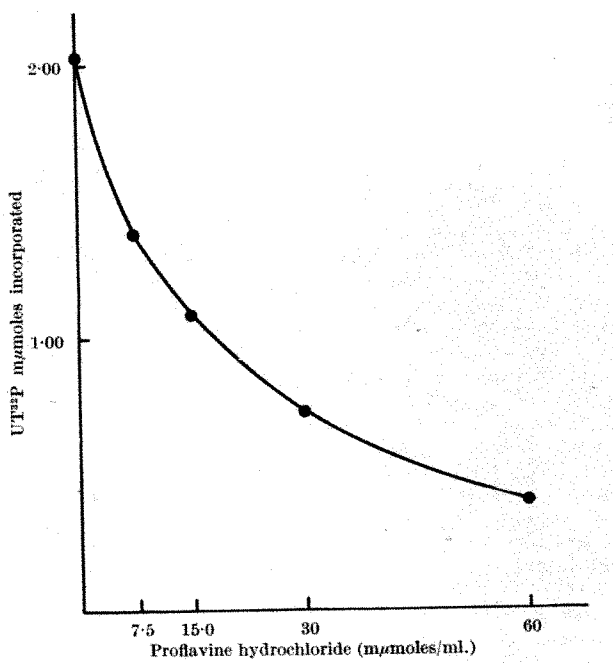


Fig. 1. Effect of proflavine on RNA synthesis primed by dAT. The reaction mixture contained 0·005 O.D. units (260 mμ) synthetic dAT and 4·1 μg enzyme protein in a final volume of 0·25 ml. UT^{32}P ($1·1 \times 10^6$ c.p.m./μmole) was the radioactive precursor. Incubation was for 15 min, 37°

tions from animal[2] and bacterial[4] sources. In view of the specificity of actinomycin action, which depends on the presence of guanine in DNA primers[6], it was of interest to establish whether proflavine effects demonstrated similar, if any, specificity for inhibition of RNA synthesis. The results in Fig. 1 and Table 4 show that this is not the case; proflavine inhibits both dAT- and- dGC-primed syntheses, whereas actinomycin affects only the latter. It is known that actinomycin which is bound to one strand of the dGC inhibits RNA synthesis from both. The results presented here do not establish whether this is the case for proflavine since the priming action of only one strand—the polydeoxycytidylic—has been assayed in this experiment. In view of the suggestion[9] that proflavine molecules are intercalated between the base pairs of DNA, it might be expected that all enzymatic reactions involving either strand of DNA would be inhibited by this dye.

From previous work it has been concluded that the inhibition of RNA synthesis by actinomycin is based exclusively on the binding of the antibiotic to priming DNA in some manner involving the guanine residues[6]. The experiments reported in this article support these conclusions. The tight binding to DNA may be visualized as obstructing polyribonucleotide chain growth, without affecting the ability of the enzyme, RNA-polymerase, to catalyse the synthesis of RNA from primers (such as dAT or poly-A) which do not bind the antibiotic. The inactive derivatives tested are not bound to DNA in the manner of the parent compounds and, consequently, do not compete with the latter. The effect of actinomycin, its specificity, and, therefore, the principles of its binding to DNA, are thus differentiated from those of proflavine. The failure of actinomycin-inhibited RNA synthetic reactions to switch to resistant homopolymer formation remains unexplained. It has been shown[10] that the presence of other nucleoside triphosphates reduces DNA-directed poly A synthesis in the absence of actinomycin. As anticipated, poly A synthesis is intrinsically very actinomycin-resistant since the supposed priming sequences in DNA would not bind the antibiotic[6]. Therefore, it is of interest that the presence of all four NTP is incompatible with homopolymer synthesis even in the presence of high concentrations of actinomycin[6]—a condition in which RNA synthesis is virtually undetectable. The results presented here exclude the possibility that an actinomycin-mediated immobilization of enzyme might be responsible for this finding, and suggest that the presence of all NTP is itself incompatible with homopolymer growth independently of concurrent RNA synthesis. This effect of NTP may be due to interference with a 'slippage' mechanism as previously proposed[10].

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* Abbreviations used in this paper are : dAT, deoxyadenylic-thymidylic copolymer ; dGC, deoxyguanylic-deoxycytidylic homopolymer mixture ; C.T., calf thymus DNA ; Poly A, polyadenylic acid ; Poly U, polyuridylic acid; AMP, CMP, GMP, UMP, 5'monophosphates of adenosine, cytidine, guanosine, uridine; ATP, CTP, GTP, UTP, 5'triphosphates of adenosine, cytidine, guanosine, uridine ; DNA, deoxyribonucleic acid ; RNA, ribonucleic acid ; NTP, nucleoside triphosphates.

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ASPIDIN : THE PRECURSOR OF BONE

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It has previously been believed that aspidin[1], the calcified tissue forming the bulk of the plates of the carapace of the heterostracan ostracoderms—the earliest known vertebrates—was acellular[2]. In consequence, it was thought by Stensiö and Ørvig[3] that like the bone of modern teleosts it had been derived secondarily from normal bone. Since, however, aspidin precedes the first incontrovertible evidence of bone in the fossil record by about 100 million years, and since bone is just as easily preserved as aspidin, until definite evidence to the contrary is produced, it seems reasonable to take aspidin as the most primitive calcified tissue, rather than bone. Aspidin of successive geological ages has therefore been examined, and its development has been traced in time through the various heterostracan groups.

In forms from the Ordovician Period (about 500 million years ago) such as *Astraspis* and *Eriptychius*, this tissue was deposited as successive lamellæ in concentric rings or aspidones[4] around vascular spaces, by cells named aspidinoblasts by Bystrow[5]. These must have originated in the vascular tissue and retreated as calcification occurred, since no cells seem to have become trapped in the hard tissue. Occasionally, however, very fine tubules are visible normal to the lamellæ, which can best be explained as canaliculi formed around the processes of the aspidinoblasts[6]. Tubules of this type are more numerous in the superficial tubercules of the carapace of *Astraspis*, and it may well be that it was at this stage that the tissue dentine became differentiated from aspidin. In later forms such as the early Devonian genera *Kallostrakon*, *Tesseraspis* and *Weigeltaspis*, the aspidin is permeated by randomly arranged spindle-shaped structures, although at the growing edges of the plates these are seen to be aligned parallel to the direction of growth, and are greatly elongated. Such structures have long been observed[2]; but they have been identified as representing the former position of bundles of collagen, and in fact the general practice has been to name them fibres of Sharpey[5,7], regardless of their position or alignment. However, fibres of Sharpey are not a component of internal systems, but are usually found anchoring one tissue to another, and this is not the case with the spindle-shaped structures in aspidin.

In more advanced heterostracans such as *Tartuosteus*, *Psammolepis*, and *Psammosteus*, these structures are no longer scattered throughout the tissue, but are now organized in a well-defined pattern. They are very abundant in trabeculæ which seem to have been laid down before the concentric lamellæ of the aspidones were apposited (Fig. 1), and can now be seen to be spindle-shaped cell spaces crossing each other in all directions. But it should be noted that they are not in direct contact with one another (Fig. 2). The spaces can thus be best understood

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Fig. 1. *Psammosteus*. Vertical section of advanced aspidin showing aspidinocytes with random arrangement in trabeculæ, and with orientation parallel to lamellæ of aspidones. (× 120)



Fig. 2. *Psammosteus*. Enlargement of single aspidinocyte. (× 400)

as marking the former position of aspidinocytes which became trapped in hard tissue, after being responsible during their role as aspidinoblasts for the production of the organic matrix on which calcification took place. The cells can thus be compared directly with osteoblasts which can also be spindle-shaped, and it may well be that in aspidin, which appears to represent a primitive stage in the development of hard tissues, both aspidinoblasts and aspidinocytes were normally spindle-shaped.

The cells in the trabeculæ do not appear to have moved, but instead, as the lamellæ of the aspidones were laid down, some of the aspidinocytes seem to have developed cytoplasmic extensions at one end, which became further and further drawn out in an attempt to maintain contact with the source of nutriment. This seems to parallel closely the situation in cementum, where cementocytes frequently behave in much the same way. Although in general the aspidinoblasts responsible for the production of the lamellæ in advanced aspidin do not seem to have become enclosed, nevertheless spindle-shaped spaces can also be seen occasionally between successive layers of the aspidones, orientated parallel to them (Fig. 1). These presumably mark the position of the few cells which did become trapped during calcification, and which thus resemble the osteocytes of primary osteones in bone.

When aspidin of successive geological ages is studied under polarized light, it is evident that there is also a gradual change in the organization of the organic matrix towards the condition found in modern bone. In the Ordovician genera the tissue consists of simple lamellæ in which the apatite crystallites appear to have been aligned parallel to the surface of apposition, and it seems reasonable, therefore, to infer that the original collagen fibrils were also so aligned. This same basic arrangement occurs in modern dentine, and supports the idea that dentine could have been derived from an aspidin-like tissue at an early stage of evolution. In forms from the Lower Devonian such as *Kallostrakon* and *Tesseraspis*, a very broad black-and-white banding is visible, suggesting that the collagen fibrils had become aligned alternately in broad zones, while in the Upper Devonian *Psammosteus*[1] and *Obruchevia* (*Aspidosteus*), a much finer black-and-white banding can be seen which is virtually indistinguishable from that well known in mammalian bone (Fig. 3). Thus, the most advanced heterostracans demonstrate that, in the organization of its organic matrix, aspidin reached the same condition as is known in true bone.

Furthermore, it was believed in the past that, since remodelling did not occur in aspidin, there was a fundamental difference between this tissue and bone. Nevertheless, Gross[4] was convinced that resorption must have taken place in the Heterostraci, and suggested the base of the middle layer of the plates as a likely site, although he was unable to produce any concrete evidence for his theory. Now, however, it is possible to demonstrate resorption in aspidin, but it is seen to occur in the more superficial part of the plates beneath the tubercles, and not where suggested by Gross. Initially, dense aspidin seems to have been laid down which was then opened out to form larger vascular spaces, indicating the presence of cells which can be called aspidinoclasts. Areas of resorption can be seen clearly where the concentric lamellæ of the aspidones have been either partially eroded or completely breached (Fig. 4). Occasionally, also, very simple secondary aspidones clearly delineated by a marked cement line are found filling in formerly resorbed areas



Fig. 3. *Obruchevia* (*Aspidosteus*). Advanced aspidin under polarized light (crossed nicols), showing alternate light and dark banding. (× 75)

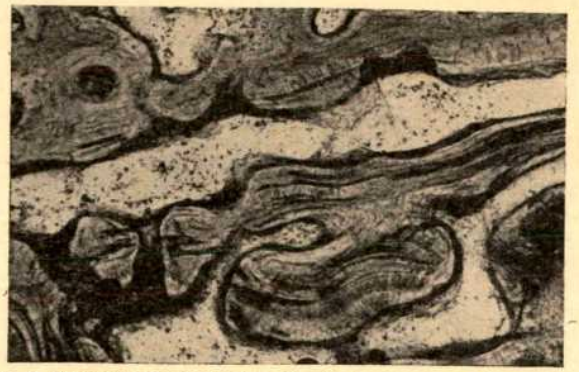


Fig. 4. *Ganosteus*. Vertical section showing irregular areas of resorption cutting into and through lamellæ of aspidones. (× 75)

(Fig. 5). These are obviously directly comparable with secondary osteones in the higher vertebrates.

Aspidin, therefore, seems to have had the essential attributes of bone even though it was not as highly organized in some respects. With its similar basic composition[8], its almost certain possession of cells with the same role as osteoblasts, osteocytes and osteoclasts, with the gradual organization of its collagen fibrils into alternate layers, and with its ability to remodel, aspidin must surely be considered as a primitive type of bone, or as the precursor of true bone. This seems much more reasonable than that it was a highly specialized and unusual tissue derived from bone, particularly in view of the fact that, in time, the gradual evolution of aspidin can be followed towards true bone, and not in the opposite direction.

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Fig. 5. *Ganosteus*. Vertical section showing simple secondary aspidone delimited by clear cement line, in formerly resorbed area. (× 75)

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SUCCESSFUL RE-INTRODUCTION OF THE MURPHY–STURM LYMPHOSARCOMA AFTER METHYLCELLULOSE-INDUCED REGRESSION OF THIS TUMOUR IN RATS

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IN our laboratory, and in others[1], complete, spontaneous regression of the Murphy–Sturm lymphosarcoma[2] in rats results in life-long resistance, on the part of such an animal, to the successful re-introduction of this tumour. If re-introduced, the tumour will not grow.

Over a period of three years we have maintained this tumour, by serial passage, in a total of more than 350 adult, Sprague–Dawley rats. Growth of the tumour, after its subcutaneous inoculation, occurred in 99 per cent of the rats and, after attaining a moderate size in 10–13 days, spontaneous regression of the tumour occurred in 20 per cent of the rats. The rest of the rats died, with progressively growing tumours, in 21–35 days. We have observed no association of symptoms in these animals, such as might be due to an infectious agent[3], in any significant degree to lead us to suspect a causal relationship between such an agent and regression of the tumour.

We define regression as the absence of tumour by palpation during life and followed by absence of tumour in a complete gross and histopathological post-mortem examination which includes careful dissection and multiple histological sections from the site of injection of the tumour.

Methylcellulose, which has been found to induce regression of some transplantable tumours in rats[4–6], is a long-chained polymer; a methyl ether of cellulose which forms a colloidal suspension in water. It is not absorbed in appreciable amounts from the alimentary tract[7,8]. Anatomical and physiological changes, after parenteral administration of amounts of methylcellulose comparable with those used in our investigations, include: hypersplenism and hæmolytic anæmia[9,10]; quantitative and qualitative changes in circulating γ-globulins[6,9]; changes in serum lipoproteins[11]; decrease in the formation of hæmolysin[9,12]; decreased coagulability of the blood[13]; storage of methylcellulose in the glomeruli and a nephrosis-like syndrome[14]; and storage of large amounts of methylcellulose in macrophages, especially in the liver and spleen[5].

We have no evidence for a direct cytotoxic effect by methylcellulose. Its action in the regression of tumours requires the presence of the spleen[5]; the mode of this action is probably an enhancement of the homograft reaction by some mechanism, as yet, not clear.

In the work recorded here we transplanted the Murphy–Sturm lymphosarcoma subcutaneously into rats which had had a methylcellulose-induced regression of a similar tumour a few weeks before. The injections of methylcellulose were continued but the animals were not 'conditioned' by any of the usual means of abrogating immunological resistance (total-body X-irradiation, adrenal cortical steroids, etc.). After the usual latent period, the second tumour grew and, again, regressed. Subsequent attempts to transplant the tumour into these rats resulted in no growth. Adequate controls demonstrated the potency of all the tumours used for transplantation.

Non-litter-mate, male, adult Sprague–Dawley rats, 300–360 g, were divided into 4 groups averaging 340–350 g. All the animals were fed Purina laboratory rat chow and water, *ad lib.*, throughout the study.

The methylcellulose ('Methocel 400 CPS', U.S.P., Dow Chemical Co.) was prepared as a clean, but not sterile, 2·5 per cent aqueous suspension and kept under refrigeration; aliquots were removed from this stock solution as needed.

Each Murphy–Sturm tumour used for transplantation had been given to its respective host (donor) rat 14 days previously. These donors were killed, while under ether anæsthesia, by cutting the aorta. The tumour was quickly removed and homogenized in cold saline using a clean but not sterile technique. A cell-count was done and the volume of homogenate containing 2×10^7 tumorous cells was inoculated subcutaneously with as little delay in the procedure as possible. The site of injection was different for the first, second and third transplantations; the control animals were always inoculated in the same site as the experimental group inoculated at the same time. The first transplantation was in the area of the right scapula; second in the area of the left scapula; third in the lumbar area.

Measurement of each tumour was carried out daily with a caliper and the volume of the tumour was calculated[6].

The treated group (*A*) of 40 rats received 2 c.c. of 2·5 per cent aqueous suspension of methylcellulose intraperitoneally 3 times per week (Monday, Wednesday, Friday) starting 10 days before transplantation of the tumour. Once started, the injections were continued, on this same dosage schedule, as long as the animal remained alive.

The control group (*B*) of 26 rats received the same tumour at the same time as group *A*, but were otherwise untreated.

Control groups *C* and *D* of 20 rats each were from the same shipment as the foregoing groups and comparable in sex and size. They were untreated and used for testing the potency of the tumours utilized for the second and third transplantations.

The tumour was transplanted into all the animals in groups *A* and *B*, became established in all these animals, and grew to approximately the same average size for both groups in the first 11 days. Thereafter, regressions began in 38 (95 per cent) of the rats in the treated group (*A*), and 6 (23 per cent) of the rats in the control group (*B*).

Considering as sub-group *A′* only those 38 rats in group *A*, and as sub-group *B′* only those 6 rats in group *B*, in which there was subsequent regression of the first tumour, the following differences were noted between these two sub-groups: (1) although the maximum average size of the tumour in both these sub-groups was reached 11 days after transplantation, the average size of the tumours was 100 per cent larger in the 6 untreated control rats (*B′*); (2) the regressions were completed by the twenty-first day after transplantation of the tumour in the 38 treated rats (*A′*), but not until the twenty-eighth day in the 6 control rats (*B′*).

Forty days after the first transplantation, 12 of the 38 rats in sub-group *A′* which had methylcellulose-induced regression of the first tumour, and all 6 of the rats in sub-group *B′* which had spontaneous regression of the first tumour, were re-inoculated with Murphy–Sturm tumour, in the same manner as here, but in the second site. In addition, control group *C* was inoculated with this second tumour.

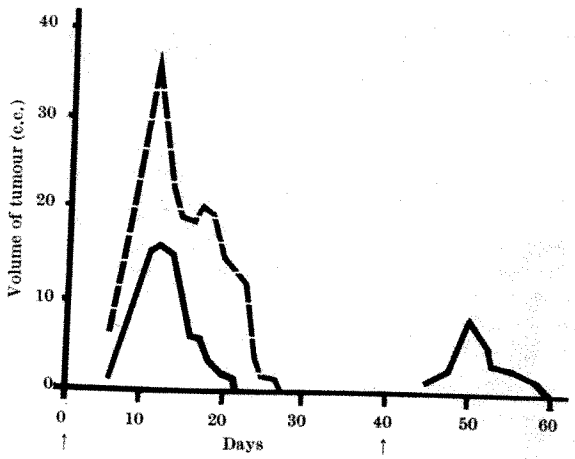


Fig. 1. Arrows indicate transplantation of tumour. Comparison of average size of tumours which regressed after first transplantation (spontaneously and induced by methylcellulose) and after second transplantation (methylcellulose-induced)
——, Treated sub-group *A′*; – – –, control sub-group *B′*

The tumour used for the second transplantation was of usual potency; it established in all 20 of the rats in control group *C* and caused the death of 17 of them by the thirtieth day after transplantation; 3 had spontaneous regression.

As expected, there was no growth of the second tumour in the 6 rats in sub-group *B′*.

In 9 of the 12 rats from sub-group *A′* the tumour established, grew to its maximum average size in 10 days, and then completely regressed in all 9 of the rats by the twentieth day after transplantation. Fig. 1 shows the average size of the tumours in all 38 rats in sub-group *A′* during the first methylcellulose-induced regression as compared with the 9 rats from sub-group *A′* in which there was methylcellulose-induced regression of the second tumour. The larger size of the tumours which regressed spontaneously in the 6 rats in sub-group *B′* is shown for comparison.

All the rats which had regression of the tumour, either spontaneously or induced by methylcellulose, maintained a generally healthy appearance and gained weight.

A third subcutaneous inoculation of the tumour was performed, 40 days after the second transplantation, in: the 9 rats in sub-group *A′* which had had methylcellulose-induced regression of both previous tumours; the 3 rats in sub-group *A′* which had methylcellulose-induced regression of the first tumour, but no growth of the second tumour; the 6 rats in sub-group *B′* which had spontaneous regression of the first tumour and no growth of the second tumour; and the 20 previously unused rats in control group *D*.

The tumour established and grew in all the rats in control group *D*, killing 16 of them by the twenty-eighth day after transplantation; 4 had spontaneous regression. None of the other rats which were inoculated with this tumour had any detectable growth of the tumour 20 days after inoculation, at which time they were killed for internal examination.

All the animals in this investigation, which remained alive 20 days after the third inoculation of the tumour, were killed at that time and a complete post-mortem examination was made of each. Tissues were fixed in formalin, embedded in paraffin, cut at 6μ, and stained with hæmatoxylin and eosin. Multiple cut surfaces and at least one histological section were made from each of the following organs from each rat: heart, lung, liver,

spleen, abdominal lymph nodes, mesentery, pancreas, testis, small intestine, and femoral bone-marrow. The sites of inoculation of tumour were all examined grossly and multiple histological sections were made from each site. In addition, the peripheral lymph nodes draining the sites of inoculation of tumour were also examined histologically. None of the gross or histological sections of internal organs, sites of injection, or peripheral lymph nodes, contained tumour. The changes due to storage of methylcellulose were similar to those reported elsewhere[5].

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TONIC STRETCH REFLEXES IN THE CALF MUSCLES OF NORMAL HUMAN SUBJECTS

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A REFLEX response to muscle stretch, the tendon jerk, can be demonstrated in most individuals by a tap on one of several suitably placed tendons. Should the responses be small or difficult to evoke it has long been known that they may be temporarily exaggerated by any prolonged voluntary muscle contraction occurring elsewhere in the body. The usual method when examining the leg reflexes is to ask the subject to perform Jendrassik's[1] manœuvre of sustained traction exerted between the linked right and left hands. The increase in amplitude of the reflex responses is brought about indirectly by a heightening of the sensitivity of the muscle stretch receptors which is thought to be caused by the activation of the γ-motoneurones supplying the intrafusal muscle fibres of the muscle spindles. Contraction of the intrafusal fibres leads to a tensing of the annulo-spiral ending and thus an increased response to stretch. The excitability of the large motoneurones supplying the leg muscles does not seem to be directly affected[2–4].

In a normal relaxed person the response to muscle stretch is dependent on the abruptness of the stimulus for if the same amount of stretch is applied slowly no active opposing contraction occurs. Continuously exaggerated responses to slow stretch are only seen in man in certain disorders of the nervous system, just as they may become obvious in experimental animals with certain brain lesions, notably intercollicular decerebration[5]. In both man and animals it has been shown that in some, though not all, of these cases the sensitization of the stretch reflex arc is due to increased activity of the γ-motoneurones acting on the intrafusal fibres supporting the annulospiral ending of the muscle spindles[6,7]. This is the same mechanism as is known to underlie the temporary augmentation of tendon jerks by the performance of the Jendrassik manœuvre in normal man. It was therefore thought of interest to determine whether the Jendrassik manœuvre had any effect on the response of human muscles to slow or maintained stretch.

The experiments were performed on six apparently normal young adults, men and women. They lay face downward on a couch with their right foot fixed to a flat plate mounted on a pivot allowing full flexion and extension of the ankle joint. A system of springs kept the foot in mid-position from where it could be moved through a variable number of degrees of dorsiflexion by a chain attached to a heavy piston moving vertically in an oil-filled cylinder.

The standard passive stretch of the triceps suræ muscles resulted from a dorsiflexion of the ankle joint of 5° in 1·5 sec which was accomplished by the free descent of the piston. At the end of the passive movement the foot remained dorsiflexed until the piston was pumped back towards the starting point. The triceps suræ could also be stretched without simultaneous movement of the ankle joint by pressing slowly and steadily on the Achilles tendon while holding the foot still, although the rate and degree of muscle stretch could not then be controlled accurately from trial to trial as in the case of dorsiflexion of the foot. Surface electrodes were placed over the triceps suræ muscles and over the antagonistic tibialis anterior muscle on the anterior surface of the leg. Another pair of electrodes were fixed over the extensors of the right wrist to indicate the timing and the intensity of the Jendrassik manœuvre. Muscle action potentials were amplified and displayed on a pen-writing Offner dynograph. A potentiometric device for measuring the position of the foot was connected to one of the channels of the dynograph so that electromyographic activity could be observed in relation to the passive movement of the ankle joint.

In the normal relaxed subject a passive dorsiflexion of the ankle joint of 5° in 1·5 sec was not accompanied by any recordable electromyographic activity of the calf muscles or of their antagonists. Occasionally in the first few trials subjects would tend to follow the applied movement of the foot with a contraction of the dorsiflexors of the ankle joint, but when they became used to the conditions of the experiment and relaxed completely this was no longer seen. Subjects were then asked to perform a series of Jendrassik manœuvres of different intensities as judged by the electromyogram of the extensors of the wrist joint. It was found that a manœuvre performed with all the subject's strength would frequently be associated with contraction of the muscles of the legs including those monitored. With the expenditure of somewhat less effort there was no movement of the legs and no electromyographic activity of the triceps suræ or of the tibialis anterior.

Several seconds after the beginning of one of the Jendrassik manœuvres which did not evoke any overt activity of the leg muscles the descent of the piston was triggered and the passive dorsiflexion applied to the foot. As soon as the foot began to move from the neutral position an irregular shower of action potentials appeared in the

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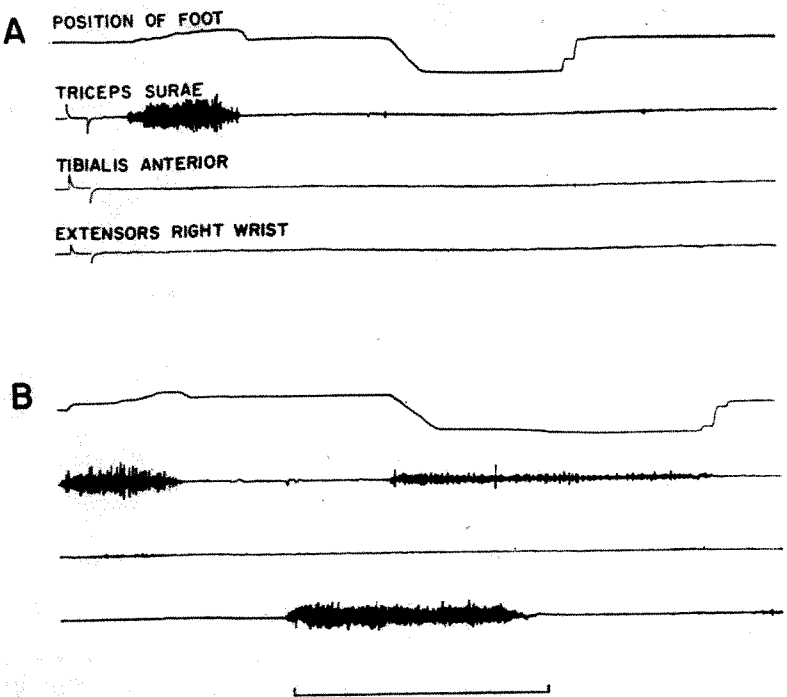


Fig. 1. Electromyographic responses of the triceps suræ to slow passive dorsiflexion of the foot

A, Top line indicates the position of the foot with dorsiflexion signalled by a depression of the trace. Five degrees between starting position and full dorsiflexion. Lower three traces are surface electromyograms each beginning with a calibration mark of 500 μV. Record shows a short voluntary contraction of the triceps suræ which is followed several seconds later by passive dorsiflexion of the foot. Note the lack of electromyographic response of the triceps suræ to dorsiflexion of the foot in the relaxed subject

B, Recordings made immediately following *A*. After a preliminary voluntary contraction of the triceps suræ (at left) a Jendrassik manœuvre is begun, signalled by the electromyogram of the extensors of the right wrist. During the Jendrassik manœuvre the foot is again passively dorsiflexed. Electromyographic activity begins in the triceps suræ as soon as the dorsiflexion of the foot starts and continues with diminishing intensity after the end of the Jendrassik manœuvre until the foot is rotated back towards the initial position. There is no activity recorded from the tibialis anterior
Time marker, 10 sec

record from the calf muscles and continued after the end of the movement while the foot was held steady in the dorsiflexed position. Provided the Jendrassik manœuvre was maintained at an approximately constant level the activity of the calf muscles could be interrupted by rotating the foot back towards the neutral position and would begin again in response to a second dorsiflexion. If the Jendrassik manœuvre was discontinued while the foot was held in the dorsiflexed position the activity of the calf muscles declined, in some cases abruptly and in some cases over a period of several seconds until eventually the muscles showed no action potentials.

There was no activity of the tibialis anterior during the movement of dorsiflexion of the foot. Fig. 1 shows a typical series of recordings during passive dorsiflexion of the foot in the relaxed subject and during the performance of the Jendrassik manœuvre. The apparatus used for rotating the foot about the ankle joint was virtually silent and was placed outside the subjects' field of view. There was no way for them to tell which among the many Jendrassik manœuvres they performed would be combined with the movement of the foot and no way of telling at what moment during any manœuvre the dorsiflexion of the foot would begin. When questioned about the activity of their leg muscles they were generally quite unaware, at least during the first experiment, that any muscle contraction had occurred apart from the voluntary contraction of the arm muscles. Often they did not realize that their foot had been moved. A similar tonic response to muscle stretch could also be evoked from the calf muscles during the performance of a Jendrassik manœuvre by holding the foot still and pressing firmly and steadily on the Achilles tendon just behind the ankle joint. Presumably in this case as with the dorsiflexion of the foot the essential stimulus was the elongation of the calf muscles. Pinching the tendon between the fingers without pulling on the tricep suræ muscles did not cause any activity. The antagonistic tibialis anterior muscle remained without recordable electrical activity throughout the manipulations.

After a passive dorsiflexion of the foot to which there was no active response it was noticed that succeeding manœuvres of dorsiflexion tended to be less effective than usual in evoking a contraction of the calf muscles. Provided the subject lay quite relaxed this apparent increase in the threshold of the stretch response would last indefinitely. Responsiveness to stretch could be immediately restored by either a short voluntary contraction of the calf muscles or by a prolonged and intense Jendrassik manœuvre. In subjects showing this effect strongly each passive dorsiflexion of the foot was preceded by either a short contraction of the calf muscles (as in Fig. 1) or by a standard number of cycles of dorsiflexion and release of the foot. When this was done it became clear that the electromyographic response of the calf muscles to slow stretch was highly dependent on the vigour with which the subjects performed the accompanying Jendrassik manœuvre. Relatively sparse activity of the arm muscles was associated with no stretch response of the calf muscles or perhaps with a response which was delayed until the movement of dorsiflexion of the foot neared completion. Increasingly intense electromyograms from the arm muscles led to stronger calf muscle responses appearing progressively earlier in the dorsiflexion of the foot until action potentials could be recorded while the foot was held stationary in the neutral position. Passive dorsiflexion would then cause an abrupt increase in activity.

The contraction of the calf muscles in response to dorsiflexion of the foot is probably dependent on the monosynaptic segmental stretch reflex arc. No measurements of latency could be made to a satisfactory degree of accuracy, but under suitable conditions the muscle contraction begins with very little delay once the dorsiflexion of the foot begins, and it ceases in a dead-beat manner when the foot is passively plantar flexed. Contraction is restricted to muscles stretched by dorsiflexion of the foot and occurs in response to stretch applied directly through the tendon of the triceps suræ with no movement of the ankle joint. The appearance of a response to slow muscle stretch during the Jendrassik manœuvre is thus thought to depend on the same mechanism of activation of γ-motoneurones as accounts for the increase in the amplitude of the tendon jerks. γ-Motoneurone activity brought about in this manner therefore appears to affect both the dynamic and static sensitivities of the muscle spindle receptors responsible for the afferent impulses generated by both fast and slow stretch.

The very long-lasting increase in stretch reflex threshold that follows a passive muscle stretch which does not evoke any active response is similar to a phenomenon encountered in experiments on human subjects by Hugon and Paillard[8]. They noticed that passive dorsiflexion and release of the foot would greatly reduce the amplitude of the ankle jerks and that reflex responsiveness was not restored until the subjects voluntarily contracted the calf muscles or performed a vigorous Jendrassik manœuvre. The explanation they offered was that passive stretch

produces a deformation of either the muscle spindle or of its linkage with the body of the muscle, and this persists after release when the rest of the muscle tends to regain its original length. Subsequent stretches would therefore need to be more extensive to have the same effect on the receptor endings. Re-setting the receptors after deformation is presumably brought about by contraction of the intrafusal muscle fibres which occurs in isolation in the Jendrassik manœuvre or in conjunction with the contraction of the extrafusal fibres in a voluntary innervation of the stretched muscle.

It was also noticed quite frequently that once the contraction of the triceps suræ was established by the combination of passive dorsiflexion of the foot and the Jendrassik manœuvre, the electromyographic activity would continue for an appreciable time, perhaps many seconds after the Jendrassik manœuvre was interrupted. Since the appearance of tonic stretch reflexes probably depends on the activation of the γ-motoneurones, this observation may mean either that γ-motoneurone discharge produces long-lasting changes in the sensitivity of the muscle spindle endings, or that γ-motoneurone discharge may outlast the Jendrassik manœuvre. It is not unlikely that in intrafusal muscle fibres contraction and relaxation are slower processes than those in extrafusal fibres. However, such differences could only be inferred if the intensity of γ-motoneurone activity was accurately measured, and this was certainly not possible in these experiments. Investigations of tendon jerks have shown that fluctuations in reflex excitability due to changes in γ-motoneurone activity are correlated with many subjective and environmental factors in addition to the well-known effect of voluntary muscle contraction[9]. The electromyogram of the arm muscles provides a guide to only one of the mechanisms which may influence the discharge of the lumbo-sacral γ-motoneurones; we have no sure way of ruling out fluctuations dependent on other factors. Nevertheless, a fairly constant relationship was seen between the intensity of the electromyographic record of contraction of the wrist extensors and the intensity (as opposed to the duration) of the stretch response of the calf muscles. A similar experiment using the tendon jerks was reported by Courts[10], who showed that the amplitude of the knee jerk varied in proportion to the weight that subjects supported by steady contraction of the arm muscles.

The graded sensitization of the stretch reflex of the triceps suræ muscles accompanying voluntary contraction of the arm muscles is probably part of an important mechanism involved in the organization of movement in man. If a similar sensitization of stretch reflexes occurs in other limb muscles the overall effect would be to stabilize the position of the limb in space by tending to fix the length of the muscles. This would be done in a very efficient way, for active muscle contraction would be automatically concentrated in muscles which, in any body position, were those subjected to stretch. The threshold of the stretch reflexes and therefore the degree of stability of the limbs would vary with the intensity of the contraction of the arm muscles. Delicate manipulations involving minimal effort would scarcely alter the stretch reflex threshold whereas a massive mobilization of the arm musculature for lifting heavy weights or for pushing or prising large objects would automatically be accompanied by such fixation of the lower limbs as would be necessary for the effective execution of the movement.

In normal intact man it is usually difficult to distinguish the contribution of simple reflexes to overall behaviour because of the complexity of most movements and the complicated nature of the stimuli that evoke them. The experimental situation used here is of particular interest because it allows the demonstration of one function of the stretch reflex in comparative isolation, making it possible to infer what part this mechanism is likely to play in the control of posture and movement in other circumstances.

I thank Prof. J. Paillard for providing the facilities, support and discussion in connexion with this work.

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DISTRIBUTION OF ALLOTYPIC SPECIFICITIES AMONG RABBIT γ-GLOBULIN MOLECULES GENETICALLY DEFINED AT TWO LOCI

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THE 7*S* γ-globulins of individual rabbits are antigenic for other rabbits and yield precipitating antibodies[1–3]. These isoantibodies identify antigenic specificities on the γ-globulin molecules in the sera of some, but not all, rabbits. Such antigenic specificities are called allotypic specificities and distinguish among 7*S* γ-globulins not thus far separable by chemical criteria. The allotypic specificities A1, A2 and A3 appear to be controlled by three allelic genes at one locus, *a*; the allotypic specificities A4, A5 and A6, by three allelic genes at a second locus, *b* (refs. 4–10). These two loci, *a* and *b*, are not closely linked[10]. Agar-gel tube analysis of sera from individual rabbits indicated that the non-allelic controlled Aa1 and Ab6 are found on the same molecule whereas the allelic controlled Aa1 and Aa3 were on different molecules[11].

A method for quantitative analysis of allotypes based on successive precipitations of specific allotype from ^{131}I-labelled 7*S* γ-globulins with anti-allotype sera was developed[12]. This method was used to study the contribution of allelic genes $A\frac{4}{b}$ and $A\frac{5}{b}$ to the formation of 7*S* γ-globulins in homozygous and heterozygous rabbits[12]. It was found that in homozygotes or heterozygotes, 80–90 per cent of the γ-globulin molecules have Ab4 or Ab5; 10–20 per cent of the γ-globulin molecules lacked Ab4 and Ab5. In the heterozygote, the quantities of molecules precipitated by anti-Ab4 or anti-Ab5 were

lependent of the order of precipitation, indicating that ›4 and Ab5 are not found on the same γ-globulin mole-ıles but only on separate molecules. In two hetero-ygotes, 64 and 66 per cent of the γ-globulin labelled with iodine-131 was precipitated by anti-Ab4 and 27 and 24 per cent by Anti-Ab5.

This article presents a study of the contribution of genes at two loci to the formation of 7*S* γ-globulin molecules, by the quantitative analysis for allotypes[12] and by agar gel investigations[2,13].

In brief, the quantitative method[12] consisted in labelling a purified preparation of γ-globulin (ref. 14) with iodine-131 (ref. 15) and determining the amount of label precipitable by monospecific anti-allotype sera. The γ-globulin preparations used give a single precipitin line by immuno-electrophoresis with a sheep anti-rabbit serum which has precipitins to several β-globulins (including β_2A) as well as to γ-globulins. More than 97 per cent of the radio-activity in each preparation was precipitated by a sheep antiserum monospecific for rabbit γ-globulin; more than 99 per cent was precipitated by trichloroacetic acid (5 per cent).

Although the reactions with anti-allotype sera are carried out in the region of antibody excess, unlabelled carrier γ-globulin together with additional antibody are added to a portion of the supernatant to ensure complete precipitation. Depending on the particular antiserum, 2–4 successive precipitations are necessary to remove completely the corresponding allotype. After complete precipitation of one allotype, small aliquots of the super-natant can be analysed in the same manner with anti-allotypic antibodies of different specificities. To achieve uniform conditions for successive precipitations, only a portion of each supernatant is used. To this is added unlabelled carrier γ-globulin of the appropriate allotypic specificity and additional antiserum. The amount of antigen and antibody present is nearly the same in successive precipitations and the volume is held to less than 1 ml. Mixtures are allowed to stand in the refriger-ator for 1 day (anti-Ab4 and anti-Ab5) or 2 days (anti-Aa1). The percentage of the radioactivity precipitated in the successive steps is used to calculate the amount of γ-globulin of a particular allotypic specificity that was initially present[12].

Fig. 1 is a photograph of an Ouchterlony experiment which compares the reactions of anti-Ab5 and anti-Aa1 with serum of an Aa1, Ab5 double homozygote as well as with a mixture of two sera obtained from Aa1, Ab4

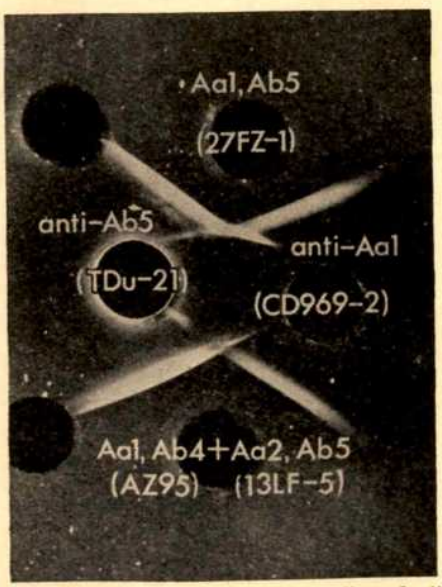


Fig. 1. Ouchterlony experiment comparing reactions of anti-Aa1 and anti-Ab5 with sera from an Aa1, Ab5 double homozygote and with the mixture of two sera from Aa1, Ab4 and Aa2, Ab5 double homozygotes

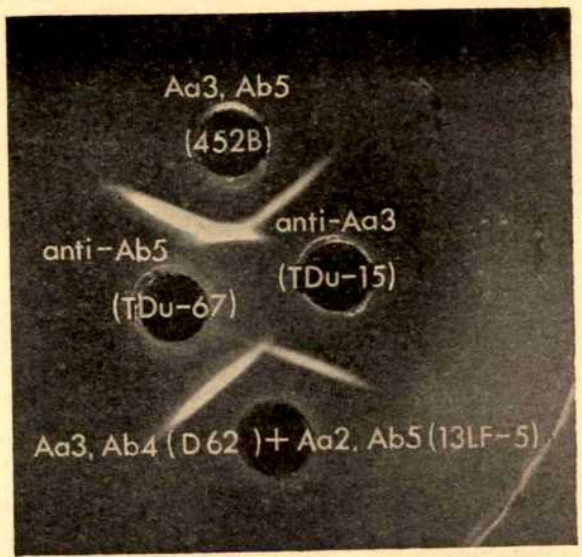


Fig. 2. Ouchterlony experiment comparing reactions of anti-Aa3 and anti-Ab5 with sera from an Aa3, Ab5 double homozygote and with mixture of two sera from Aa3, Ab4 and Aa2, Ab5 double homozygotes

and Aa2, Ab5 double homozygotes. In the mixture of the two sera, Aa1 and Ab5 are *a priori* not on the same molecules; the agar-gel plate accordingly shows a typical reaction of non-identity as expected for antisera which do not cross-react. The reaction of anti-Ab5 and anti-Aa1 with the serum of an Aa1, Ab5 double homozygote is quite different; there is partial coalescence and double spur formation, indicating that Aa1 and Ab5 are on separate molecules as well as on the same molecules. This hypothesis is supported by quantitative precipitin data, which are discussed later.

Fig. 2 shows a similar experiment comparing the reactions of anti-Aa3 and anti-Ab5 with the serum of an Aa3, Ab5 double homozygote as well as with a mixture of two sera from Aa3, Ab4 and Aa2, Ab5 double homozygotes. Again, the artificial mixture gives a typical reaction of non-identity whereas the serum of the Aa3, Ab5 double homozygote gives a reaction of partial coalescence and double spur formation. This suggests that some molecules have either the Aa3 or Ab5 specificity while others have both.

The γ-globulin preparations, when substituted for the sera of the double homozygotes (27FZ–1 and 452B) in the Ouchterlony experiments of Figs. 1 and 2, also showed the same result of partial coalescence and double spur formation in accordance with the idea that the reactions shown are due to the γ-globulins in the sera.

In previous work[12], quantitative analysis for Ab4 and Ab5 yielded the following results: (1) in the double homozygote (31FZ–2) 82 per cent of the γ-globulin labelled with iodine-131 was precipitable by anti-Ab5; (2) in the double heterozygote (20FZ–2), 64 per cent of the labelled γ-globulin was precipitable by anti-Ab4 and 27 per cent by anti-Ab5.

Fig. 3 shows the results of quantitative analysis for Aa1 in 7*S* γ-globulin from the same Aa1, Ab5 double homo-zygote (31FZ–2, top curve) and the same Aa1–Aa3, Ab4–Ab5 double heterozygote (20FZ–2). Four pre-cipitations with anti-Aa1 removed 66 per cent of the labelled γ-globulin from the double homozygote (31FZ–2) and 38 per cent from the double heterozygote (20FZ–2). After precipitation from the double heterozygote of Ab5 (27 per cent of the molecules), 32 per cent of labelled γ-globulin was then precipitable by anti-Aa1. After precipitation from the double heterozygote of Ab4 (64 per cent of the molecules) only 12 per cent of the γ-globulin labelled with iodine-131 was precipitable by anti-Aa1 (as compared with 38 per cent when the anti-Aa1 was used first). In each case the percentages given are based on

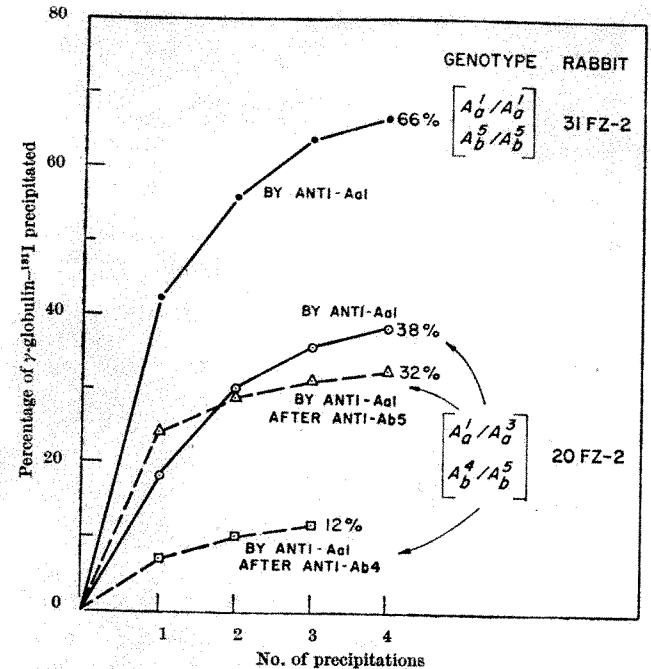


Fig. 3. Quantitative analysis of purified γ-globulin from two rabbits for the amount precipitable by anti-Aa1

the total amount of protein originally present. Three or four precipitations were necessary before the removal of Aa1 approached completion in contrast to the two precipitations generally sufficient for removal of Ab4 or Ab5. This suggests that Aa1 is a 'weak' antigen, perhaps due to an insufficiency of antigenic sites and/or that the amount precipitable may depend to some extent on the antibody preparation used[16].

Since, in the double homozygote, 66 per cent of the labelled γ-globulin is precipitated by anti-Aa1 and 82 per cent by anti-Ab5 (ref. 12), at least 48 per cent (66–18 per cent) of the radioactivity must be associated with molecules having both specificities Aa1 and Ab5. In another experiment (not shown) after precipitation of Ab5 (82 per cent of the molecules), 7 per cent was precipitable by anti-Aa1. Thus at least 7 per cent of the molecules have Aa1 but not Ab5 in accord with the Ouchterlony analysis shown in Fig. 1. In another Aa1–Ab5 double homozygote, 35FZ–1, 17·8 per cent of the labelled γ-globulin was precipitable by anti-Aa1 after removal of 70·3 per cent of the radioactivity by anti-Ab5, confirming the existence of Aa1 molecules lacking the Ab5 specificity (by the criteria of precipitation).

It had been shown previously[12] that in the heterozygote Ab4 and Ab5 are on separate molecules, and that 91 per cent of the molecules have one or the other specificity. Therefore, at least 29 per cent (38–9 per cent) of the radioactivity must be associated with molecules having two specificities, Aa1 plus either Ab4 or Ab5. In the double heterozygote, precipitation by anti-Ab5 reduced the amount of Aa1 from 38 to 32 per cent of the total, whereas precipitation by anti-Ab4 reduced the amount of Aa1 from 38 to 12 per cent. This indicates that 68 per cent (26/38) of the molecules with Aa1 also have Ab4 specificity and that 16 per cent (6/38) of the molecules with Aa1 also have Ab5. If the results presented in Fig. 1 were consistent with completely random association of the non-allelic specificities, then the fraction of Aa1 molecules associated with Ab4 would be the same as the fraction of Ab4 molecules in the total population (64 per cent). The actual value observed was 68 per cent. In the case of association of Aa1 with Ab5, the predicted and observed values were 27 and 16 per cent. However, the value of 16 per cent is sensitive to small errors in percentages of Aa1 observed before and after removal of Ab5. It is of particular interest that the Aa1 and Ab5 specificities were under control of genes derived from different parents. Thus, the molecules in the double heterozygote which have both Aa1 and Ab4 are γ-globulins which are present in neither parent. That such hybrid protein molecules have antigenic specificities (or other properties) differing from the γ-globulins in either parent is suggested also by the work of Oudin[16].

It is of interest to compare the relationship of genetic control of the biosynthesis of γ-globulin to that of hæmoglobin[17,18]. So far, the results obtained for γ-globulin are consistent with those obtained for hæmoglobin. Allelic genes for γ-globulin, as well as for hæmoglobin, do not contribute to the same molecules, whereas non-allelic genes do contribute to the same molecules. Moreover, allelic genes function in the same cell to produce two forms of hæmoglobin. For the γ-globulin, the evidence obtained also indicates that allelic genes function within the same cell to produce two varieties of γ-globulin[19]. It is of interest to note that analogous studies of mucopolysaccharide molecules for human blood group specificities yield somewhat different results: the A and B specificities determined by allelic genes as well as the A and Le specificities determined by genes at two loci appear on the same mucopolysaccharide molecule[20]. Incorporation of the antigenic specificities into the mucopolysaccharide molecule is considered to occur late in its biosynthesis and to be a result of enzyme action rather than a result of primary gene action[20].

Since the facts obtained so far for γ-globulin are in accordance with those for hæmoglobin[17,18] with respect to the contribution of allelic genes[11,12], we suggested[12] that in the double heterozygote, polypeptide chains carrying the Ab4 specificity form pairs immediately after synthesis and that the Ab4 pairs do not combine with similarly formed Ab5 pairs but do combine with pairs of polypeptide chains the synthesis of which is controlled by genes at the a locus, such as by $A\frac{1.}{a}$. This hypothesis further suggests the possibility that the structural model of γ-globulin may differ from that proposed by Porter[21] in that the B-chains as well as the A-chains may exist within the molecule as dimers similar to the α- and β-chains of hæmoglobin. A distinction between γ-globulin and hæmoglobin would be the importance of disulphide bonds in holding together polypeptide chains in γ-globulin. Chemical investigations are needed to evaluate this hypothesis.

If we assume that the synthesis of polypeptide chains and their interaction to form the completed γ-globulin molecule occurs within a single cell, then the foregoing findings lead to the conclusion that in a double heterozygote such as 20FZ–2 (Aa1–Aa3, Ab4–Ab5), at least four allotypes of γ-globulin molecules are synthesized within the same cell; these allotypes are A–a1–b4, A–a1–b5, A–a3–b4 and A–a3–b5. Also, since the parents were Aa1, Ab5 and Aa3, Ab4 double homozygotes, then the A–a1–b5 molecules correspond to those in one parent, the A–a3–b4 molecules to those in the other parent, and finally the other two allotypes A–a1–b4 and A–a3–b5 are hybrids present only in the offspring but not in either parent.

Since more than one allotypic specificity, that is, Ab5, Ab5′ (refs. 4 and 10), is 'functionally' determined by a single allele, that is, $A\frac{5}{b}$, and since the number of genetic loci involved in γ-globulin synthesis is at least three[10], the number of allotypes synthesized within a single cell in a double heterozygote is likely to be much more than four. The question then arises as to the relationship (if any) of this information to the number of antibodies of different specificities formed within single cells[22,23]. Work is continuing to determine the extent of the variation of γ-globulin molecules (number of allotypes) synthesized within a single cell.

In conclusion, the results presented in this article show that genes at the two loci *a* and *b* contribute to the synthesis of the same γ-globulin molecules but not to all of them. Thus, in the Aa1–Ab5 double homozygote there are at least four kinds of γ-globulin molecules: (1) those with Aa1 not precipitable by anti-Ab5; (2) those with Ab5 not precipitable by anti-Aa1; (3) those with both Aa1 and Ab5; (4) those not precipitable by either anti-Aa1 or anti-Ab5. The explanation of this distribution of allotypic specificities among γ-globulin molecules must lie partly in the fact that additional loci are involved in the control of γ-globulins. For example, it is now known that allotypic specificity *P* (ref. 10) is present in each of the γ-globulin preparations analysed and its distribution among the γ-globulins is under investigation.

Finally, in the Aa1–Aa3, Ab4–Ab5 double heterozygote, the polypeptide chains carrying the Aa1 specificity associate with polypeptide chains carrying either Ab4 or Ab5. About two-thirds of the molecules with Aa1 also had the Ab4 specificity even though the genes controlling these specificities came from different parents. Thus, these A–a1–b4 molecules present in the offspring are not present in either parent. The quantities of A–a1–b4 and A–a1–b5 molecules found are consistent with the possibility that the assortment of polypeptide chains under control of the two loci is random, that is, that the fraction of Aa1 molecules also bearing the Ab4 or Ab5 specificity is equal to the fraction of molecules with Ab4 or Ab5, respectively, in the total population[24].

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EFFECT OF STARVATION ON ADENOSINE TRIPHOSPHATE CONCENTRATION IN *AEROBACTER AEROGENES*

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WHEN washed stationary phase *Aerobacter aerogenes* are starved in aerated phosphate–saline buffer, *p*H 6·5 (about 10^{10} bacteria/ml.), the viability remains high (90–98 per cent) for at least 40 h (ref. 1). During this period, degradation of endogenous protein, ribonucleic acid and, if present, glycogen occurs and the products are excreted. Synthesis of various compounds also occurs in starved bacteria, for example, during protein and nucleic acid turnover[2], and many of the reactions involved will require adenosine triphosphate (ATP). Dawes and Ribbons[3] have pointed out that energy will also be utilized by starved bacteria during osmotic regulation and for the maintenance of the intracellular *p*H value. Although ATP will be formed during the metabolism of reserves, the rate of formation is likely to fall as the reserves decline during prolonged starvation. Because the amount of endogenous ATP and the ability to synthesize this compound may be of importance for bacterial survival, we have investigated the effects of starvation on its concentration in *A. aerogenes*.

A. aerogenes, grown in an aerated carbon-limiting mannitol–ammonia–salts medium[1] at *p*H 7·2–7·4, were gathered 0·5 h after growth had ceased and washed twice with phosphate–saline buffer by centrifuging. Suspensions (3–10 × 10^9 cells/ml.) in this buffer (0·11 M NaCl containing K_2HPO_4 + KH_2PO_4 (0·02 M PO_4), *p*H 6·5) were starved in gas wash-bottles with vigorous aeration. Changes in the viability were determined by viable plate counts or a slide culture technique[4].

Extracts were prepared by adding perchloric acid (0·25 ml. of 72 per cent w/v) to bacterial suspensions (5 ml.), allowing each mixture to stand at 20° for 0·5 h and then removing the cell debris by centrifuging. Extraction at 20° rather than at 0°–4° gave more reproducible results for the ATP content and did not cause a significant breakdown of standard ATP. The acid extract was neutralized with 2 N potassium hydroxide solution. Precipitated potassium perchlorate was separated after cooling the mixture to 0° and the clear solution was diluted with water to 10 ml. Neither mechanical disintegration of the bacteria nor their dissolution in potassium hydroxide solution before acid extraction increased the ATP concentration in the extract. Hot water extracts of intact bacteria gave low results due partly to the presence of substances which were inhibitory to the assay.

Adenosine triphosphate was determined with the firefly-luminescence technique[5] using a scintillation counter constructed by our colleague Mr. S. Lovett. Reaction mixtures (4 ml.) contained 0·02 M sodium arsenate buffer (*p*H 7·4), magnesium sulphate (3 mM), sample (0–0·3 μg ATP) and an extract of 2·5 mg firefly tails; luminescence was measured for 0·5 min, starting 1 min after the addition of enzyme, and the response was nearly linear within this range. When testing a series of extracts, standard and blank (no ATP) mixtures were assayed frequently. Perchlorate ions in bacterial extracts inhibited the reaction but interference was small when 0·05 ml. or less of extract was assayed. The separation of ATP from acid extracts by gradient elution from ion-exchange resin[6], followed by ultra-violet absorption measurements or firefly luminescence assay of the appropriate eluates, indicated that direct measurements on extracts were 20–25 per cent high. The loss of added ATP labelled with phosphorus-32 during this fractionation was less than 10 per cent of the quantity applied to the column. Of the various fractions obtained from the extracts, only adenosine-5′-diphosphate,

and deoxyadenosine-5′-triphosphate gave a significant response in the assay and these compounds probably accounted for the discrepancy.

The ATP content of freshly gathered and washed bacteria varied with the oxygen tension and solute concentration of the suspending solution; both anaerobic conditions and high solute concentration markedly reduced the amount of ATP extracted (Fig. 1). Considerably less ATP was found when the acid was added directly to a centrifuged pellet of bacteria rather than to a suspension of the pellet in aerated water or phosphate–saline buffer. Presumably the anaerobic condition of the bacteria in the pellet was responsible for the low ATP concentration since resuspension of the pellet in de-aerated buffer did not increase the ATP content. The synthesis of ATP which occurred when the bacteria were transferred from anaerobic to aerobic conditions (Fig. 2) or from a solution of high to one of low solute concentration was extremely rapid. The concentrations found in freshly gathered bacteria suspended in phosphate–saline buffer under aerobic and anaerobic conditions by direct assay of acid extracts were 1·6–2·2 and 0·2–0·5 μg of ATP/mg bacterial dry weight, respectively.

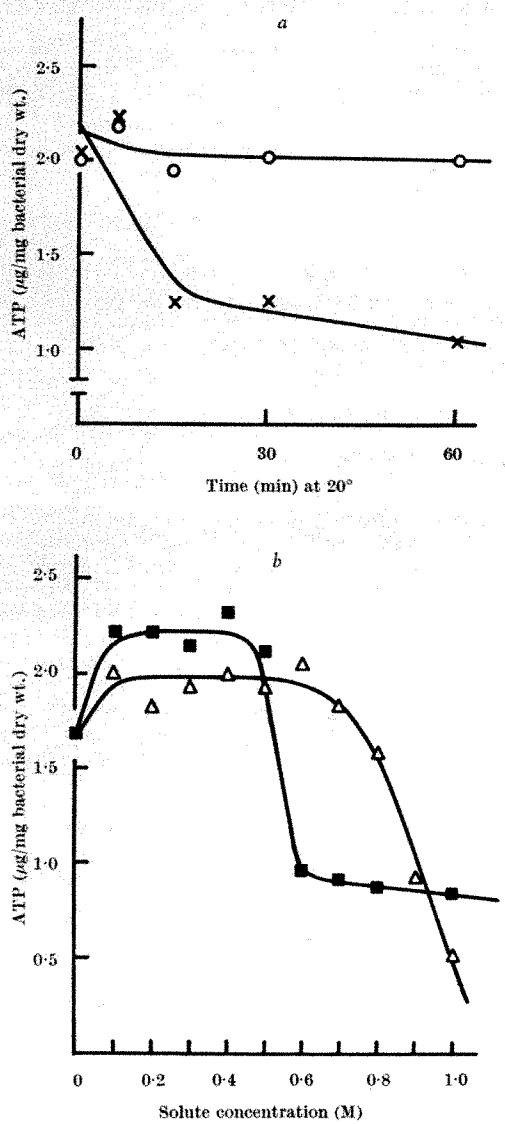


Fig. 1. Effect of oxygen tension and solute concentration of suspending fluid on the ATP content of washed stationary phase *A. aerogenes* (*a*) One portion of an (aerated) bacterial suspension (4·8 mg dry wt./ml.) was aerated (○), and through the other nitrogen was passed (×). Samples taken at intervals from each suspension were acidified and neutralized extracts were prepared for ATP assay. (*b*) Bacteria separated from 5 ml. volumes of a washed suspension (4·1 mg dry wt./ml.) were resuspended in aerated solutions (5 ml.) of various concentrations of sucrose (△) and sodium chlorine (■). After standing for 5 min, acid extracts of the suspensions were prepared for ATP assay

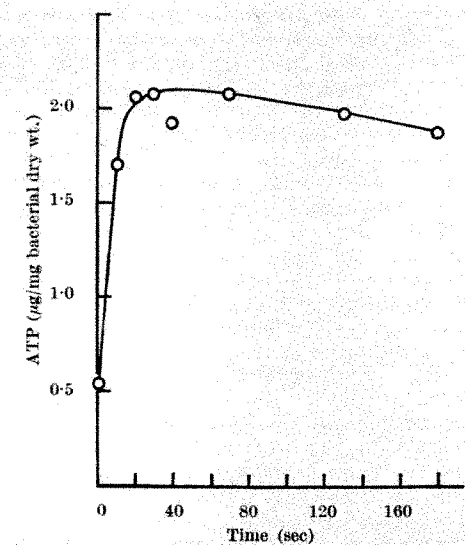


Fig. 2. Synthesis of ATP on transfer of *A. aerogenes* from anaerobic to aerobic conditions. Bacteria separated from 5 ml. volumes of a washed bacterial suspension (4·6 mg dry wt./ml.) were resuspended in 5 ml. of aerated phosphate–saline buffer (*p*H 6·5) and the suspensions were acidified (perchloric acid) at intervals. As a control ($t = 0$) the bacteria were resuspended in acidified phosphate–saline

The analysis of cold perchloric acid extracts of bacteria on ion-exchange resin showed that, although the concentration of ATP was different under aerobic and anaerobic conditions, the total amount of adenosine-5′-mono-, di- and tri-phosphates was the same. For example, the amounts found in 1 g dry weight of bacteria were 1·39, 2·04 and 2·24 μmoles respectively under aerobic conditions and 3·04, 1·81 and 0·8 μmoles respectively under anaerobic conditions. Thus the aeration of an anaerobic suspension causes a regeneration of ATP at the expense of adenosine-5′-phosphate. The concentration of ATP found in this experiment under aerobic conditions was lower than usual, probably because air was passed into a very thick suspension and aeration was not complete. Nevertheless, the value obtained is similar to that reported by Franzen and Binkley[7] for exponentially growing *Escherichia coli*. Using ion-exchange fractionation, these authors found the relative amounts of adenosine-5′-mono-, di- and triphosphates to be about 1 : 5 : 50. These ratios, which were very similar in *E. coli* gathered from six different growth media, are very different from our ratios for stationary phase *A. aerogenes*. However, the relative amounts of these compounds in bacteria appear to vary widely: Kuriki and Okazaki (ref. 8, Fig. 1) showed a high ratio of adenosine-5′-phosphate to ATP in an acid extract from exponentially growing *E. coli* strain 15*T*⁻ (thymine requiring mutant), and Smith and Wheat[9] reported a high ratio of adenosine-5′-phosphate to adenosine-5′-diphosphate in an acid extract from *Chromobacterium violaceum* (NCTC 7917).

The effects of starvation on the ATP content and viability of *A. aerogenes* in aerated phosphate–saline buffer were investigated at 20°, 37° and 44° (Fig. 3). At 20° both viability and ATP remained fairly constant for 55 h; at 37° there was a small initial increase in ATP after which the level fell to less than the initial value before there was a significant loss in viability; at 44° there was a pronounced initial increase in ATP after which the level fell with the viability. The degradation of ribonucleic acid, accompanied by a leakage of ultra-violet-absorbing substances, also increased rapidly with temperature in the range 20°–50°; the initial increase in ATP at the higher temperatures may therefore be due to a synthesis of ATP from adenosine-5′-phosphate or adenosine-5′-di-

phosphate liberated from ribosomes by autodegradation[10] or by some other route which is stimulated by higher temperatures.

Although no direct relationship was found to exist between ATP concentration and viability or survival prospects of a bacterial population, we obtained some evidence to suggest that the ability of bacteria to synthesize ATP in the absence of exogenous nutrient is related to survival. Starvation progressively reduced the magnitude of the increase in ATP which occurs when bacteria are transferred from anaerobic to aerobic conditions; after starvation at 37° for 42 h when viability was 85 per cent, there was no increase when this transfer was made and from then on there was a progressive loss of viability (Fig. 4). Similarly, during starvation at 37° in aerated M sodium chloride the ability of bacteria to synthesize ATP on transfer to aerated 0·15 M sodium chloride declined at a faster rate than viability (Fig. 5). After 4 h, loss in viability was 10 per cent and the amount of ATP synthesized on transfer of the bacteria was less than 50 per cent of the 2-h value; after 18 h loss in viability was 92 per cent, and no ATP was synthesized on transfer of the bacteria. The results obtained for bacteria starved in 0·15 M sodium chloride are also shown (Fig. 5).

These results indicate that the ATP concentration in non-growing *A. aerogenes* depends largely on the oxygen tension and solute concentration of the environment and is not necessarily related to the viability or the survival prospects of a population. Starvation eventually reduces both the ATP concentration and the ability of the bacteria to synthesize ATP when transferred from anaerobic to aerobic conditions or from solutions of high to those of low solute concentrations.

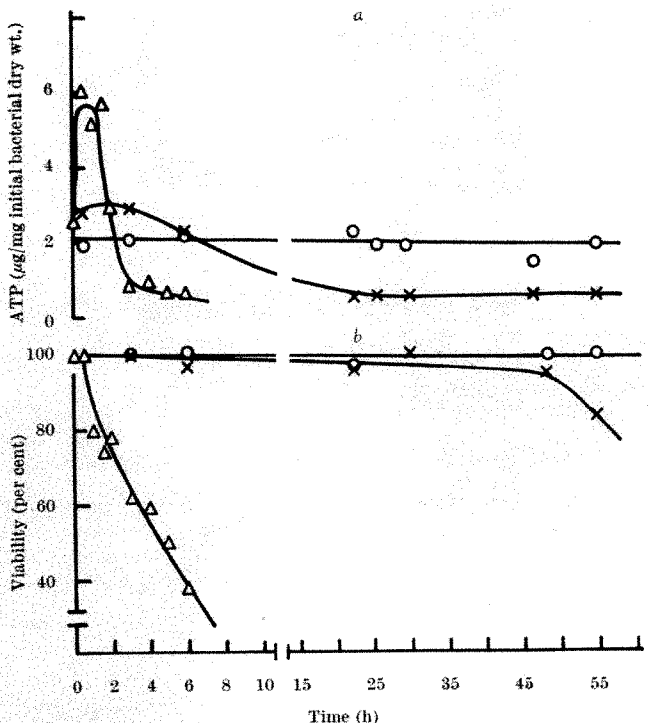


Fig. 3. Effect of starvation on the ATP concentration and viability of *A. aerogenes*. Portions of a washed suspension of stationary phase bacteria (4 × 10⁹/ml.) were held at 20° (○), 37° (×) and 44° (△) in aerated phosphate–saline buffer and samples removed at intervals for (*a*) ATP and (*b*) viability determinations

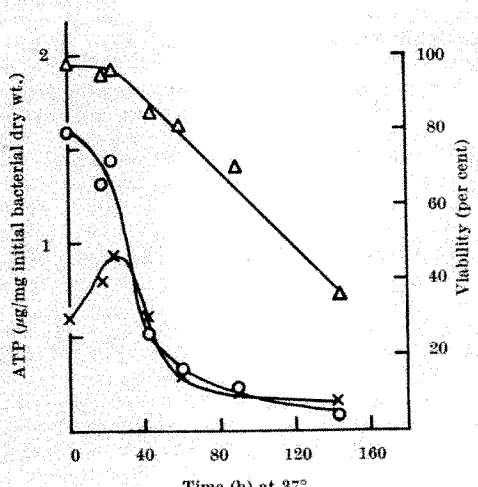


Fig. 4. Effect of starvation on the ability of *A. aerogenes* to synthesize ATP. A washed, aerated suspension of bacteria (4 × 10⁹/ml.) in phosphate–saline was held at 37° and duplicate samples (5 ml.) were removed at intervals. Both samples were centrifuged and the pellet from one resuspended in 5 ml. of 0·5 N perchloric acid (anaerobic, ×); the other pellet was resuspended in aerated phosphate–saline and the suspension acidified after 5 min (aerobic, ○). Extracts were prepared and assayed for ATP. Viability of suspension (△)

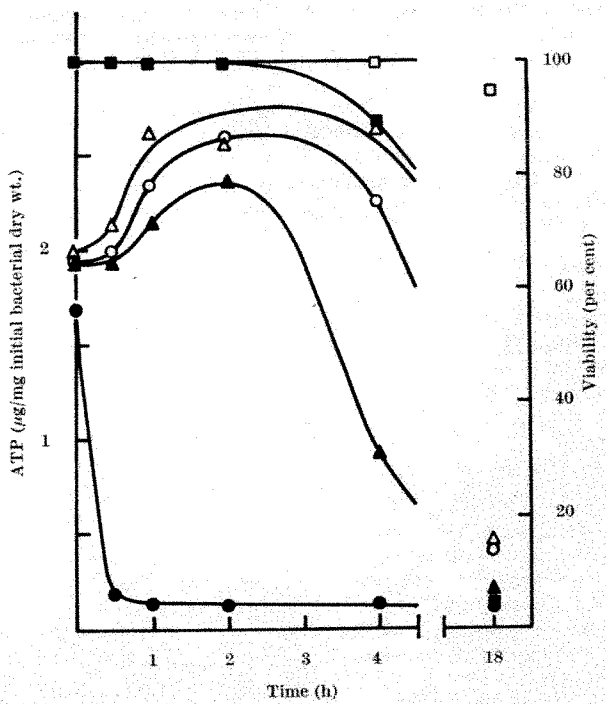


Fig. 5. Effect of starvation in solutions of high and low solute concentration on the ability of *A. aerogenes* to synthesize ATP. Suspensions of washed bacteria (7 × 10⁹/ml.) in 0·15 M sodium chloride (open symbols) and M sodium chloride (closed symbols) were aerated at 37°. Duplicate samples (5 ml.) were removed at intervals from each suspension and one of each pair was acidified directly (○, ●), while the other was centrifuged and the pellet resuspended in aerated 0·15 M sodium chloride before acidifying (△, ▲). Extracts were prepared for ATP assay. Viability of suspensions (□, ■)

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LETTERS TO THE EDITOR

PHYSICS

Liquid Explosives with Transparent Detonation Products

In an attempt to observe events behind the shock front of detonating explosives we have investigated the possibility of finding materials having transparent reaction products. Explosives of this sort might then simplify the problems since visible light photography could be used instead of X-ray techniques. Preliminary results obtained using a high-speed framing camera have been reported[1] for 18 molal hydrazine mononitrate in hydrazine. This explosive was chosen since it was believed that the opacity commonly observed[2] in detonation gases was due to free carbon. The reaction products of this carbon-free explosive transmitted light, and strong turbulent patterns could be seen. We can now present evidence showing that explosives containing carbon, specifically mixtures of nitromethane and tetranitromethane, can also have transparent products.

An observation container for liquid explosives was made by cementing thin aluminium ribs between two opposite edges of a pair of $6 \times 6 \times 1\frac{1}{2}$ in. 'Plexiglas' blocks. A 1/8-in. aluminium plate was cemented over one of the open ends, forming a tank open on one side and having an internal thickness equal to the thickness of the metal ribs. The cameras, either a high-speed framing or rotating-mirror streak type, observed events occurring within the loaded tanks by the use of diffused backlight from an argon flash-bomb. Most of the tests were on explosive sheets $\frac{1}{4}$ in. thick since at this thickness the pure explosive components, nitromethane or tetranitromethane, would initiate but later fade after propagating only part way through the sample. Opacity of the pure explosive reaction products could then be clearly seen since our intense backlight could not penetrate the cloud. The test liquids were line initiated through the 1/8-in. aluminium plate. The streak camera photographs were velocity synchronized, that is, the mirror speed was adjusted to produce a stationary image on the film of the event sweeping past the 0·020-in. camera slit.

Fig. 1 is a framing camera photograph looking through a sheet of nitromethane at the time of failure. Early frames of this event show the detonation proceeding uniformly along a straight line. After a time, roughness develops and at points where failure has occurred the shock wave is detached from the product cloud. The emerging shock wave always has the contour of the product cloud but quickly becomes smooth as it pulls away.

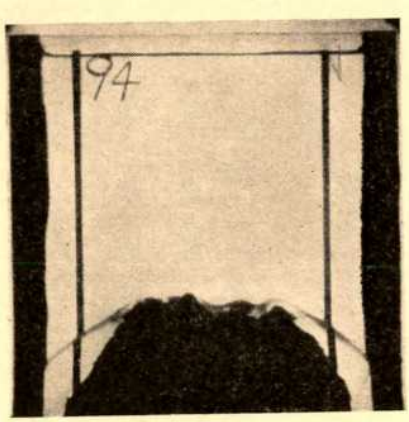


Fig. 1. Framing camera view through a $\frac{1}{4}$-in. thick layer of nitromethane. Exposure time $\frac{1}{4}$ μsec

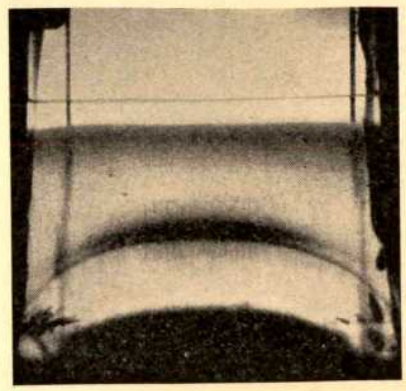


Fig. 2. Framing camera view through a $\frac{1}{4}$-in. layer of detonating nitromethane–tetranitromethane mixture. Exposure time $\frac{1}{4}$ μsec

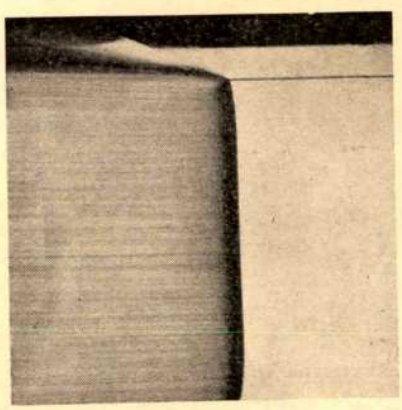


Fig. 3. Streak camera photograph of detonation (left to right) in a nitromethane–tetranitromethane mixture

The two vertical lines which are refracted by the pure shock in the liquid are silhouettes of 1/8-in. wide metal ribbons on the back outside surface of the tank. When such ribbons were placed on the back inside surface, the failing detonation tended to climb them as might be expected. Tetranitromethane at these dimensions shows a somewhat similar failure pattern.

Fig. 2 is a photograph looking through a 1/8-in. sheet of a detonating mixture of 70/30 (by vol.) nitromethane/tetranitromethane. This mixture is sensitive and will easily detonate in 1/32-in. sheets. The two grid lines are visible through the reaction products. The curved arc is due to a shock in the 'Plexiglas' windows from the plane wave booster which overlapped the tank edges. Toward the detonation front some opacity can be seen. In both Figs. 1 and 2 the liquid surface can be seen toward the top of the tank.

Fig. 3 is a streak camera view through an identical 1/8-in. tank filled with 80/20 nitromethane/tetranitromethane. The striations behind the zone are due to turbulence in the gas products[1]. The reaction zone region is abruptly opaque and gradually clears. The air-liquid interface forms a dark horizontal line near the top of the photograph. An air shock followed by a shock in the 'Plexiglas' window can also be seen. The rarefaction at the unconfined interface appears to have produced a region of transparency in the opaque zone and forms an angle of about 45° with the direction of propagation. This opaque zone is roughly twice as long in a 1/4-in. thick tank as in a 1/8-in. one, that is, about $1\frac{1}{4}$ μsec compared with 1/2 μsec.

A 'dark space' of 0·5 sec duration has been observed[3] at the detonation front in pentolite and it was suggested that this was due to a highly ionized region which acted as a reflector and blocked the transmission of internal light. We favour this mechanism to explain the absence of transmitted backlight in our experiments. However, Johansson and Sjölin[2] believe that carbon particles may form a light-absorbing screen at the surface layer of the detonation gas and they have data indicating this as a possibility. Our experiments show that the opaque reaction products from nitromethane, ostensibly free carbon, can be burned to produce clear products if the oxygen balance is adjusted by the addition of tetranitromethane to anywhere within the carbon monoxide–carbon dioxide stoichiometric limits. In line with the Johansson–Sjölin explanation it is of interest to note that self-luminosity is markedly higher in those mixtures having transparent products and reaches a peak within the carbon monoxide–carbon dioxide limits. Detonation luminosity is at its lowest with pure tetranitromethane but in this case, as with oxygen-rich mixtures, the opacity may be due to nitrogen oxides.

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Transmission Parameters of Porous Sound Absorbers (with Particular Reference to the Flow Resistance)

Flow resistance, being relatively easy to measure, is commonly used as a rough guide in choosing sound-absorbing materials for specific purposes. This practice is based on the tacit assumption that, when there is an alternating air-flow through the sample, the resistance encountered is identical with the flow resistance. It can be shown, however, that in the case of several materials this assumption is false, and that the acoustical resistance under alternating conditions is substantially greater than the flow resistance.

To establish the variation of effective resistance with frequency the characteristic impedance (Z_0) and the specific transmission constant ($\gamma = \alpha + j\beta$) of a number of commonly used porous sound-absorbing materials have been measured over the range 100–1,300 c/s using samples 1 in.–3 in. thick inserted into a rigid tube 3·47 in. in diameter. At each frequency sound is passed into the sample under two conditions in succession: first, when the sample is terminated by an impedance effectively infinite, and second when the terminal load is purely capacitive. By adopting an electroacoustic artifice the effect of the input impedance of the probe microphones, which measure the sound pressure, is completely eliminated, and by using an a.c. potentiometer to measure microphone voltages, the pressure ratio is derived as a complex number. Assuming that conventional transmission theory is applicable, this ratio is $\cosh(\gamma l)$ (l = sample thickness); whence γl. Knowing γl, the second test yields Z_0 in terms of the terminal capacitance.

After analysing the variation with frequency of these two parameters, it is found that in all the materials examined the series arms of the equivalent-tee network which represents the performance of the acoustical structure contain resistance and inductance in series, the shunt arm being a capacitance in series with a small resistance. Where the test samples have been sufficiently stable to yield very precise data it has been possible to investigate the series arms of the tee network in detail; it is then found that each branch consists of two series combinations of R and L in parallel which have impedances $(R_1 + j\omega L_1)$ and $(R_2 + j\omega L_2)$, L_2, say, being greater than L_1. Re-analysis of the data published in ref. 1 confirms this curious disposition. The same data also confirm the series arrangement of the shunt branch of the tee-network, a fact apparently unrecognized by the authors. It is to be noticed that a later publication[2] discusses the possibility of a rather similar disposition, using arguments of quite a different kind. Yet another publication[3] presents data which on re-analysis confirm the series combination of R and C, in the shunt arm, mentioned here.

If then we assume that the series arm of the equivalent tee-network has the dual form described, with $L_2 > L_1$, it is clear that flow resistance measures the value of R_1 in parallel with R_2, whereas when frequency rises from zero the effective resistance rises towards R_1—a quantity which in various materials has been found to be of the order of 1·5 up to 3 or more times the flow resistance. Moreover, as L_2 may be many times greater than L_1, the frequency at which the transition from the lower to the higher effective resistance is substantially complete may be as low as 150 c/s; usually it is in the region of 200–300 c/s.

Knowing both Z_0 and γ it has been possible to go a stage further and to build electrical artificial lines, the input impedance of which imitates the acoustical impedance, at normal incidence, which sheets of any thickness of the tested materials would present when mounted on the walls of a room as sound absorbers. The real part of the electrical input impedance is—subject to the usual corrections—a measure of the acoustical resistance in which the sound would be absorbed. By modifying the length of the artificial line and its terminal impedance it becomes possible to predict the effect of altering the thickness of the absorber and of its method of mounting.

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Dielectric Properties of Cross-linked Polystyrene Film formed in the Glow Discharge

Irradiation of a monomer vapour frequently causes polymerization leading to the formation of a solid deposit on any convenient surface. Electrons[1], ultra-violet, X-, γ-radiation or an electrical discharge[2] can be used.

In the work recorded here. a glow discharge at 180 kc/s is used to form uniform 1μ polystyrene film. The discharge is maintained between parallel cylindrical aluminium electrodes in several mm mercury pressure of styrene vapour contained in a pre-evacuated chamber. Aluminium foil, in contact with one electrode, serves as the substrate for the film. This electrode rotates uniformly during polymerization. Aluminium is afterwards evaporated on to the surface of the polymer film to provide a second electrode.

Fig. 1 shows power factor and capacitance against temperature at several frequencies. The wide distribution of relaxation times, apparent from the shape of the curves, results from the complex structure of the film. Traces of oxygen and water vapour, which give rise to highly polar groups, are especially significant since only milligrams of polymer are formed. Further, electron spin resonance measurements made at room temperature *in vacuo* show the presence of free radicals which presumably peroxidize on exposure to air.

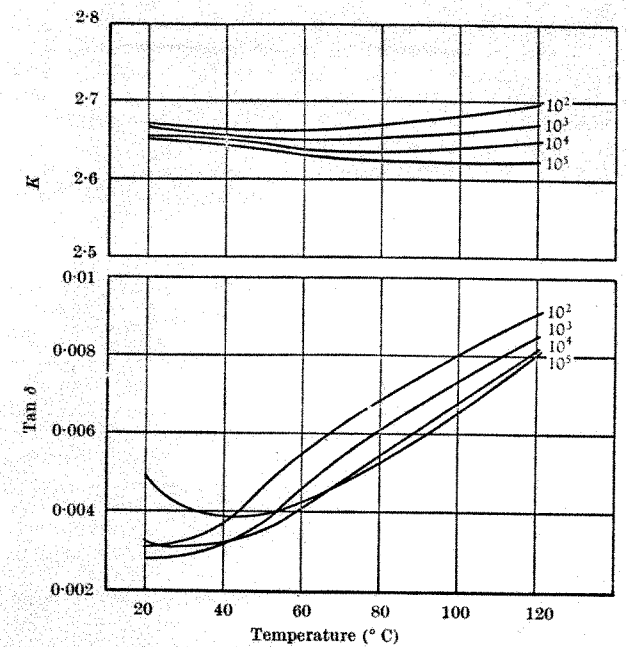


Fig. 1. Permittivity and loss tangent versus temperature at four frequencies

An interesting feature of the films is their insolubility, indicating a high degree of cross-linking. Dipolar loss due to the presence of combined oxygen is evidently spread over a wide frequency range. The frequency/loss angle relationship depends on the flexibility of the polymer chain to which the oxygen is attached. This is greatly restricted in a cross-linked material.

I thank the Ministry of Aviation for permission to publish this work.

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GEOPHYSICS

Tritium Content of Antarctic Snow

The abundance of tritium in Nature is of much geophysical interest[1–3]. In view of the present paucity of data on Antarctic tritium, we are reporting here determinations made on three Antarctic snow samples collected by one of us (H. A. C. N.) during 1960–61.

Three samples of several litres each were collected from the Ross Ice Shelf in the general vicinity of Ross Island (77·5° S., 168° E.). Determinations of tritium were made in August 1962 by a laboratory specializing in low-level tritium assay of geological samples (the tritium laboratory of Isotopes Incorporated, Westwood, New Jersey; we thank M. Trautman for carefully supervising the tritium determinations, for discussions of the techniques used, and for the precautions taken to prevent contamination of the samples by tritium from other sources). The method used was the standard one of electrolytic enrichment of tritium followed by low-level counting[4]. The counting was performed on two portions of each enriched sample, following check-runs using 'blank' (< 10 T.U.) water. One T.U. is an abundance of 1 tritium atom per 10^{18} hydrogen atoms. The results are shown in Table 1 (samples *A*, *B* and *B′*). The third column gives the tritium content extrapolated to the time of precipitation of the snow. The tritium content at the time of assay is given in parentheses in the same column. The errors shown for the values in parentheses are the overall standard deviations of the tritium analyses.

Table 1. Tritium Contents of Antarctic and Southern-Hemisphere Precipitation

Description of sample	Time of precipitation	Tritium content in T.U.*	Ref.
A. Ross Ice Shelf snow, near Cape Crozier; collected early Dec. 1960; top 15 cm	Sept. to Dec. 1960	82 ± 8 (82 ± 8)	This communication
B. Ross Ice Shelf firn, near Scott Base; collected mid-Jan. 1961; 6·6–6·8 m depth	~1941	17 ± 3 (5·2 ± 0·5)	This communication
B′. Ross Ice Shelf ice, near Cape Crozier; collected early Dec. 1960; ~8 m depth (contaminated *in situ*; see text)		– – – – (43 ± 4)	This communication
Antarctic snow; collected Feb. 19, 1955; top 0·3 m; average of 4 samples	1953–54	16 ± 3	Table 2 of ref. 5
Antarctic snow; collected Jan. 15, 1955; 0·3–1 m depth; average of 4 samples	~1952–53	17 ± 3	Table 2 of ref. 5
Antarctic snow; collected Jan. and Mar. 1956; depths covering 5 years; several samples	~1951–55	Little or no artificial tritium (see ref. 6)	Ref. 6
Akatarawa Stream (Wellington, N.Z.) water; yearly average of monthly samples	1956	5·3	Ref. 7
	1957	6·6	Ref. 7
	1958	9·8	Ref. 7
	1960 (May–Sept.)	10	Ref. 7
Average rains in middle and low southern latitudes	1958–mid 1960	~10	Ref. 2

* Values given in parentheses in this column are tritium contents at time of assay; these are preceded by values extrapolated to time of precipitation.

Sample B. This firn sample was collected near the bottom of a glaciological pit 6·8 m deep from a point about 5 cm inside the pit wall. The stratigraphy of the pit walls and of a vertical core taken from below the pit floor shows that this sample represents the snow of approximately the year 1941[8]. The Ross Ice Shelf at this point is 43 m thick, the bottom 28 m of which is soaked by brine from the sea below[8]. The value of 17 T.U. for this single sample is in good agreement with the average of eight shallower Antarctic samples measured by Begemann and Libby[5] and listed in Table 1.

Sample B′. This sample was taken from the side of a deep fissure in the seaward front of the Ross Ice Shelf at a point about 8 m below the Shelf surface. Although some material was removed from the side surface before collecting, the sample very likely had been contaminated *in situ* by seepage of recent precipitation. With this in mind, one sees that its measured tritium content is consistent with those of samples *B* and *A*.

Sample A. This sample, representing the average snow of September–December 1960, has a tritium content five times the level for 1941 as given by sample *B*. For comparison, Table 1 summarizes other published tritium data known to us for southern-hemisphere precipitation after 1956. The doubling of the tritium-level during 1956–60 in New Zealand precipitation (as measured in the fast-flowing waters of Akatarawa Stream) has been ascribed to injection of artificial tritium into the northern-hemisphere stratosphere by thermonuclear explosions[7]. Individual rains at other middle and low southern latitude localities fluctuated considerably around 10 T.U. from 1958 to mid-1960[2], but in no case approached the 82 T.U. measured in sample *A*. It is also likely[9] that much natural tritium was introduced into the north and south polar stratospheres during and following the unusually active 1958 solar maximum, both by local production due to solar cosmic rays and by accretion of solar tritium. However, the global fall-out pattern of artificial strontium-90 introduced at any latitude into the northern-hemisphere stratosphere is strongly peaked in latitudes 30°–60° in both hemispheres[1,9] though the total fall-out is much less in the southern hemisphere. There is evidence that the fall-out mechanism of stratospheric tritium is similar to that of stratospheric strontium-90 (ref. 2). One would then expect the concentration of natural or artificial tritium at the south polar region to be lower (rather than higher) than that at middle southern latitudes. Thus, according to this view, neither artificial nor natural injection of excess tritium into the stratosphere seems to explain the high tritium content of the single sample

reported here. It is conceivable that some special meteorological or hydrological condition was responsible.

One might also consider the possibility of substantially increased tritium porduction in the polar 'troposphere' by the high-energy (> 500 MeV) end of the solar-cosmic-ray spectrum (especially the November, 1960 events just preceding collection of samples). Since the polar tropospheric residence time of tritium is perhaps of the order of weeks only, intense polar fall-out could thus result. However, without knowledge of Antarctic hydrology, no quantitative statement can be made with regard to this possibility. It must be emphasized that the tritium-level given here is based on a single sample (though not on a single snowfall); further Arctic and Antarctic tritium data would be most desirable.

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MINERALOGY

Electron Spin Resonance Spectra in Various Clay Minerals

In the course of studying the mechanism of vinyl polymerization on clay mineral substrates[1] by means of electron spin resonance it became evident that strong electron spin resonance signals were associated with the original clays themselves. A survey of five different montmorillonites, two kaolinites and an illite has shown that this is a general phenomenon.

The electron spin resonance spectra for montmorillonites at room temperature have a single, characteristic, asymmetric peak near the region of free spin ($g = 2{\cdot}00$), as shown in the top trace in Fig. 1 for a sample from Clay Spur, Wyoming (A.P.I., No. 26). In some of the other montmorillonites which we have examined, this characteristic peak is superimposed on multiple peaks from transition metal elements, principally iron and manganese, that are present as impurities in the crystal lattice of the clay mineral. This comparatively narrow signal near the region of free spin we attribute to the presence of appreciable quantities of an organic free radical.

The spectrum for a sample of illite from Fithian, Illinois (A.P.I., No. 35), is shown in the middle trace in Fig. 1, and is seen to be a symmetrical peak near the free spin region.

The two kaolinites yield a different signal, an asymmetrical double peak as shown in the bottom trace in Fig. 1 for a sample from Macon, Georgia (A.P.I., No. 4). This double peak indicates restricted rotation of free radical species with an anisotropic g-value[2]. The sample of vermiculite from Libby, Montana, failed to show any signals other than those attributable to transition metal elements.

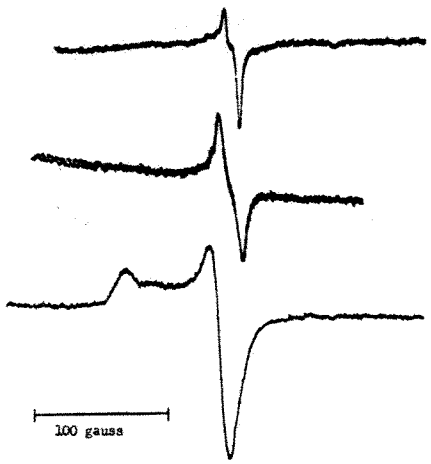


Fig. 1. Characteristic electron spin resonance spectra for clay minerals: top trace, montmorillonite (Clay Spur, Wyo.); middle trace, illite (Fithian, Ill.); bottom trace, kaolinite (Macon, Ga.)

These clay mineral samples which we have examined are all from mineral deposits usually assumed to be substantially free of organic matter. We estimate, by comparison with standardized solutions of DPPH, that the free radical content of these clays is of the order of 10^{16} spins/g. This concentration of free radicals, while large, can be attributed to mere traces of organic matter which are found[3] in most clay deposits. The lack of any organic free radicals in the vermiculite sample may be attributable to the geological nature of vermiculite deposits, which tend to be massive rock-like formations quite impervious to water. The other clay deposits could presumably acquire their organic contaminant by percolation of water-carrying organic matter from surrounding areas.

Identification of these organic free radicals is, as yet, speculative. One is tempted to relate these free radicals to humic acid, which is known to contain free radicals and is adsorbed by clay minerals. Our electron spin resonance spectra for two commercial samples of humic acid (K. and K. Laboratories, New York; Alpco Co., Ione, California) resemble closely those found for humic acid from Alaskan peat[4] and from the *B* horizon of two podzolic soils as well as another commercial sample[5]. All six of these humic acids give an electron spin resonance peak about 7 gauss wide. The clay samples, however, give varying asymmetrical peaks: about 13 gauss for the montmorillonites, and about 24 gauss for the kaolinites and illite. Therefore, we suggest that free radicals are present which cannot be explained in terms of humic acid alone.

Since clay is often added or present in both biological and chemical systems, we deem it important to point out that appreciable and variable concentrations of organic free radicals exist in clay minerals hitherto considered relatively pure. For example, when clays are used in studies of microbial activity at solid–liquid interfaces, the effects are both appreciable and variable, as evidenced by a recent review[6], which cites equal numbers of investigators who have found either stimulation or depression of growth. Similarly, the varying ability of minerals to stabilize soil organic matter[7] is still under investigation. Finally, it seems that the presence of these potentially active organic free radicals should be considered in studies of the reactions of antibiotics in soils[8] and in the present very active investigations of the persistence of herbicides and pesticides[9].

Clays are, of course, used as additives in non-enzymatic systems, and here again we would expect that an organic free radical may play an important part. Catalysis of polymerization[1], a scavenging action for halogens, or interference in any one-electron mode of oxidation or

reduction are among the reactions one might expect from such an impurity. Further work is under way in our laboratories aimed at furthering our knowledge of these potentially reactive materials.

We thank Dr. W. G. Hodgson, Stamford, Connecticut, and Dr. R. G. Wheeler, Yale University, for helpful discussions.

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ENGINEERING

Yield Front in the Propagation of Plastic Deformation in Normalized Mild Steel

It is generally accepted that slip occurs in polycrystalline materials along planes subject to the maximum or principal shear stress, inclined at 45° to the principal stress directions. In tensile tests on parallel specimens it has been reported[1] that plastic working advances behind a front inclined at an angle of 35° to the non-zero principal stress. This is not applicable to specimens having varying cross-sectional area where work-hardening appears to occur simultaneously with the progression of a plastic front which has been observed to be normal to the axis of the specimen. In biaxial stress systems plastic working along slip lines has been observed not to be contained within a front[2]. Bijlaard[3] has examined the simple tension case. Using a similar analysis it can be shown that for non-dilatational incremental plastic strains a 'front' exists at 35° to the cross-section behind which deformation can take place to produce zero normal strain along the front. Such deformation would be free from restraint arising from the material ahead of the front, and it is plausible to believe that the propagation of a front and also fracture by necking may be determined by this property.

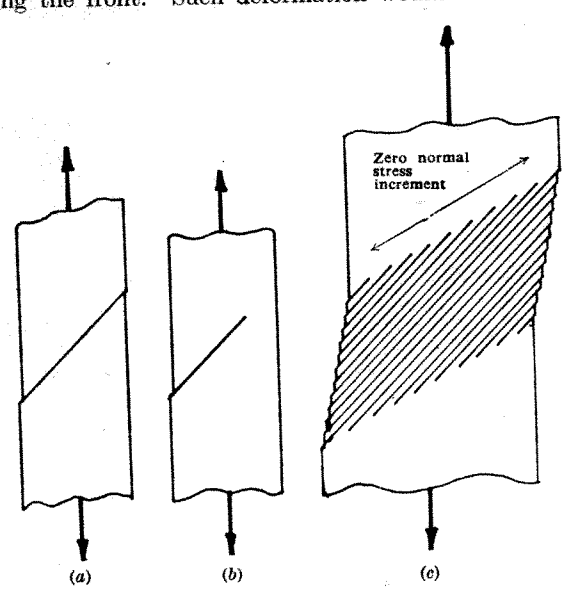


Fig. 1. *a*, Free slip line; *b*, locked slip line with local strain field at head of slip; *c*, stabilized yield front

The plastic strain, however, is produced by a very large number of dislocations, and in a polycrystalline structure these dislocations must occur mostly at 45° to the applied stress. It has been shown that initially slip occurs along the 45° direction, but that as yield continues a well-defined front at 55° to the axis develops. The two effects may be reconciled by a pictorial representation of an idealized slip line and of a system of slip lines. No explanation is offered of the mechanism whereby the front is formed, but it is demonstrated that a front, if formed, would be stable and could propagate.

If these concepts are reconcilable it is necessary to show why in pure shear no such front can exist. Taking principal stresses P, Q, R, with $R = 0$. Suppose $P = -Q$, then for incremental plastic strains the principal strains will be ε and $-\varepsilon$ and the normal strain inclined at an angle β will be:

$$\varepsilon_\beta = \varepsilon \cos^2\beta - \varepsilon \sin^2\beta$$

Thus,

$$\varepsilon_\beta = 0 \text{ when } \cos^2\beta = \sin^2\beta, \text{ or } \beta = 45°$$

There is, therefore, no front behind which principal slip lines can be preferentially arrested.

These effects have been confirmed using strain optically sensitive coating techniques, but further tests are required on other states of biaxial stress.

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Effect of Some Organic Additives on Conduction Current and Breakdown of Insulating Liquids

Previous work on the gassing properties of insulating liquids revealed beneficial effects of some organic additives on gassing rate of these liquids[1]. The results presented here show that these additives also affect the conduction current and breakdown voltage of insulating liquids.

Test liquid used was *B*30 transformer oil supplied by Gulf Oil (Great Britain), Ltd., and the additives chosen were: azobenzene, azoxybenzene, diphenyl, benzophenone, benzil, anthracene, naphthaquinone and anthraquinone. The concentrations of additives in solutions varied from $2{\cdot}3 \times 10^{-6}$ to $4{\cdot}59 \times 10^{-3}$ mole/100 g of the basic liquid. Test samples, filtered through a No. 5 sintered glass filter, were carefully degassed (10^{-3} mm mercury) and stress conditioned at about 600 kV/cm, for not less than 2 h, before each test. Spherical nickel electrodes 5 mm in diameter were used in all tests. Conduction current and breakdown voltages were measured for four gap settings, namely: 28, 51, 81 and 125μ, and it was possible to measure the conduction current up to breakdown fields. Reported values of breakdown voltages are mean values of 14 runs.

It was found that all additives used did affect the conduction current and breakdown voltage of transformer oil. For the ranges of concentrations used most additives produced a reduction of conduction current and in general an increase in breakdown voltage. Moreover, the results showed that there was an 'optimum' concentration of the additive which gave maximum reduction of conduction current and another, somewhat higher concentration, which resulted in a maximum increase of breakdown voltage. Exceeding these optimum concentrations produced a smaller reduction in conduction current and the gain in electric strength of the solution became less. For

still higher concentrations even an increase in conduction current and a reduction of breakdown voltage were possible.

Typical results are shown in Fig. 1 for 125μ gap setting. Table 1 gives the optimum concentrations for maximum breakdown voltages and for comparison, also, the concentrations which produced maximum reduction of conduction current.

The beneficial effect of all additives increased with the volume of the solution under stress (gap setting). Additives containing oxygen in their molecular structure gave also a considerable increase of breakdown voltage for small gap settings, an effect which was not observed for other additives, namely: azobenzene, diphenyl and anthracene. Breakdown voltage/gap setting characteristics, given in Fig. 2, show, for solutions containing azoxybenzene,

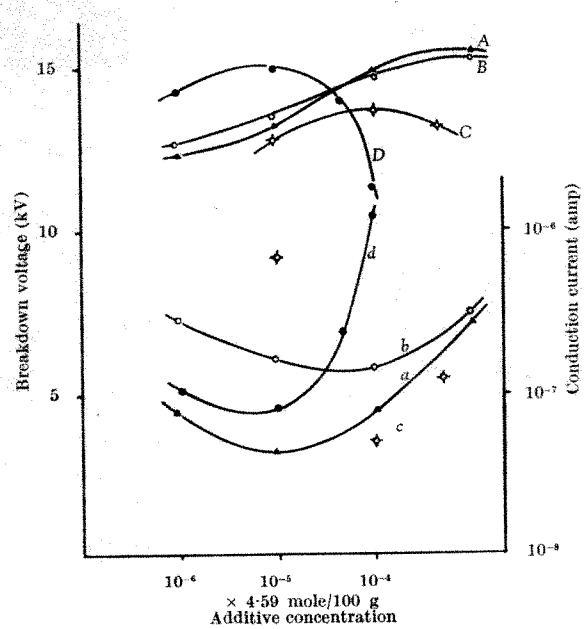


Fig. 1. Breakdown voltage and conduction current in solution as a function of additive concentration for 125μ gap setting. *A*, *B*, *C*, *D*, Breakdown voltage; *a*, *b*, *c*, *d*, conduction current in solutions containing azobenzene, azoxybenzene, benzophenone and benzil respectively. Pure liquid: breakdown voltage, 12·2 kV; conduction current, 0·922 × 10⁻⁶ amp.

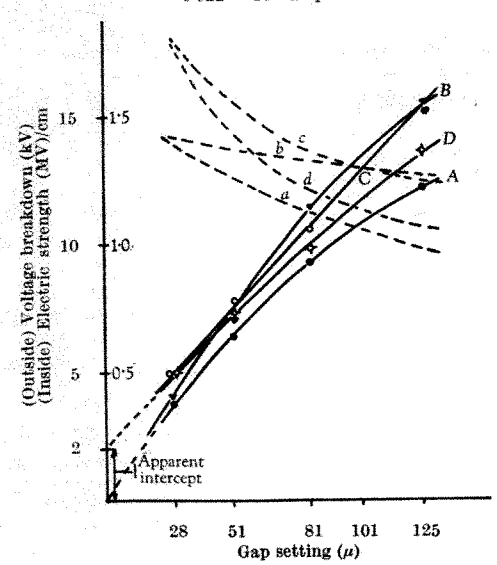


Fig. 2. Breakdown voltage and electric strength of solutions containing various additives as a function of gap setting. Breakdown voltage/gap setting characteristics: *A*, pure transformer oil; *B*, transformer oil + azobenzene, concentration 4·59 × 10⁻³ m/100 g; *C*, transformer oil + azoxybenzene concentration 4·59 × 10⁻³ m/100 g; *D*, transformer oil + benzophenone, concentration 4·59 × 10⁻⁴ m/100 g. *a*, *b*, *c*, *d*, Corresponding electric strength/gap setting characteristics

Table 1. Optimum Concentrations for Maximum Increase of Breakdown Voltage and Maximum Reduction of Conduction Current in Transformer Oil Solutions containing Various Additives

Additive	Optimum concentration (mole/100 g) for: Maximum reduction of conduction current		Maximum increase in breakdown voltage	
	28-μ gap	125-μ gap	28-μ gap	125-μ gap
Azobenzene	$4·59 \times 10^{-4}$	$4·59 \times 10^{-5}$		$4·59 \times 10^{-3}$
Azoxybenzene	$4·59 \times 10^{-6}$	$4·59 \times 10^{-4}$	$4·59 \times 10^{-4}$	$4·59 \times 10^{-3}$
Diphenyl	$2·30 \times 10^{-2}$	$4·59 \times 10^{-3}$		$2·30 \times 10^{-2}$
Benzophenone	$4·59 \times 10^{-5}$	$4·59 \times 10^{-4}$	$4·59 \times 10^{-4}$	$4·59 \times 10^{-4}$
Benzil	$4·59 \times 10^{-6}$	$4·59 \times 10^{-5}$	$4·59 \times 10^{-5}$	$4·59 \times 10^{-5}$
Anthracene	$2·30 \times 10^{-5}$	$2·30 \times 10^{-6}$		$2·30 \times 10^{-4}$
1,4-Naphthaquinone	$4·59 \times 10^{-6}$	$2·30 \times 10^{-5}$	$4·59 \times 10^{-5}$	$4·59 \times 10^{-5}$
1,4-Anthraquinone	$4·59 \times 10^{-6}$	$4·59 \times 10^{-6}$	$2·30 \times 10^{-5}$	$2·30 \times 10^{-5}$

benzophenone, benzil and quinones an apparent intercept for zero gap setting. This intercept was practically constant, at a value of about 2 kV, and independent of the nature of the additive and its concentration.

I thank Prof. M. W. Humphrey Davies for providing the facilities to carry out this work and Dr. H. Tropper for his advice.

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CHEMISTRY

Physical Transitions of the Alkali Metal Nitrates as Revealed by Differential Thermal Analysis

Differential thermal analysis experiments have been carried out on the alkali metal nitrates, lithium nitrate, sodium nitrate, potassium nitrate, rubidium nitrate and cæsium nitrate. These experiments were conducted in air at the nominal heating rate of 10° C/min. Differential thermal analysis curves of the alkali metal nitrates were previously reported[1]; however, the use of smaller sample masses, bare thermocouples and the different thermocouple location and arrangement used in the present work considerably improved the resolution of the differential thermal analysis curves. This was also found to be true in the case of potassium perchlorate[2]. The results of the nitrate experiments are shown in Figs. 1, 2, 3, 4 and 5. Generally, the essential features of the curves for lithium, sodium and cæsium nitrates are in agreement with previous work[1]. In the case of potassium nitrate a slight exothermal rise was reported to occur immediately prior to fusion[1]. This is not observed in our work as may be seen in Fig. 3.

The marked increase in the resolution of the differential thermal analysis peaks was revealed to be especially important in the case of rubidium nitrate. This resulted in the observation of a previously unreported endotherm at 302° C. The differential thermal analysis curve for rubidium nitrate shows good resolution of all the endotherms. Five endotherms are clearly revealed. The first three due to crystalline transitions and the fifth due to fusion have been reported[1], although they did not appear as discrete individual bands as recorded here. The fourth endotherm, which involves a very small heat change, estimated from Fig. 4 to be 50 cal/mole, may easily be obscured in a poorly resolved curve, and consequently was not previously observed. The maxima of the five differential thermal analysis bands, which represent the transition temperatures and melting point, for rubidium nitrate occur at 167°, 236°, 290°, 302° and 314° C. The corresponding peaks occur at 172°, 241°, 293°, 303° and 315° C. Thermogravimetric analysis shows that decomposition does not occur during these endotherms, consequently the fourth endotherm at 302° is almost certainly due to a crystalline transition, previously unknown for

rubidium nitrate. Replicate experiments confirm these results. Plyushchev, Markina and Shklovev identified the first three crystalline transitions from phase transformation diagrams at 164°, 219° and 291° C (ref. 3) and reported the melting point at 314° C (ref. 4). These values are in fair agreement with the results of the present work with the exception that the temperature of the second transition is 17° lower than our value of 236° and also the crystalline transition at 302° was not reported.

The differential thermal analysis apparatus used has already been described[2]. Briefly, it consists of a metal block for the reference and sample containers, and 'Pyrex' test-tubes which are heated simultaneously at a pre-

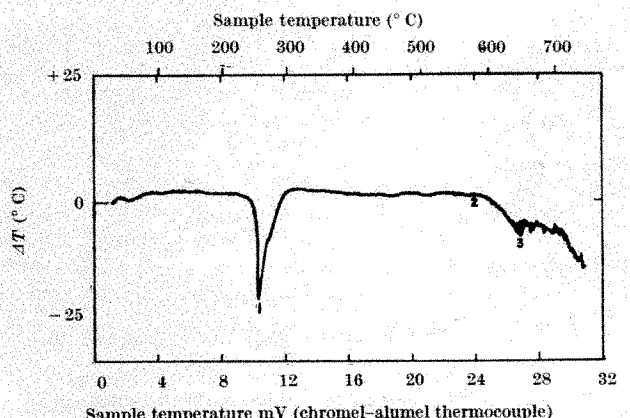


Fig. 1. Differential thermal analysis, $LiNO_3$, 500 mg, in air. Heating rate, 10° C/min

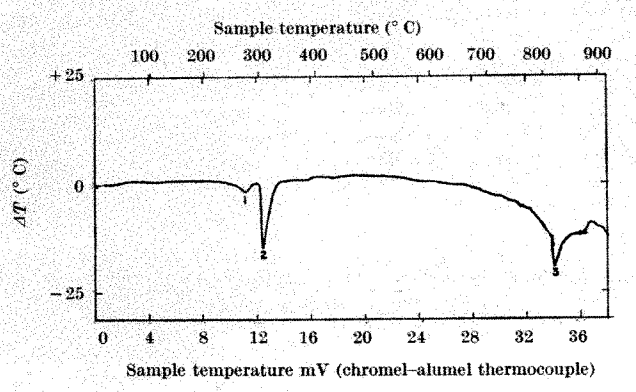


Fig. 2. Differential thermal analysis, $NaNO_3$, 500 mg, in air. Heating rate, 10° C/min

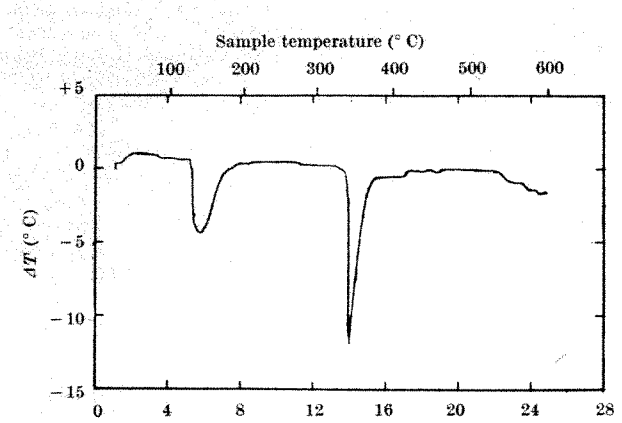


Fig. 3. Differential thermal analysis, KNO_3, 500 mg, in air. Heating rate, 10° C/min

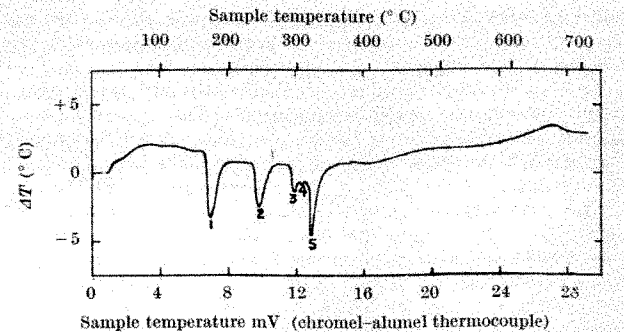


Fig. 4. Differential thermal analysis, $RbNO_3$, 500 mg, in air. Heating rate, 10° C/min

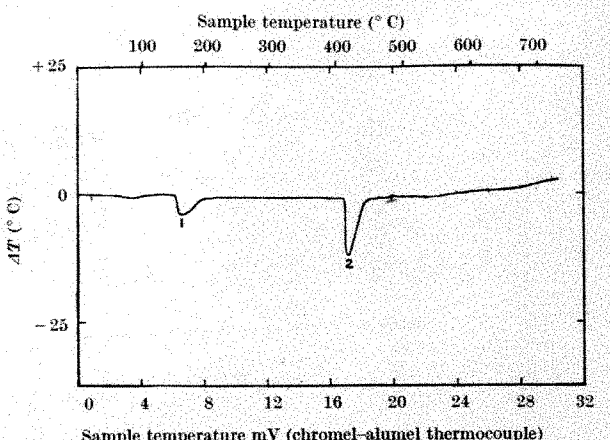


Fig. 5. Differential thermal analysis, $CsNO_3$, 500 mg, in air. Heating rate, 10° C/min

determined heating rate, in this case 10° C/min. The rate of heating was regulated by a West Gardsman controller. Temperature differences between the sample and thermally inert reference compound, calcined alumina, were plotted as a function of sample temperature on a Mosely *X*—*Y* autograph recorder. 500 mg samples were run at atmospheric pressure in air. The 28-gauge chromel–alumel thermocouple wires were spread apart and shaped to fit along the bottom of the sample container. All the samples with the exception of rubidium nitrate were twice recrystallized from triply distilled water. The rubidium nitrate was four times recrystallized. All the starting materials were of C.P. grade.

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Effect of Alkyl and Halo Substituents on the Homolytic Dissociation of Dibenzyl Hyponitrites

Polar effects showing consistent trends with the nature of substituents and satisfactory correlations with the Hammett equations have been observed in the homolytic dissociations of benzoyl[1] and thenoyl peroxides[2], *t*-butyl perbenzoates[3], phenylperacetates[4] and peroxycarbamates[5], and *m*-substituted phenylazotriphenylmethanes[6]. In all these reactions electron-repelling substituents have been found to increase reaction rate whereas electron-with-

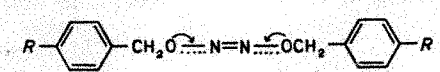
drawing substituents lower rates, the magnitude of the effects being much smaller than in ionic reactions.

A consistent trend with substitution, but in the reverse sense to that already noted, is now reported for the first-order homolytic dissociation of some symmetrically substituted dibenzyl hyponitrites. The final products of decomposition, the respective benzaldehydes, benzyl alcohols and nitrogen, are similar to those reported for the parent dibenzyl hyponitrite[7], where the activation energy for the first-order decomposition, about 25 kcal, obtained manometrically at maximum inhibition has been confirmed by radical counting methods using iodine and diphenylpicrylhydrazyl[8]. Table 1 lists rates for the first-order decomposition of 0·05 M solutions of the substituted compounds in nitrobenzene obtained manometrically in the presence of *p*-benzoquinone (0·8 M) in excess of that required for maximum inhibition.

Table 1. First Order Constants for the Decomposition, at 35·4°, of Substituted Dibenzyl Hyponitrites (0·05 M) in Nitrobenzene in Presence of 0·8 M *p*-Benzoquinone

Substituent	*m*-CH_3	*p*-CH_3	H	*p*-Cl	*p*-Br	*m*-Cl
$10^5\,k$ (sec^{-1})	6·22	6·31	6·50	6·80	6·93	7·11

An extreme variation of 15 per cent in the value of the rate constant is observed in changing from the *m*-CH_3 to the *m*-Cl substituent, the electron-attracting halo substituents increasing the rate whereas the weakly electron-releasing methyl groups decrease the rate, relative to the unsubstituted compound. The effects of substitution may be understood from the nature of electronic influences in dibenzyl hyponitrite, where the known electron-withdrawing tendency of the azo-group[9,10] should lead to an increase in the N—O bond order as depicted in the formula:



This tendency is strongly marked in the hyponitrite anion where infra-red studies of the N—O stretching frequency by Le Fevre *et al.*[11] and by Kuhn and Lippincott[12] indicate an N—O bond order greater than one. Relative to the unsubstituted compound, the effect of electron-repelling and electron-attracting substituents would be, respectively, to increase and decrease the N—O bond order. The relative order of the rates is therefore consistent with a mechanism involving the simultaneous rupture of the two N—O bonds[7] with the formation of the stable nitrogen molecule. A similar mechanism has been invoked in other radical reactions involving the elimination of nitrogen[13]. A Hammett plot (Fig. 1) is satisfactory with the exception of the *p*-methyl substituent. The small value of the reaction constant as compared with other radical dissociations is expected from the damping of pure inductive effects and the absence of any direct resonance interaction with the locus of reaction.

A wider range of substituents is being examined.

This work was initiated at the University of Hong Kong. I thank Prof. J. E. Driver for his interest.

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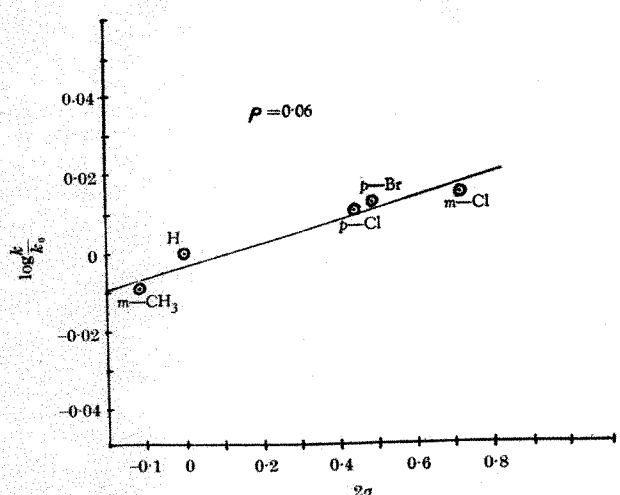


Fig. 1

The Partition Law in Solvent Extraction

The thermodynamic law for the partition of a solute between two immiscible or partially miscible solvents is:

$$a = Ka'$$

where a and a' are the activities in the two phases, and K is a constant. This law is frequently used to determine a' when a is known[1]; but we are unaware of any instance in which independent determinations of a and a' have been compared with measured partition data.

We have now carried out such an investigation of the system HCl–H_2O–TBP (tri-*n*-butyl phosphate) at 25° C. Activities of hydrochloric acid in water are of course well known, partition data have been obtained by various authors[2], and we have determined activities of hydro-

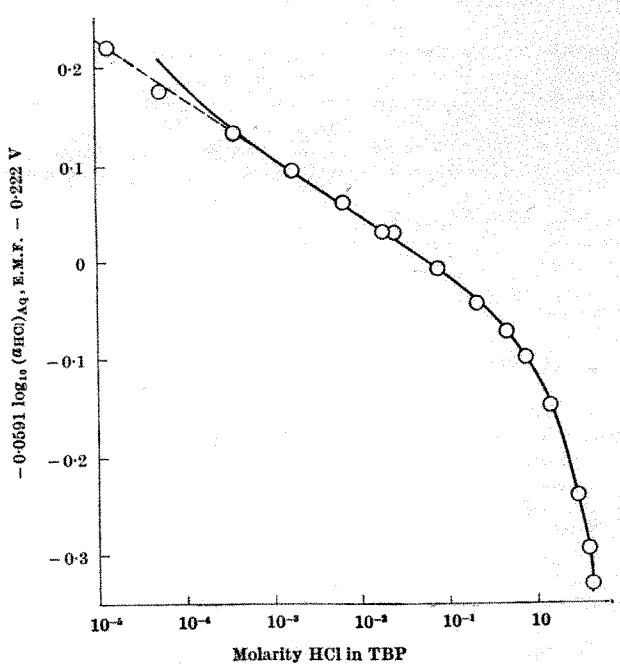


Fig. 1. Comparison of electrochemical and distribution data for activity of hydrochloric acid in tri-*n*-butyl phosphate

chloric acid in the TBP phase by E.M.F. measurements on the cell:

$$H_2,Pt|HCl(m) \text{ in TBP}|AgCl,Ag$$

We have shown that the hydrogen and silver/silver chloride electrodes are reversible in TBP.

For the corresponding aqueous solution cell, the E.M.F. is given by:

$$E = E_0 - 0{\cdot}05914 \log a_{HCl}$$

For the TBP solution cell just mentioned it is given by:

$$E' = E_0' - 0{\cdot}05914 \log a'_{HCl}$$

If the solutions in the two cells are in partition equilibrium, then from the first law of thermodynamics, $E = E'$. Hence:

$$E' - E_0 = -0{\cdot}05914 \log a_{HCl}$$

The accepted value of E_0 is 0·222 V (ref. 3).

Fig. 1 shows $E' - E_0$ (circles) and $-0{\cdot}05914 \log_{10} a_{HCl}$ (solid line based on Hesford and McKay's results[2], which cover the required concentration range), plotted against the concentration of hydrochloric acid in the TBP phase. The agreement is good except at very low concentrations where the measurements become difficult.

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Pertechnic Acid : an Aperiodic Variation in Acid Strength

For a given oxidation level the oxy-acids in a given sub-group of the periodic table are normally found to exhibit a regular variation in acid strengths. In group VII, for example, Cobble[1] lists the logical pK values as $-2{\cdot}25$, $(-1{\cdot}5)$ and $-1{\cdot}25$ for $HMnO_4$, $HTcO_4$ (estimated, with due regard for the lanthanide contraction) and $HReO_4$. The general behaviour of pertechnic acid in aqueous neutralimetry certainly is consistent with that of a monoprotic and 'relatively' strong acid.

On the other hand, Kertes and Beck[2] conclude a paper on the tributyl phosphate extraction of Tc(VII) with the dilemma: "Consequently, the origin of the different extractabilities of $HTcO_4$ and $HReO_4$ found, lies in their different acid strengths and it follows that $HReO_4$ is a stronger acid than $HTcO_4$. The respective position of the elements in the Periodic Table definitely suggests the opposite. This discrepancy shows that, although the acid strength is an important factor governing the extractability of acid by oxygen containing extractants, it is probably not the only one. Consequently, some other factors not yet known seem to be more determining".

The sometime red colour of $HTcO_4$, the unexplored possibility of mesopertechnetates, the (H^+)-dependence of certain liquid-liquid extractions of Tc(VII) (refs. 3 and 4), the nature of Re/Tc separations on anion-exchange resins, the polarographic and the spectrophotometric behaviour of pertechnetate ion versus pH (refs. 3 and 4)—all have been examined or are now being examined in this laboratory. Each of these phenomena has an obviously requisite, or a more subtly probable, dependence on the ionization constant of pertechnic acid.

The acid ionization constant itself is now being measured precisely and as a function of temperature. From our earlier results[4], K_{ion} of $HTcO_4$* at 25·4° C is about $5 \pm 2 \times 10^{-1}$. This value of 0·5 is nearly one-hundred-fold less than the anticipated value of 32, and is well below the K_{ion} of 18 for $HReO_4$. We also find that this low value is requisite for a logical explanation of the ether extraction of pertechnetate from aqueous media of various pH (ref. 4).

Of course, this surprisingly aperiodic behaviour may possibly be explained in terms of dimerization, hydration, or similar, factors. Present research is directed toward seeking this explanation, and the expression $HTcO_4$* may represent an over-simplification respecting the measured acidity. Our acid was prepared by the dissolution in distilled water of Tc_2O_7, made by the combustion in oxygen of Tc[99] obtained in 99·9 per cent purity from the Oak Ridge National Laboratory.

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Trace Analysis by Combined Chromatographic Techniques

The application of gas chromatography to the determination of trace impurities may be considerably extended by using a combination of chromatographic techniques. We may illustrate this by the following example in which concentrations of alkenes in alkanes as low as 1 in 10^8 have been readily and precisely determined.

n-Heptane was first freed from alkenes by passing the liquid through a column of alumina modified with silver nitrate (about 10 g of packing per 100 g of heptane). In the first series of experiments, quantities of 1-hexene and 1-heptene ranging from 1 to 10 mg each were added to 100 g of the purified heptane. The resulting samples were then forced under slight nitrogen pressure and at room temperature through a similar modified alumina column (3 mm internal diameter and 1 g packing). When the column was free of liquid its temperature was raised to 75° C and nitrogen passed through to elute the last traces of heptane. The hexene and heptene were then displaced from the column by a stream of nitrogen saturated with 1-octene. The resulting chromatogram (gas-density detector) is shown in Fig. 1, the dotted lines representing the hexene and heptene steps. The lengths of these steps were found to be proportional to the quantities of alkenes introduced into the heptane, so that this method may be used directly for concentrations down to 1 in 10^5.

In the second series of experiments 1 μg quantities of 1-hexene, 1-heptene and 2-heptene were added to 100 g of purified heptane. Such samples were treated as in the first

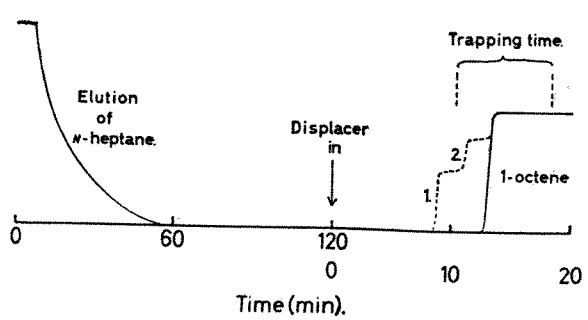


Fig. 1. Elution of last traces of n-heptane and displacement of alkenes (step 1 = hexene, step 2 = heptene) from a column of alumina modified with silver nitrate

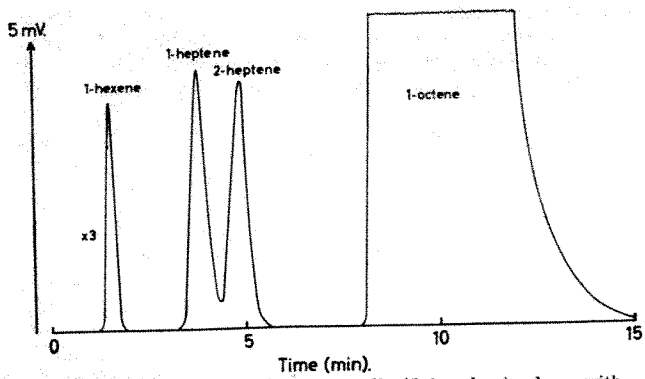


Fig. 2. Analysis of octene-front on gas-liquid (squalane) column with flame-ionization detector. 1 μg each of 1-hexene, 1-heptene (b.p. 94·9°), and 2-heptene (b.p. 98·1°) from 100 g *n*-heptane (b.p. 98·5°)

series to produce the chromatogram shown in Fig. 1 but without distinct hexene and heptene steps. The front of the octene step was trapped out over the period shown and analysed with a gas-liquid elution chromatograph to give the chromatogram shown in Fig. 2. The same peak areas were obtained when either 1 or 2 g of packing was used in the displacement column, indicating that there was no significant irreversible adsorption.

In a preliminary series of experiments, 100 g samples of unadulterated pure heptane were treated as in the second series of experiments. The final gas-liquid elution chromatograms contained no discernible peaks before the emergence of the octene.

For the second series of experiments and for the 'blanks', the octene displacer was itself prepared by a pseudo-displacement technique in which 25 g of the modified alumina was first saturated with commercial octene at room temperature. This was then purged with nitrogen, and the column temperature finally raised to 120° C to release about 1·5 g of purified octene, which was trapped out at low temperature.

There would appear to be no inherent difficulty in extending the method to much lower concentrations, and to other types of trace impurities by appropriate choice of selective column material. The displacement method lends itself to preparative work, thus quantities of an alkene of the order of 2–5 g (depending on molecular weight) could be extracted pure from alkanes with a 100 g silver nitrate modified column. The purification procedures outlined here for heptane and octene should also have application in the preparation of solvents suitable for the extraction of trace materials. We propose to illustrate these further developments in future publications.

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Polymerization of a Thermosetting Polymer in an Electric Field

There has recently been a considerable amount of discussion and speculation[1] as to the possible effects on a polymer from polymerization in a relatively strong electric field. The possibility of using this technique to yield polymers which would have unusual physical and/or electrical properties has been considered. Presented in this communication are the results obtained during an investigation of the polymerization of a thermosetting polymer in constant electric fields of various strengths.

The monomer consisted of 30 per cent styrene and 70 per cent of a linear condensation polymer of maleic and phthalic acids with propylene glycol. The source of free radicals was 1 per cent methyl ethyl ketone peroxide. Polymerization was conducted at 80° ± 2° C at field strengths varying from 125 to 25,000 V/cm in small concentric cylindrical electrodes[2]. High voltage was supplied by a DuMont type 263-*B* power supply which was connected directly to the electrodes.

In the initial experiments the conductivity of the polymer was followed continuously during polymerization by a continuous current monitoring device[3] in a manner very similar to that previously described[2,4]. However, at higher field strengths (> 2,500 V/cm) the electric field produced unusual changes and accelerations in the rates of polymerization. Since these changes were not reproducible, attempts to monitor the conductivity during these polymerizations were not continued. Similar effects have been noted recently by Khaletskii and Sukhorukov[5].

We have found[4,6,7] that the dependence on temperature of the electrical volume resistivity is a sensitive index of the extent of polymerization and is indicative of structural changes in the polymer[8]. For comparative purposes the activation energy for the electrical conduction process E_c, which is calculated from the temperature dependence of the resistivity, is a more convenient parameter. Therefore, immediately after polymerization in the electrical field the temperature dependence of the resistivity was determined. This determination was made at a low (~ 200 V/cm) field strength.

The results of this work are shown in Fig. 1 where the E_c values obtained after isothermally polymerizing a series of samples at 80° ± 2° C in electric fields of various strengths for four hours are plotted against the electric field strength. It will be noted that E_c decreases as the field strength is increased. For example, $E_c = 46$ kcal/mole for a polymer polymerized at ~ 200 V/cm while E_c decreases to 32 kcal/mole for a sample polymerized at 25,000 V/cm (5,000 applied volts). Since smaller E_c values indicate less temperature dependence of the resistivity we see that with increasing field strength during polymerization the temperature dependence of the resistivity decreases. Thus, the effect of polymerizing in an electric field is to produce a polymer which is somewhat more conductive in a direction parallel to that of the applied

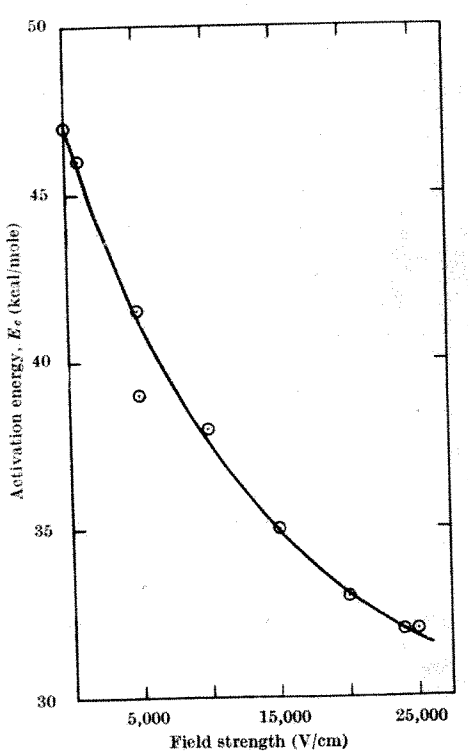


Fig. 1. Dependence of activation energy for electrical conduction in the solid polymer on the field strength during polymerization

field. This effect is more pronounced at higher field strengths.

This effect is relatively small and probably results from minor structural changes. This is evidenced by the fact that the magnitude of the resistivity exhibited only a slight decrease and that the glass transition temperature, T_g, of a polymer polymerized at a field strength of 16,000 V/cm is ~ 123° C as compared with a T_g of 126° C for the same material polymerized in the absence of an applied field.

We conclude that the application of relatively high electrical fields during polymerization produces some degree of alignment or orientation of the polymer structure and that one of the effects of this form of ordering is to reduce the temperature dependence of the resistivity.

We thank Mr. T. J. Wade for assistance in the experimental phase of this investigation.

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Isomerization of 1,2-dichloroethylene induced by Chlorodifluoromethyl Radicals : Formation of Metastable Radicals

It has been reported that radicals produced by the decomposition of benzoyl peroxide in the liquid phase cause the rapid isomerization of *cis* and *trans* 1,2-dichloroethylene in the liquid phase in the temperature-range 80°–135° C (refs. 1 and 2). It is supposed that the isomerization is brought about by chlorine atoms which react with the olefines to form 1,2,2-trichloroethyl radicals which rapidly decompose to give the original 1,2-dichloroethylene or its isomer. This reaction has recently been shown to intervene in the chlorination of 1,2-dichloroethylene, even at lower temperatures[3]. The chlorine atoms in the first-mentioned isomerization process are supposed to be formed by displacement from the olefine by a free radical derived from benzoyl peroxide. No direct evidence has previously been obtained for such a process.

In the present investigation the products of the reaction of chlorodifluoromethyl radicals, produced by the photolysis of 1,3-dichlorotetrafluoroacetone[4] with the isomers of 1,2-dichloroethylene, were examined using gas chromatography. The major reaction brought about was the isomerization of the olefine. In addition to unreacted ketone, and the isomers of the olefine, the reaction products were found to contain 1,2-dichlorotetrafluoroethane and an additional major component. No dichlorodifluoromethane was detected, although this compound is known to be formed in small amounts by the abstraction of a chlorine atom from 1,3-dichlorotetrafluoroacetone by chlorodifluoromethyl radicals[5]. The additional component was isolated from a number of gas chromatography runs in sufficient amount to allow its identification by mass spectrometry as 1,3-dichloro-3,3-difluoropropene.

The absence of dichlorodifluoromethane and of 1,2,3-tetrachloro-1,1-difluoropropane shows that the named propene could not have been formed in disproportionation reactions. Although gas chromatography revealed a minor product having a retention time close to that expected for the appropriate substituted propane, this component was present in too small an amount either to permit its identification or to account for the formation of much of the substituted propene. We must suppose, therefore, that the observed propene is formed by the decomposition of 1,2,3-trichloro-3,3-difluoropropyl radicals. No data are available to enable the estimation of the energy required for the dissociation of the propyl radical to give the olefine, but energies for such processes are commonly about 20 kcal/mole (for thermally equilibrated radicals). The rapid decomposition of the radicals in this case at temperatures in the range 25°–60° C can be understood only if the decomposing species are excited. Dainton[3] has recently shown that the 1,2,2-trichloroethyl radicals formed by the addition of a chlorine atom to 1,2-dichloroethylene are formed in an excited state which may easily decompose with a small or zero energy of activation by loss of a chlorine atom. Dainton supposes that the decomposition involves nascent radicals containing the energy with which they are formed. The decomposition of the substituted propyl radical examined in the work recorded here is not the reverse of that leading to its formation, and the decomposition must follow at least a redistribution of energy within the radical. It may be significant that the carbon–chlorine bond in the propyl radical is likely to be weaker than the carbon–carbon bond that would be broken in the reaction that is the reverse of that by which the radical is formed.

This work has provided evidence of the formation of a radical in a relatively long-lived metastable state, and has confirmed a mechanism postulated for the isomerization of the isomeric 1,2-dichloroethylenes by free radicals. Whether all the observed isomerization proceeds through the chain reaction involving chlorine atoms, as has been proposed by previous workers, or whether some results from the breakdown of the substituted propyl radicals to regenerate chlorodifluoromethyl radicals, will be difficult to decide. Further investigation of these phenomena is intended.

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BIOCHEMISTRY

A Possible Mechanism of Synthesis of Nucleic Acids

In the organism, *B. megaterium*, it is possible to separate from the cytoplasm a nuclear fraction which contains all the DNA of the cell, some protein and about 3 per cent of the cellular RNA[1]. It has been found that the DNA polymerase is present both in the nuclear fraction and in the cytoplasm, but the whole of the RNA polymerase is present in the nuclear fraction[2].

These enzymes are not easily removed from the nuclear fraction by mild procedures; in particular it has been found that in order to liberate the RNA polymerase it is necessary to disrupt the DNA with DNase[2] and it is necessary to conclude that this enzyme is firmly bound to DNA in the complex. In the case of mammalian nuclei, it has also been found[3] that certain enzymes are active when present in the nucleus in complexes; for example, in regenerating liver, thymidylate kinase cannot be detected in *in vitro* assays until an hour or two after the onset of DNA synthesis although it must have been

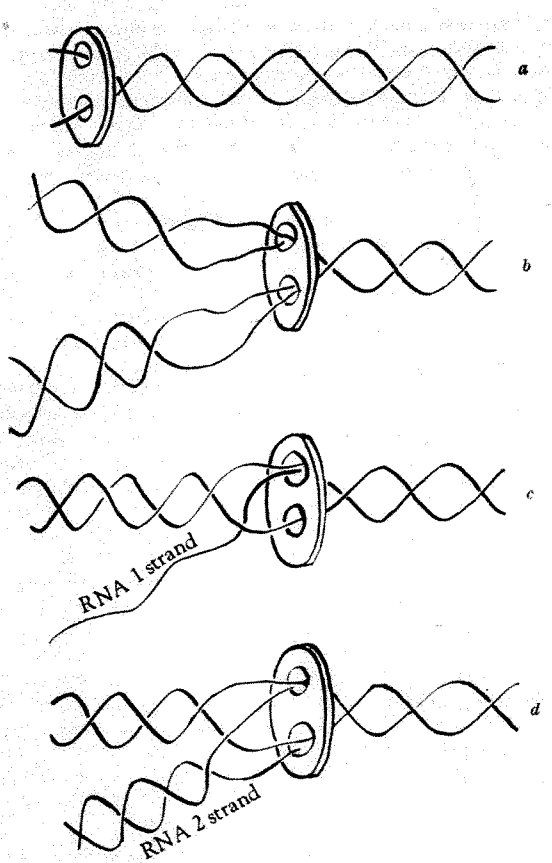


Fig. 1. Schemes of synthesis of DNA and RNA

present previously to produce the necessary DNA precursors. On the basis of this evidence it has been suggested by Bianchi *et al.*[3] that such enzymes only appear in the cytoplasm when the stage of the cell-cycle at which they are active in the nucleus is finished.

The facts suggest a highly organized arrangement of the enzymes concerned with the nuclear synthesis of DNA and RNA. The following mechanism is suggested as a possible mode of association of the DNA and RNA polymerase which require DNA as a primer. The enzyme is envisaged as a kind of disk (Fig. 1) with two holes or slots each of which can accommodate one of the strands of the primer DNA. The disk will first attach itself to one end of the DNA fibre with the end of one strand in each hole, as in (*a*). The complementary nucleotide for the given position in the sequence will now take its place, and as these are attached one by one to the growing chain the disk will move forward along the DNA fibre, rotating as it goes and thereby unwinding the double-stranded helix. This avoids the difficulties which have been suggested[4] of finding a mechanism for unwinding the helix. The energy required to separate the strands will come from the synthetic reaction involving the precursor triphosphates. The new double strands emerging can be expected to wind up in a helix, the energy required being provided by the short-ranged attractive forces between the bases. The new fibres would also be loosely wound around each other; a feature which is often seen in photographs of chromosomes. In this scheme the enzyme molecule is located at branching points, where the division of the fibres occurs. It may be observed that electron micrographs of DNA have often been obtained which show such branching and that a thickened blob is sometimes seen at the branching point which may well be due to an enzyme molecule.

Evidence has been given by Kornberg that the two strands of the DNA fibre are of opposite polarity[5]. This means that looking down the fibre in one direction, one strand has the phosphates attached to deoxyribose in the 3 and 5 positions in the order *p*, 5, 3, *p*, 5, 3, etc., while in the other strand the reverse is the case, that is, *p*, 3, 5, *p*, 3, 5, etc. On the proposed mechanism the enzyme must be capable of bringing about the polymerization of strands having both polarities simultaneously. This will, of course, be necessary in any mechanism in which the replication of a DNA fibre occurs, starting at one end. That replication in the DNA occurs sequentially has been shown by Cairns[6] with *E. coli* DNA, who observed 'forks' similar to those predicated here.

A similar mechanism with the appropriate enzyme may be presumed to operate in the case of synthesis of RNA on a DNA template. In this case two possibilities exist: (1) a complementary thread of RNA is attached at one of the holes only, as in Fig. 1*c*, giving a single-stranded RNA, while the DNA fibre coils up again behind the disk; (2) a double-stranded RNA could be formed as in Fig. 1*d*. Insufficient is known at present about nuclear RNA to distinguish between these possibilities.

There has been some uncertainty whether the DNA polymerase in fact makes use of (*a*) single-stranded (or denatured DNA) or (*b*) double-stranded. Bollum[7] prepared a calf thymus polymerase which used (*a*) but not (*b*). On the other hand, the enzyme of Lehman *et al.*[8] from *B. coli* undoubtedly uses both. In the mechanism proposed, anomalies of this kind might easily occur. Some special configuration, for example protein, may be required at the end of the fibre to enable the enzymes to become attached and in its absence it may be easier for the enzyme to cope with single-stranded than double-stranded DNA.

Note added in proof. At the recent Cold Spring Harbor symposium on "Nucleic Acid Replication", Cairns showed pictures of the circular chromosome of *E. coli* in the course of replication in which the new and old strands of DNA are certainly not coiled around each other, and to account for this suggested that the original strand was capable of rotation. If this applied to the model shown here, that is, if the original strand of DNA to the right of the disk is allowed to rotate, the necessity of coiling of the new and the old fibres around each other will be avoided.

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Carbamylation of Oxytocin and Arginine-Vasopressin

Synthetic analogues of oxytocin and vasopressin in which the terminal amino group is absent or modified exhibit remarkable differences in biological activity. Whereas desamino-oxytocin is highly active[1,2], analogues of oxytocin in which the amino group is substituted by acylation or alkylation retain little pharmacological activity[3–5], and some act as antagonists[4,6]. Similarly, 1-desamino-8-lysine-vasopressin has a relatively high

pressor potency[1] while 1-*N*-acetyl-8-lysine-vasopressin is inactive[7].

A recent report[8] that cyanate reacts with the terminal amino groups of proteins and peptides under mild conditions to form carbamylated derivatives has suggested to us the possibility of preparing 1-*N*-carbamyl analogues of oxytocin and vasopressin. Such analogues would be of particular interest since the carbamyl group differs chemically from both acyl and alkyl substituents. In order to determine optimal experimental conditions for the preparation of *N*-carbamyl analogues, we have treated oxytocin and vasopressin with cyanate and studied the effect on pharmacological activity.

The procedure used was to allow synthetic oxytocin or arginine-vasopressin to react with potassium cyanate at 30° C; the solutions were maintained at *p*H 5·0 or 6·0 by automatic addition of 2·5 M acetic acid. When the reaction was allowed to proceed for 120 min at *p*H 5, additional cyanate was added after 60 min to restore the initial concentration, the reagent having a half-life of 32 min under these conditions. At intervals, aliquots of the reaction mixture were removed and the reaction was stopped by acidification with concentrated hydrochloric acid to *p*H 2, which destroys cyanate. Residual activity in neutralized samples was determined by bioassay. Oxytocic activity was assayed on the isolated rat uterus, milk-ejecting activity on the lactating guinea pig, pressor activity on the blood pressure of the rat, and antidiuretic activity in the water-loaded rat under ethyl alcohol anæsthesia[9]. In control experiments, a solution containing 0·04 M cyanate was acidified to *p*H 2; oxytocin or vasopressin was added after 30 sec and the reaction mixture incubated for 2 h. Full recovery of activity was obtained with both hormones.

On treatment with 0·2 M potassium cyanate at *p*H 6, oxytocin (1 U./ml.) was rapidly inactivated. The residual oxytocic activity was 6 per cent at 10 min, and 1·5 per cent at 30 min. In order to obtain more information about the rate of inactivation and to provide greater protection for the disulphide bond, the concentration of cyanate was reduced to 0·04 M and the *p*H adjusted to 5. Under these conditions a slower rate was observed; but after 120 min, as shown in Table 1, both oxytocin and vasopressin were almost completely inactivated. No significant dissociation of biological activities could be detected since the rates of inactivation measured on different assay systems were similar. During the assays there was no inhibition of the responses to threshold doses of standard oxytocin or vasopressin.

To establish the specificity of the cyanate reaction, desamino-oxytocin (0·2 μg/ml.) was treated with 0·04 M cyanate for 120 min under the same conditions. No inactivation was observed; the recovery of oxytocic activity was 102 per cent and that of milk-ejecting activity 96 per cent. This strongly suggests that the inactivation of oxytocin and, by inference, vasopressin, is the result of a specific reaction of cyanate with the terminal amino group.

The finding that oxytocin and vasopressin are rapidly inactivated by 0·04 M cyanate directs attention to the irreversible inactivation of oxytocin by urea[10]. Since a concentration of 0·02 M cyanate is present at equilibrium in 8 M urea[11], this inactivation could be attributed to a chemical reaction with cyanate rather than to a physical effect involving a change in molecular conformation. Our results emphasize the necessity for caution in the use of urea in biochemical investigations[12].

Table 1. Residual Activity (%) in Samples of Oxytocin (2 U./ml.) and Arginine-vasopressin (1 U./ml.) treated with 0·04 M Potassium Cyanate at *p*H 5 and 30° C

Reaction time (min)	Oxytocin		Arginine-vasopressin	
	Oxytocic activity	Milk-ejecting activity	Pressor activity	Antidiuretic activity
5	51	69	—	—
6	—	—	51	—
10	31	50	44	—
30	10	15	11	—
60	3	3	2	2
120*	0·1	0·07	< 0·2	0·04

*The values for oxytocin at 120 min were obtained with an initial concentration of 50 U./ml.

Experiments are now in progress to isolate the *N*-carbamyl derivatives of oxytocin and vasopressin for detailed chemical and pharmacological examination. The reaction of cyanate with angiotensin and bradykinin is also under investigation.

The preparation of *N*-carbamyl analogues of biologically active peptides may prove useful both in elucidating the functional importance of their terminal amino groups and in producing potential antagonists.

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A New Amino-acid, γ-Hydroxyhomoarginine, in *Lathyrus*

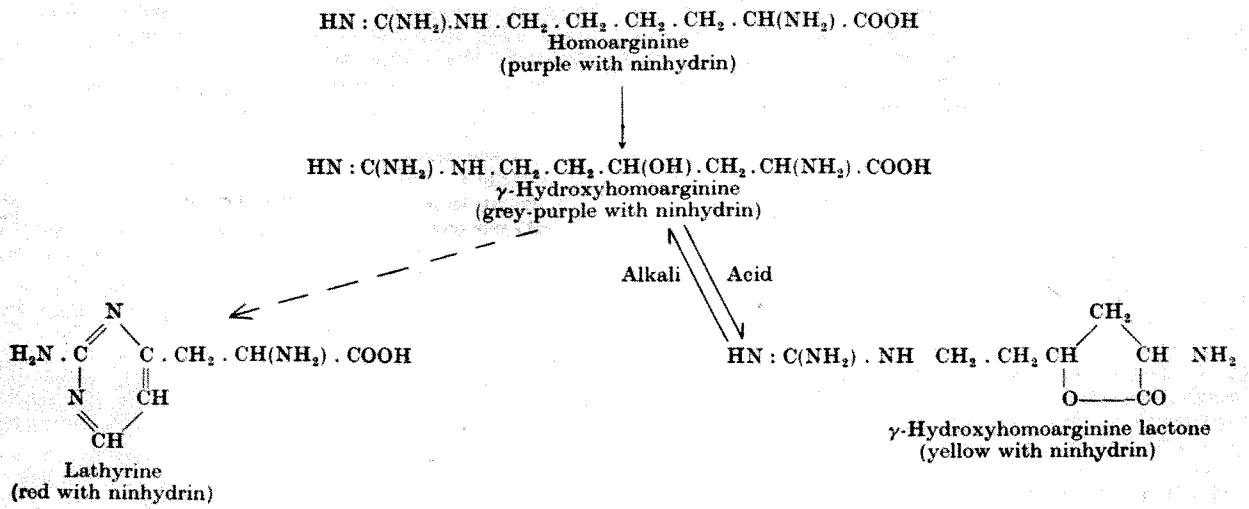
A study of free amino-acids and related compounds in *Lathyrus* revealed the presence of lathyrine[1] and two other ninhydrin-reacting compounds of unknown constitution, designated B_1 and B_3, in the seeds of *L. tingitanus* and three other species, *L. aphaca*, *L. cyanus* and *L. sphaericus*[2].

The identities of lathyrine and B_1 were afterwards established as 2-aminopyrimidin-4-ylalanine[3] and L-homoarginine[4] respectively. It is now reported that B_3 has been isolated from seeds of *L. tingitanus* and shown to be α-amino-γ-hydroxy-ε-guanidinocaproic acid (γ-hydroxyhomoarginine).

After removing lathyrine from finely ground seed of *L. tingitanus* with methanol, the guanidino amino-acids (homoarginine and B_3) were extracted with 50 per cent aqueous ethanol, the extract concentrated and passed successively through columns of 'De-Acidite *FF*' and 'Zeo-Karb 226'. The basic amino-acids were eluted from the cation exchange resin with ammonium hydroxide and the unknown (B_3) freed from homoarginine by the repeated recrystallization of its flavianate. The purified flavianate was decomposed with concentrated hydrochloric acid, excess acid removed with aniline and the hydrochloride recrystallized from hot ethanol.

As in the isolation of γ-hydroxyarginine from *Vicia sativa*[5] this procedure induced a transformation, and a more basic guanidino compound giving a yellow reaction with ninhydrin was isolated. This artefact (which reverted to the original compound in alkaline solution) analysed as the dihydrochloride of a hydroxyhomoarginine lactone or isomer, m.p. 214°. Found: C, 32·71; H, 6·41; Cl, 27·47; N, 21·95. $C_7H_{16}O_2N_4Cl_2$ requires C, 32·44; H, 6·18; Cl, 27·41; N, 21·62 per cent.

The free hydroxy acid behaved as an α-amino-monocarboxylic acid when treated successively with cupric nitrate and ninhydrin[6], while the failure of the original compound or the product of its alkaline hydrolysis (the corresponding diamino-acid) to yield ammonia when



treated with periodate indicated that the hydroxyl group was vicinal neither to the α-amino-group nor to the guanidino group.

Lactone formation ruled out the possibility of the hydroxyl group being in the ε position and the possible structures resolved themselves to α-amino-β-guanidino-δ-hydroxycaproic acid or α-amino-γ-hydroxy-ε-guanidinocaproic acid (γ-hydroxyhomoarginine). On lactonization these compounds would form δ- and γ-lactones respectively. The infra-red spectrum of the isolated lactone showed a strong peak at a frequency of 1,762 cm^{-1}, which was absent from the spectrum of the hydroxy acid itself. This characteristic absorption confirmed the presence of a γ-lactone and the identity of the original compound as γ-hydroxyhomoarginine.

Comparisons made between the spectra (infra-red and nuclear magnetic resonance) of the isolated lactone and those of γ-hydroxyarginine lactone were consistent with this conclusion.

Using L-homoarginine labelled with carbon-14 it has been shown this amino-acid is the precursor of γ-hydroxyhomoarginine in *L. tingitanus*, and it would seem probable that hydroxylation is the first step in the postulated[4] conversion of homoarginine to lathyrine in the plant.

I thank Prof. H. Harris for his interest, Mr. G. E. Hirst for determining the infra-red spectra and Mr. R. G. Foster for determining the nuclear magnetic resonance spectra.

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Pigment Formation by *Penicillium rubrum* Stoll

During an investigation on the appearance of red-pigmented patches on chrome-tanned hide it is found that this pigmentation is caused by the growth of *Penicillium rubrum* Stoll on it. Though such pigmentation is a common phenomenon, its occurrence is not frequent. *Penicillium rubrum* Stoll, like other members of *Penicillium purpurogenum* series, represents almost a common mycoflora of soil and other organic matter and produces red pigmentation on the substrate on which it grows. Therefore, the rarity of occurrence of such pigmentation on chrome-tanned hide and, in fact, on other organic matters is surprising and obviously suggests some strict specificity involved in pigment formation by *Penicillium rubrum* Stoll. Some of these specificities have been determined on laboratory culture media. Sabouraud's medium with 1·0 per cent 'Bactopeptone' (Difco), 4·0 per cent glucose A.R. (B.D.H.), 2·5 per cent agar, *p*H 5·0–5·5, is taken as the basal medium on which *Penicillium rubrum* Stoll produces intense red pigmentation in 4–8 days at 23°–25°. Substitution of one or the other constituent of the medium was made for specific studies. Abbreviations used for visual assessment of the formation of pigment are *P*− =pigment nil; *P*+ = pigment positive; each additional plus (+) sign indicates proportional increase in intensity of the pigment.

Ability of a number of different nitrogenous materials, and amino-acids when individually used as the sole source of nitrogen and of various 'sugars' and carbohydrates to produce pigment in Sabouraud's medium, have been determined. The extent of pigmentation observed after 8 days is given in parentheses.

Nitrogenous materials (1·0 per cent w/v): 'Bactopeptone' Difco (*P*++++); 'Proteose peptone' Difco (*P*++++); 'Peptone Italian' (*P*++++); 'vitamin-free casamino-acid' Difco (*P*++++); 'Bacto gelatin' Difco (*P*+); 'Bacto yeast extract' Difco (*P*+).

Amino-acids (1·0 per cent w/v; L-isomers are used unless otherwise stated): glycine (*P*−); glutamic acid (*P*+); aspartic acid (*P*−); leucine (*P*−); phenyl alanine (*P* trace); *dl*-tryptophan (*P*−); β-alanine (*P*++); arginine hydrochloride (*P* trace); asparagine (*P*−); glutamine (*P*−); histidine hydrochloride (*P*+++); proline (*P*−); hydroxyproline (*P*−); lysine hydrochloride (*P*−); cystein hydrochloride (*P*++++); *dl*-valine (*P*+); tyrosine (*P*−); *dl*-methionine (*P*−); glutathione (−SH) (*P*++++).

Sugars, etc. (4·0 per cent w/v): glucose (*P*++++); sucrose (*P*++++); maltose (*P*++++); lactose (*P*−); mannitol (*P*++): glycerine (*P*++); starch (*P*++).

Absence either of the source of nitrogen or of sugars or other carbohydrates in the medium invariably failed to show any pigmentation even though in some cases fair to

Table 1. Effect of Different Concentrations (% w/v) of Peptone and Glucose in the Basal Medium on Pigment Production by *P. rubrum* Stoll

Period (days)	'Bactopeptone' variable; glucose 4·0								'Bactopeptone' 1·0; glucose variable					
	(4·0)	(3·0)	(2·0)	(1·0)	(0·5)	(0·2)	(0·1)	(0·05)	(4·0)	(3·0)	(2·0)	(1·0)	(0·5)	(0·1)
4	*P*−	*P*−	*P*++++	*P*++++	*P*++++	*P*++++	*P*+++	*P*+++	*P*++++	*P*++++	*P*++	*P*−	*P*−	*P*−
8	*P*−	*P*−	*P*++++	*P*++++	*P*++++	*P*++++	*P*+++	*P*+++	*P*++++	*P*++++	*P*++	*P*−	*P*−	*P*−

good growth of the mould was obtained. This shows that growth and pigmentation do not always go side by side.

The question whether for a positive pigmentation 'peptone' and 'sugar' are required to be present in any definite concentration or mere presence of them, irrespective of the amount, will do the job, has been investigated and the results obtained are given in Table 1.

It is significant that while the pigmentation is fairly good when the peptone is as low as 0·05 per cent, it is nil when glucose is brought down to the level of 1·0 per cent. In all probability, it suggests that provided nitrogen is present in the medium to initiate the mycelial growth pigmentation will occur if glucose is above a certain level. It further suggests, in view of fairly high requirement of glucose for a positive reaction, that glucose, either by itself or through some intermediates, is linked chemically in pigment formation. The specificity shown by the mould for its cultural requirement of amino-acids as a source of nitrogen appears to be fairly rigid even though the pigment (phoenicine[2]) has been shown to be of a composition ($C_{14}H_{10}O_6$) containing no nitrogen[3]. Detailed work will be published elsewhere.

I thank Dr. Y. Nayudamma, director of the Central Leather Research Institute, for his interest in this work and the director of the Commonwealth Mycological Institute, Kew, England, for identifying the strain of *Penicillium*.

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Astringency of Persimmons (*Diospyros kaki*, L.)

It is a well-known fact that unripe persimmons which are fully coloured but firm are astringent, and that on ripening the astringency disappears concomitant with a softening of the fruit into a jelly-like consistency.

It has also been long known that interruption of respiration by placing the firm fruit in an atmosphere of alcohol will render the fruit non-astringent without accelerating the softening process. Traditionally, it was the practice to store the fruit in casks previously used to store sake, and the residual alcohol was sufficient to process the fruit in four or five days. This practice was examined as early as 1912 by Gore[1], and it was found that carbon dioxide as well as other gases could be used[2]. The action of sulphur fumes in preserving the astringent taste normally destroyed in drying was noted by Cruess and Joslyn in 1929 (ref. 3).

The cause of the astringency has been ascribed to tannins, and one of the mechanisms proposed for the removal of astringency involves the combination of soluble tannin with soluble pectin[4].

More recently, it has been shown by Bate-Smith[5] and Swain[6,7] that a large number of food tannins include leucoanthocyanins, also called anthocyanogens, or more recently, flavolans. These are flavanoid compounds which yield anthocyanidins on heating with mineral acid. They occur as monomers and polymers, usually in conjunction with catechins.

The tannin of persimmons appears to fall into this category. It has recently been shown that the major constituent of the tannin includes leucodelphinidin-3-glucoside[8]. The unusual nature of this tannin is that it is one of the few examples of an anthocyanogen which is a glycoside. All the observed properties of its solubility behaviour are in accord with the proposed structure.

This communication records the effects of certain processing conditions on the tannin content.

An initial experiment with the Hachiya variety was run in 1961 and concerned the changes occurring in the phenolic compounds during the ripening of persimmons under natural storage conditions. A summary of the results is shown in Table 1 (ref. 12).

Table 1

Date of sampling	Total phenolic (ref. 9) compounds (mg/g fruit)	Catechin type (ref. 10) compounds (mg/g fruit)	Anthocyanogens (ref. 11) (O.D./5 ml. extract)
Nov. 16	13	0·60	0·98
Nov. 23	7·5	0·20	0·42

The 1962 work was concerned with the correlation of the astringent factor with the leuco compounds. Preliminary work using segments of fruit showed that the juice of the astringent fruit would colour red on the application of vanillin and hydrochloric acid to a far greater degree than that of the ripe fruit. Staining of the fruit itself reveals that the amount in the juice represents only a small fraction of the total. Nevertheless, it is concluded that the soluble fraction would be more important in the astringency which is definitely different.

Firm orange fruit of the Hachiya variety were selected and divided into lots of five fruits as uniform as possible. One lot was kept at 0° C while the others were placed in large jars and exposed to alcohol vapour (11 ml./gal.), nitrogen alone and air. Another lot was coated with paraffin at approximately 50° C and cooled instantly. These were then stored for 5 days at room temperature (22° C). The fruit flesh was then subjected to shear press measurements. A second experiment using younger fruit showed that the alcohol action could be applied to firm fruits where the texture did not appreciably alter. The pulp was then recovered and tasted. The results are shown in Table 2.

Table 2. Shear Press Measurements, Relative Resistance to Shear

	Exp. 1		Exp. 2	
Initial	100	extremely astringent		
Control	32	astringent	59	astringent
Over-ripe	29	non-astringent		
Alcohol-treated	32	non-astringent	83	non-astringent
Wax-coated	47	slightly astringent		
Nitrogen	94	extremely astringent		

The results calculated as cyanidin, although the actual pigment appears to be delphinidin, are shown in Table 3.

Table 3. Anthocyanogen Content of the Methanol Extract (p.p.m.)

Initial	1,800	Alcohol-treated	8
Control	965	Wax-coated	58
Over-ripe	15	Nitrogen	1,050

The absorption curve of the developed colour showed a single maximum at 555 mμ and correlates with that found by Ito and Oshima[8].

Firm astringent and non-astringent fruit were blended in a Waring blender to give a smooth paste. 'Pectinol' (Rohm and Haas) was then added to each. Within 30 min, the non-astringent one showed signs of hydrolysis with attendant liquification of the pulp. After standing 12 h, the difference was striking. The astringent one still preserved its paste-like consistency while the non-astringent one was liquefied and had a distinct aroma reminiscent of the flavour of dried persimmons. The ability of the astringent pulp to tan enzymes is apparent here.

Astringent fruit at 0° C was peeled and placed in a humid oven at 40° C. Core samples were obtained at hourly intervals from 5 mm inside the surface. The results are shown in Fig. 1, and it is seen that a sharp decrease occurs between 2 and 6 h.

A quantity of non-astringent persimmons was obtained from the University Farm and the extract was compared with the astringent variety. The methanol extract contained no soluble leucoanthocyanins except for a small amount next to the skin. The distribution of soluble leucoanthocyanin through the fruit is shown in Fig. 2.

The pulps were then placed in hydrochloric acid–methyl alcohol, brought to boiling, and allowed to remain

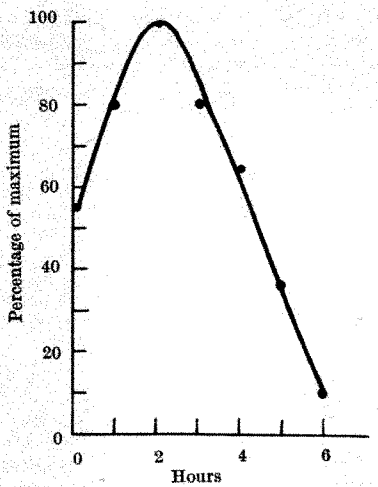


Fig. 1. Changes in soluble anthocyanogens of peeled fruit at 40° C

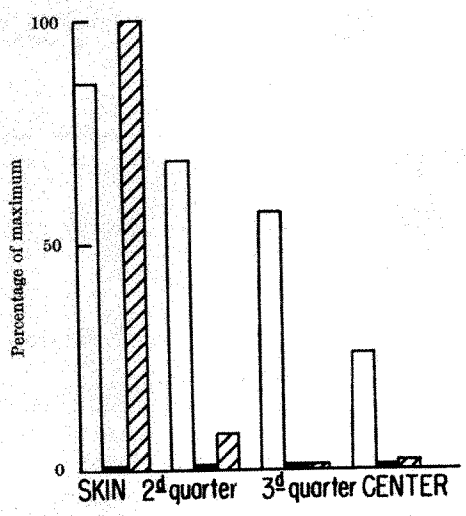


Fig. 2. Distribution of anthocyanogens in the fruit. White, astringent variety; black, astringent variety treated with alcohol; hatched, non-astringent variety

overnight. They were then extracted and the filtrate concentrated and chromatographed on Whatman No. 1 filter paper with BuOH : HOAc : HOH (4 : 1 : 5 v/v). The R_F value for the main violet-coloured spot was 0·40–0·42 on the astringent, non-astringent and combined extracts. A small spot was present at 0·62 in all samples. A large brown area remained at the origin and formed a curtain. The violet spots were eluted and the maximum in hydrochloric acid–methyl alcohol was 545 mμ. From these results it is concluded that the anthocyanidin was delphinidin, confirming the work of Oshima, and that a delphinidin generating leuco compounds is also present in the non-astringent variety.

It is concluded that a soluble form of a delphinidin-generating colourless compound is responsible for the astringent taste of unripe persimmons. These compounds can be either transformed or destroyed by interruption of the respiration. It also appears that in view of the fact that a great deal of these compounds remain in non-astringent fruit, there are astringent and non-astringent forms. The naturally non-astringent types appear to be a result of the absence of the soluble form.

The presence of the tannin appears to have some protective value as birds appear to attack only those fruits which are non-astringent. Among three trees located together—non-astringent, astringent but fully ripe, and an astringent only firm ripe—the birds had devastated the first two. This is reminiscent of the case of sorghum in which bird-proof sorghum is unpalatable due to its high content of anthocyanogens.

We thank Dr. Roy Young of the Department of Subtropical Horticulture, University of California, Los Angeles, for a discussion on post-harvest physiology.

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Hydroxylation of Phenoxyacetic Acid by Stem Tissue of *Avena sativa*

Recent work in this Department[1,2] has demonstrated that phenoxyacetic acid is rapidly converted into a number of products by the stem tissues of several plant species. Further research has now established that, under certain conditions, mesocotyl segments of *Avena sativa* within 24 h can convert phenoxyacetic acid almost completely into four main products.

These processes were followed by allowing the segments to take up phenoxy-[1-^{14}C]-acetic acid, followed by extraction of the tissue and a separation of the radioactive compounds by paper chromatography. A typical record of such a chromatogram is shown in Fig. 1, where it will be noted that the greatest amount of radioactivity is contained within the peak labelled *Z*. Under the experimental conditions used, 70 per cent of the total radioactivity extracted from the tissue was applied to the chromatogram, and of this about 40 per cent was found as substance *Z*.

In order to identify this compound the following procedures were adopted. Segments were cut from the mesocotyls of *Avena* seedlings raised in the dark at 25° C for five days. These segments were then placed in buffered aqueous solution containing the potassium salt of phenoxy-[1-^{14}C]-acetic acid at 8×10^{-4} M and incubated for 24 h in the light. The segments, after rinsing, were extracted with hot ethanol, and the extract was concentrated and applied to a polyamide[3] ('Perlonpulver') column. Fraction *Z* was adsorbed to an appreciable extent, and after washing the column with water, the compound could be eluted with methanol. Further purification of *Z* was effected by paper chromatography using as solvents

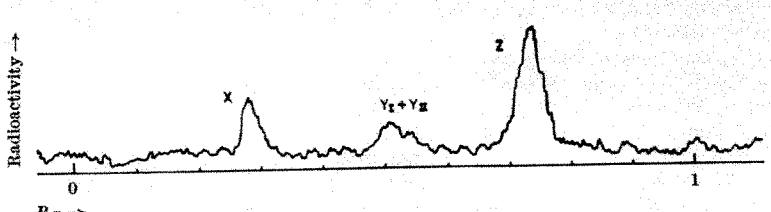


Fig. 1. Distribution of radioactivity in a chromatogram of *Avena* tissue extract on Whatman No. 3 *MM* paper developed in butanol/propionic acid/water (2 : 1 : 1·3). Phenoxyacetic acid has an R_F of 0·89

butanol/propionic acid/water (2 : 1 : 1·3) and isopropanol/ammonia (*d*, 0·88)/water (20 : 1 : 2). Finally the product was crystallized from a mixture of benzene and petroleum ether b.p. 60°–80° C. In this way 10 mg of white crystals m.p. 152°–153° C were obtained from 250 g of *Avena* tissue.

This product was stable to acid and alkaline hydrolyses and showed selective absorption in the ultra-violet region —λ_{max} 285 mμ *p*H 2 and λ_{max} 305 mμ *p*H 11. Phenoxyacetic acid itself has λ_{max} 267 mμ independent of *p*H. These facts and the adsorption of *Z* on to the polyamide suggested a phenolic structure for *Z*[3–5].

In 1957 it was reported by Byrde and Woodcock[6] that phenoxyacetic acid was converted to 2-hydroxy- and 4-hydroxy-phenoxyacetic acids by *Aspergillus niger*. Dr. Woodcock kindly supplied authentic samples of these compounds. Compound *Z* was found to be identical to 4-hydroxy-phenoxyacetic acid in the following respects. (1) λ_{max} 285 mμ *p*H 2, λ_{max} 305 mμ *p*H 11; (2) m.p. 152°–153° C (authentic sample 152°–154° C); (3) R_F 0·42 in isopropanol/ammonia/water (20 : 1 : 2); (4) R_F 0·75 in butanol/propionic acid/water (2 : 1 : 1·3); (5) The same purple colour was developed with diazotized sulphanilic acid.

The foregoing results are considered to be sufficient to distinguish between 4-hydroxy- and 2-hydroxy-phenoxyacetic acids[6].

Little is known of the enzymatic mechanisms involved in such reactions. Klämbt[7] has recently shown that benzoic acid is hydroxylated by *Helianthus annuus*, while Harborne and Corner[8] have described hydroxylation of cinnamic acids in a variety of plants. The existence of this system, which may well be a mechanism for the detoxification of phenoxyacetic acid, suggests that enzymes capable of introducing hydroxyl groups into aromatic rings may be widely distributed in the plant kingdom. The characteristics of the enzyme involved in this particular case are now being investigated.

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PHYSIOLOGY

Effect of Cortisone on Extra-adrenal Chromaffin Tissue of the Rat

It has been assumed that the differentiation of primary sympathetic cells into chromaffin cells is stimulated by hormones produced by the adrenal cortex[1]. These hormones have also been thought to be necessary in the methylation of noradrenaline into adrenaline[2]. However, while medullary catecholamines are known to cause an increased secretion of cortical hormones[3], no direct evidence is available of the influence of cortical hormones on chromaffin cells.

In the course of an examination of the chromaffin tissue of the rat, I observed that administration of cortisone strongly affects the normally rapid post-natal disappearance of chromaffin cells from the extra-adrenal chromaffin tissue. Fig. 1 illustrates the age-dependent changes in the volume of this tissue, measured planimetrically from serial sections, in normal and cortisone-treated rats. In the normal rat, the disappearance of extra-adrenal chromaffin cells occurs very rapidly. After the first post-natal week there are a few faintly chromaffin cells left only in the main para-aortic body, frequently called the organ of Zuckerkandl. Administration of cortisone, if started during the first two post-natal days, prevents the disappearance of the chromaffin cells from this organ and brings about the appearance of numerous new strongly chromaffin cells in it, in smaller abdominal para-aortic bodies and in structures such as the sympathetic ganglia, in which normally none or only a few weakly chromaffin cells are seen. The planimetrically measured volume of the extra-adrenal chromaffin tissue therefore increases. Moreover, the intensity of the chromaffin reaction clearly increases during cortisone treatment, while it gradually weakens in untreated rats (Fig. 2).

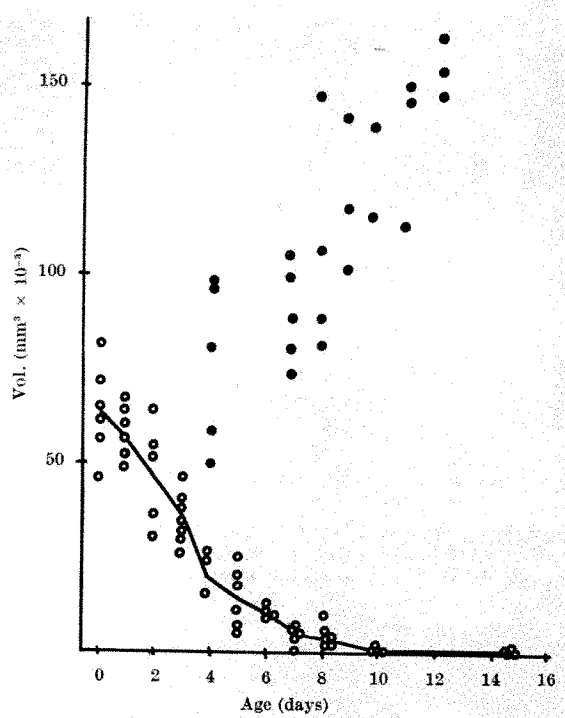


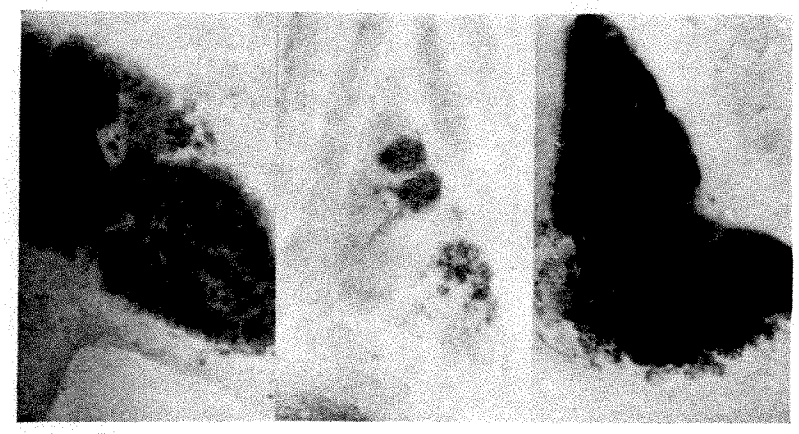
Fig. 1. Effect of age and cortisone on the volume of the extra-adrenal chromaffin tissue of the rat. Open circles, untreated controls; full circles, rats injected daily with 0·25 mg of cortisone after birth

The effect of cortisone depends on the administration being made sufficiently early. Cortisone does not cause regeneration of the cells once they have lost their 'chromaffinity' and started to degenerate. The appearance of new chromaffin cells in the sympathetic ganglia also seems to depend on their developmental stage: the less primary, undifferentiated sympathetic cells present when the cortisone administration is initiated, the less new chromaffin cells appear.

Hydrocortisone exerts an influence similar to that of cortisone described here. Adrenocorticotrophic hormone is capable of preventing the disappearance of chromaffin cells from para-aortic bodies, but does not cause an increase in the volume of extra-adrenal chromaffin tissue. Deoxycorticosterone is without any effect on the chromaffin cells.

No effects of cortical hormones on intra-adrenal chromaffin tissue were observed, which is not surprising, since most of the cells in the adrenal medulla are clearly chromaffin already three days after birth, and the intensity of the chromaffin reaction in them increases with age even in untreated rats.

So far as I know, these observations are new and provide the first direct evidence of the influence of cortical hor-



A B C

Fig. 2. Chromaffin reaction in the main para-aortic body. *A*, new-born rat; *B*, 7-days-old untreated control; *C*, 9-day-old rat treated daily with 0·25 mg cortisone

mones on chromaffin cells. Comprehensive interpretation of these observations will not be attempted in this brief report. However, they suggest that enclosure inside the adrenal cortical tissue, which provides a flow of cortical hormones, may be a condition for subsequent normal development and secretory function of chromaffin medullary cells.

This work is part of a research project on the function of the adrenal medulla directed by Prof. Olavi Eränkö and supported by research grant No. *A*–1725 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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Uptake of Iodothyronines by Erythrocytes *in vitro* in Various Species

It has been shown that the electrophoretic mobility of thyroxine labelled with iodine-131 in human serum corresponds with the inter-α zone globulins, thyroxine-binding protein (TBG). The capacity of the serum TBG for thyroxine is limited but the physiological 'saturation' of its binding sites varies with the state of thyroid activity. It is relatively saturated with endogenous thyroxine in the hyperthyroid and relatively unsaturated in the hypothyroid state[1]. Thus the binding of thyroxine added to serum varies inversely with the thyroid function. If the competition between red cells and the binding sites of TBG is expressed in terms of *in vitro* 'uptake' of radioactive tri-iodothyronine ('Trix') by red cells, the uptake is higher in thyrotoxic and lower in myxœdematous persons[2], and the binding coefficient of red blood cell uptake of 'Trix' is negatively correlated with the TBG capacity.

So far, only investigations of human serum have been described in detail, and very little is known about serum TBG capacity in animals. The presence of TBG in animals has been shown, but the precise pattern in the α-globulins differs between species[1]. This is also true with reference to the presence or absence of the thyroxine-binding pre-albumin substance of serum. No TBG has been found in chicken and duck sera[3]. It may be anticipated, therefore, that variation in the properties of the thyroxine-binding proteins in animals may be given in turn a species difference in the uptake of labelled iodothyronines *in vitro* by erythrocytes. In fact 5·4 and 2·5 per cent uptakes of labelled thyroxine by red cells of rabbit and sheep respectively have been observed previously[4]. This communication represents further data on the differences between uptakes in various animals.

The experiment involved animals of 6 species, kept under normal farm conditions. Blood samples were drawn from the jugular vein, or from the brachial wing vein in the case of hens, directly into a heparinized syringe. Thyroxine-131 and tri-iodothyronine-^{131}I ('Trix') in 50 per cent aqueous propylene glycol were obtained from the Radiochemical Centre, Amersham, England. Their purity, not less than 90 per cent, was checked by ascending chromatography, on Whatman No. 3 filter paper, in two solvent systems, *n*-butanol – dioxan – 2 N ammonia and *n*-butanol – glacial acetic acid – water. Under standardized conditions, whole blood was incubated for 2½ h at 37° C with the labelled iodothyronines in a corked flask on a rotating plate. Thyroxine-^{131}I or 'Trix' was added in quantities of 15·6–57·6 × 10^{-4} μg in 0·1 ml. isotonic saline per 1 ml. of whole blood. At the end of the period of incubation the radioactivity of 1 ml. of whole blood was estimated in a well-type scintillation counter. After 5 washes with 0·9 per cent saline the radioactivity remaining in the red cell mass was determined in the same centrifuge tube as before, with a counting accuracy of ± 2 per cent. From the proportion of counts of red blood cells to count in whole blood, the percentage of uptake was calculated. For comparative purposes the uptake was corrected to 100 per cent hæmatocrit. The results are shown in Table 1.

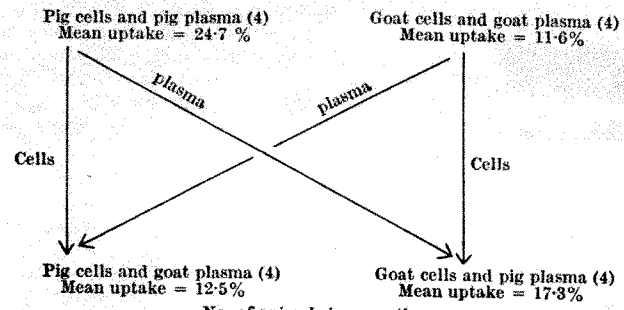
The uptake of 'Trix' varies between species; it is three times greater in hen and rabbit than in the ruminants with values for the pig occupying a middle position. On the other hand, uptake of thyroxine-^{131}I is nearly the same in the ruminant group and pig, but greater in hen and rabbit. The higher uptake of thyroxine-^{131}I in rabbit in comparison with other mammals confirms a previously noted observation[4]. A positive correlation between 'Trix' and

Table 1

Animal	Age (months)	*In vitro* red blood cell uptake of iodothyronines in % (corrected to 100 hæmatocrit)						Binding capacity of serum TBG (μg/100 ml.)	Protein-bound iodine in serum (μg/100 ml.)
		Tri-iodothyronine-^{131}I			L-Thyroxine-^{131}I				
		Mean	*S.D.*	*S.E.M.*	Mean	*S.D.*	*S.E.M.*		
Pig	3–4	24·2 (16)	± 2·32	± 0·58	3·1 (6)	± 0·26	± 0·10	?	2·3–5·6[11]
Goat	10	12·6 (16)	± 1·83	± 0·45	3·7 (8)	± 1·57	± 0·55	?	6·06[10]
Sheep	11	10·5 (14)	± 1·57	± 0·41	3·3 (6)	± 0·74	± 0·30	11·0–16·0[6]	5·5[6]
Calf	2–3	14·3 (8)	± 2·87	± 1·01	3·2 (8)	± 0·80	± 0·28	?	4·8[7]
Rabbit	24	38·6 (10)	± 2·03	± 0·83	7·1 (6)	± 1·14	± 0·46	10·0 (?)[5]	4·8[9]
Hen	18	38·2 (10)	± 9·15	± 2·89	15·9 (8)	± 4·33	± 1·53	None[3]	3·5[12]
Man	Adult (euthyroid)	13·3[3]	± 1·9					11–29·0[1]	5·3[8]

No. of animals in parentheses.

Table 2



No. of animals in parentheses.

uptake of thyroxine-^{131}I has been observed in individual hens ($r = 0{\cdot}9$; $P < 0{\cdot}01$).

For comparative purposes Table 1 includes published figures for serum TBG capacity and protein-bound iodine-level. There is clearly no relationship between levels of uptake of protein-bound iodine and 'Trix'. The same cannot be said about the relationship between TBG and uptake because of inadequate information. With reference to the hen, however, high uptake of both iodothyronines seems to correspond to an observed lack of TBG in chicken and their different response to tri-iodothyronine and thyroxine in comparison with mammals[3]. It is possible that the high uptake of 'Trix' and thyroxine-^{131}I in rabbit is partly connected with the fact that in normal conditions rabbit serum is relatively saturated with endogenous hormones[5].

Table 2 shows a criss-cross experiment on the inter-species recombination of red cells and plasma. In this experiment, unwashed red cells were mixed with plasma of different species. After 2 h of incubation, although a slight tendency to agglutination and hæmolysis was observed, the addition of goat plasma to pig red cells decreased uptake of 'Trix' from 24·7 to 12·5 per cent and the addition of pig plasma to goat red cells increased uptake from 11·6 to 17·3 per cent. This shows that goat thyroxine-binding proteins have a greater affinity for 'Trix' than those of pig plasma, and is in general agreement with present views on the competition for 'Trix' between binding sites on the red cells and those on the proteins in the serum.

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Effect of Melanocyte-stimulating Hormone on the Eye of the Rabbit

The aqueous flare is a scattering phenomenon, which may be observed when a beam of light passes the anterior chamber of the eye. It is caused by the presence of substances with large molecules (proteins). Using a photographic apparatus[1] for measuring the intensity of the flare, Anjou, Krakau and Stigmar[2] observed that an injection of chromatographically pure preparations of ACTH may cause an increase, sometimes quite impressive, of the flare density in normal eyes of rabbits. The maximum density occurred about 3 h after the injection; then the flare intensity faded slowly. The flare effect was regularly accompanied by a reduction of the intraocular pressure. No effect could be seen after vasopressin.

The investigation has been continued with a photoelectric modification of the previous device[3]. Suspecting that the effect might be elicited via the adrenals a series of adrenal hormones (cortisone, hydrocortisone, deoxycorticosterone and norepinephrine) were tested. No responses as a consequence of these were observed.

With α- and β-melanocyte-stimulating hormone (supplied by A. V. Schally and R. Guillemin, Baylor University, Houston, Texas), however, the typical effect on the flare was obtained with a dosage of about 3 μg/kg (s.c.). The effect on the flare density is due to an increased permeability of the blood-aqueous barrier. This was demonstrated by means of intravenously injected fluorescein, which passes into the anterior chamber in far greater amounts during the period of increasing flare than is normally the case.

The idea that we are dealing with an influence on the melanocytes of the eye is in agreement with the fact that ACTH shows a melanocyte-stimulating hormone effect. It also agrees with anatomical facts—thus the pigment epithelium is found in the boundary layer between the small vessels and the non-pigmented epithelium in the ciliary processes. Finally, strong support is provided by the observation that the effect can be provoked mainly in pigmented rabbits, whereas there is practically no response in albinotic animals to the doses administered (one slight reaction observed in a series of 10 albino rabbits).

The present finding may possibly have some bearing on certain previous observations on diurnal variations provocable by light in the physiological flare intensity, and on other phenomena connected with aqueous dynamics.

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Selective Stimulation of Intra-cardiac Postganglionic Fibres

It was observed by Lewis *et al.*[1] that the duration of the refractory period of the electrically stimulated auricles may be increased by atropine. They believed that the effect may be ascribed to the stimulation of the intra-auricular parasympathetic fibres by the driving stimuli. Since that report, the effects of the stimulation of the intra-cardiac parasympathetic and/or sympathetic nerve fibres superimposed on the effect of stimulation of the heart muscle or sino-auricular node have been observed by other authors[2–4]. Brady *et al.*[5] described the inotropic effect of series of impulses applied during the refractory period of the cardiac muscle.

In this communication a simple method of selective stimulation of the intra-auricular postganglionic fibres is described.

The auricles of the young rabbits anæsthetized with chloralose (0·1 g/kg intravenously) were isolated with both vagus nerves attached, following McEwen's method[6]. They were mounted in a bath of Tyrode solution containing 250 mg sodium bicarbonate/l. and saturated with 100

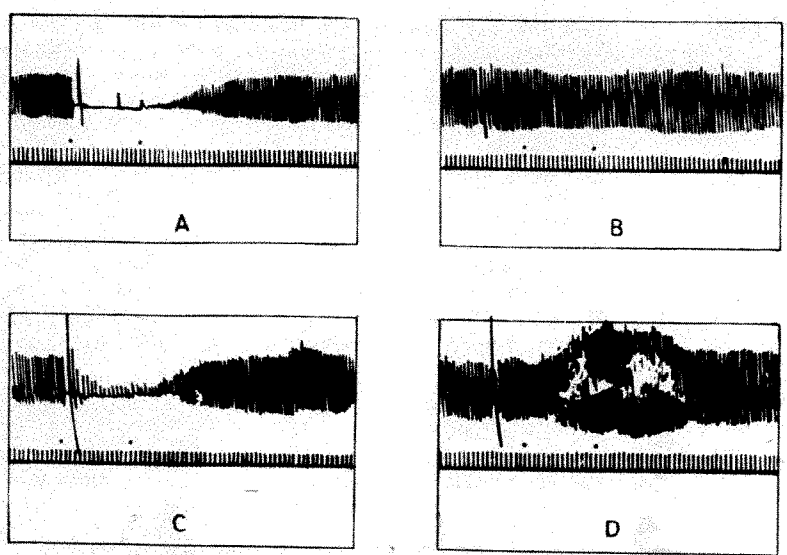


Fig. 1. From above downward: contractions of the isolated rabbit auricles, the dots indicating the nerves stimulation, time at 1-sec intervals. *A*, Response to the stimulation of both vagus nerves; *B*, stimulation of both vagus nerves after hexamethonium (2×10^{-4} g/ml.) has been added to the bath; *C*, response to the direct stimulation of the intra-cardiac postganglionic fibres; *D*, response to the direct postganglionic stimulation after atropine (1×10^{-5} g/ml.) has been added to the bath

per cent oxygen at a temperature of 35° C. Both nerves were left freely in the bath. They were placed on the electrodes for the time of stimulation only. The contractions of the auricles were recorded by means of the light lever writing on a smoked drum.

A pair of the round-plate silver–silver chloride electrodes, connected to the Grass stimulator (model *S4D*), was placed in the bath, to stimulate the intra-cardiac nerve elements. The diameter of the positive electrode was 10 mm. It was placed about 10 mm. from the auricular surface to face the orifice of the inferior vena cava. The diameter of the free surface of the negative electrode was 2 mm. It was placed about 2 mm from the auricular surface to face the area lying at the angle between the orifice of the vena cava superior and the base of the right appendix. The purpose of the placing of the electrodes was to submit the parasympathetic fibres and ganglia, situated in the sino-auricular node region, into the influence of the electric field generated in the bath by the tetanizing rectangular stimuli.

Both vagus nerves were stimulated for 30 sec in each 5 min with supramaximal rectangular stimuli of 10 msec duration at the rate of 30/sec. Stimulation was started 30 min after the auricles had been isolated. When the stable and reproducible responses had been obtained (Fig. 1*A*), hexamethonium was added to the bath to obtain a final concentration of 2×10^{-4} g/ml. This was sufficient to block the transmission in the intra-auricular parasympathetic ganglia, as was shown by McEwen[6] (Fig. 1*B*). After complete blockage had been effected, the rectangular stimuli of a duration of 0·01 msec–0·25 msec at a rate of 100/sec were applied by means of the plate electrodes. It was possible to obtain pure negative chronotropic and inotropic responses followed by the positive effects after the stimulation had been stopped. No extra systoles were observed, provided the strength of the stimuli was properly adjusted (Fig. 1*C*).

The negative responses were reversed by atropine in a concentration of 1×10^{-5} g/ml. (Fig. 1*D*). No responses to the direct stimulation by means of plate electrodes could be obtained after hemicholinium No. 3 (ref. 7) had been added to the bath at a concentration of 1×10^{-5} g/ml. and the vagus nerves stimulated up to complete disappearance of responses. These results suggest that the negative effects were due to the stimulation of the intra-auricular parasympathetic postganglionics and not to the stimulation of the auricular muscle or the sino-auricular node. Release of acetylcholine was the important link in the positive effects, since these disappeared under the influence of hemicholinium.

The optimal conditions for the selective stimulation of intra-auricular postganglionic fibres were tested. The threshold strength of the stimuli evoking the easily noticeable negative response was tested and the strength–duration curve recorded. Another strength–duration curve was recorded for the threshold stimuli evoking the auricular extrasystoles, and both curves compared. As may be seen in Fig. 2, the difference between the threshold values for nerve elements and those for auricular muscle is greatest with very short stimuli and disappears completely when the duration of the stimuli exceeds 0·20 msec.

The simple and easy method of selective stimulation of intra-auricular parasympathetic fibres is provided by these experiments. This method may be useful, if knowledge of the site of action (preganglionic endings or postganglionic endings) of some vagolytic drug is required.

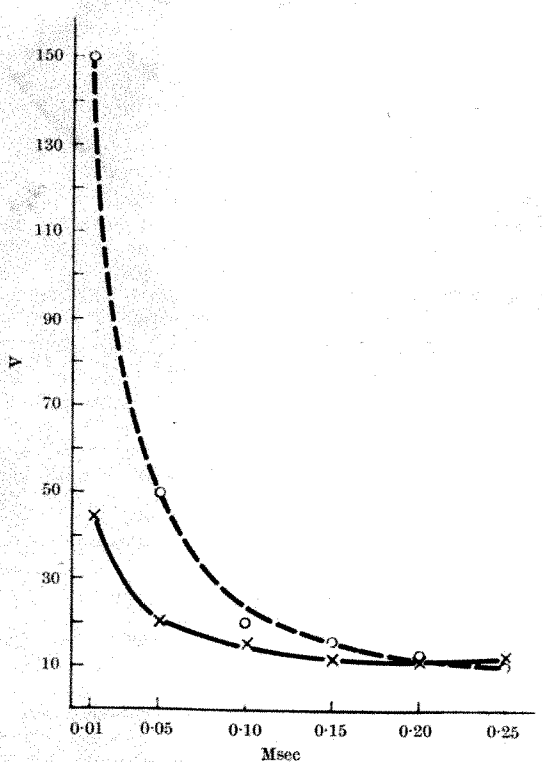


Fig. 2. The strength–duration curves of intra-cardiac postganglionic fibres and auricular muscle. ○, Auricular muscle; ×, intra-auricular postganglionic fibres

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Urine-concentrating Mechanism in Man

Although many extensive investigations in animals[1–3] have demonstrated the existence of renal medullary hyperosmolality suggestive of a counter-current mechanism of urine concentration, direct evidence of medullary hyperosmolality in man has never been obtained hitherto. We have examined the tissue concentrations of sodium, potassium, urea and ammonia in human kidneys removed at operation or shortly after death, using standard techniques[4].

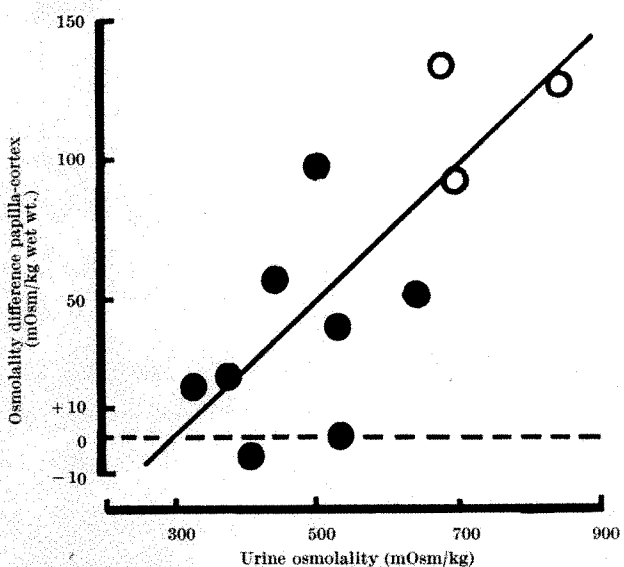


Fig. 1. Relationship of cortico-papillary tissue osmolality gradient to urine osmolality. ○, Nephrectomy specimens; ●, post-mortem specimens. Calculated regression line formula is $y = 0{\cdot}228\,x - 66{\cdot}8$; $r = 0{\cdot}76$; $0{\cdot}01 > P > 0{\cdot}001$

The results in Table 1 show that there is a rise in sodium and urea tissue concentrations from cortex to papilla in persons producing hypertonic urine; potassium and ammonia concentrations show little change. A more detailed report, including an investigation of renal solute concentration changes after death, will appear elsewhere[5]. The relationship between urinary osmolality and the cortico-papillary tissue osmolality gradient is highly significant, $r = 0{\cdot}76$; $0{\cdot}01 > P > 0{\cdot}001$, the calculated regression line formula being $y = 0{\cdot}228x - 66{\cdot}8$. The regression line reaches urinary isotonicity with plasma at a tissue osmolality gradient of zero; this differs from the findings in dogs in which there is a persistent cortico-papillary tissue osmolality gradient even in extreme water diuresis[6], but we have not had the opportunity of examining human kidney tissue under conditions of urinary hypotonicity.

The importance of sodium and urea in producing an increase in tissue osmolality in the human papilla is similar to that found in dogs[2] although the absolute values are lower; this correlates well with man's inferior maximal urinary concentrating ability when compared with the dog.

The increasing cortico-papillary tissue osmolality gradient with increasing urine concentration supports the hypothesis that urine concentration in man is carried out by a counter-current mechanism.

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Micro-injection of Trypsin into Axons of Squid

Recent work appears to show that phospholipids play a fundamental part in membrane physiology[1,2]. The participation of protein has been thought by some to be more doubtful since external application of proteases seems not to interfere with electrical function of squid, lobster or frog axons[3–5]. However, it should be recalled that externally applied protease does produce injury potentials in muscle[4]. Attempts to demonstrate that protease had entered squid axons without producing changes in electrophysiological properties may have been in error even though the controls at the time seemed appropriate[3].

This communication presents, without further interpretation, the results of five experiments which indicate that nerve proteins do play an important part in impulse generation.

Pieces of giant axons (1,000–1,500μ in diameter and 5 cm long) from the large squid *Homastrephes gigas* were used in these experiments. A micropipette (100–200μ tip diameter) was introduced longitudinally into the axon from one end. This pipette was connected to a syringe filled with mineral oil to which pressure could be applied. The pipette was also connected by means of a T tube and an agar sea-water bridge to a calomel electrode. Another calomel electrode was in contact through an agar sea-water bridge with the external medium (natural sea-water). In this way, the micropipette was used both to inject solutions into the axon and to measure resting and action potentials. Stimulation was effected with two external platinum-electrodes.

Resting potentials were within the range of 40–45 mV. Overshoots were between 20 and 35 mV. After measuring resting and action potentials, approximately 10^{-3} c.c. of

Table 1. Papilla-cortex Concentration Gradient

Patient No.	Nephrectomy or post-mortem	Disease	Hours *post mortem*	Sodium (m.equiv./kg wet wt.)	Potassium (m.equiv./kg wet wt.)	Ammonia (mM/kg wet wt.)	Urea (mM/kg wet wt.)	Tissue osmolality (mOsm./kg wet wet)	Urine osmolality (mOsm./kg)
1	N.	Renal artery stenosis	—	+ 44·8	− 1·3	− 3·0	+ 8·2	+ 89·2	694
2	N.	Renal stones	—	+ 52·1	− 1·6	+ 2·7	+ 15·9	+ 122·5	848
3	N.	Polar hypernephroma	—	+ 48·8	− 6·0	+ 1·0	+ 45·6	+ 133·2	680
4	P.M.	Cerebral hæmorrhage	2¼	+ 34·9	− 11·4	− 1·1	+ 52·8	+ 97·6	505
5	P.M.	Carcinoma bronchus	6	+ 15·7	− 22·0	− 0·6	+ 9·3	− 4·2	407
6	P.M.	Mitral stenosis	9	+ 14·7	+ 4·4	− 0·1	+ 7·2	+ 45·2	637
7	P.M.	Carcinoma bronchus	8	+ 13·2	− 15·4	− 0·1	+ 5·3	+ 0·7	531
8	P.M.	Steatorrhœa	8	+ 25·7	− 5·0	+ 0·1	+ 2·6	+ 44·2	530
9	P.M.	Diabetic coma	1	+ 32·0	− 19·8	+ 3·6	+ 25·5	+ 57·1	446
10	P.M.	Empyema. Broncho-pneumonia. Recovering from acute glomerulonephritis	8	+ 6·8	− 2·2	+ 4·7	− 1·0	+ 17·6	328
11	P.M.	Syphilitic aortitis	2½	+ 7·0	− 0·3	+ 1·6	+ 5·6	+ 22·4	374
12	P.M.	Pneumonia	15	+ 73·2	+ 25·5	− 3·6	− 1·3	+ 189·0	Urine not available

Tissue concentration gradients obtained by subtracting cortical concentrations from papillary concentrations. All figures expressed in terms of wet weight of tissue.

a trypsin solution (1 mg/c.c. at *p*H 7·3) was injected along the axon as the pipette was slowly withdrawn, the solvent being a salt solution mimicking the cytoplasmatic ionic composition. During the first minute after injection, the fibre was excitable at 60/sec and threshold was not modified. At the beginning of the second minute threshold increased, and by the end of the second minute only local responses were elicited. After five minutes the local response too had disappeared, although the resting potential remained unmodified. The control for the described experiments is a set of more than 50 micro-injections of pure solvent. After we mastered the technique, the few failures we had were always when we were introducing the pipette, never at the time of withdrawal, that is, during injection. In fact, our experience tells us that large changes in state of the axoplasm have no immediate effect on impulse genesis (see also ref. 6). This was not true of the membrane, since every time we touched it from the inside, the action potential was abolished.

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PATHOLOGY

A Quantitative Assay for the Number of Murine Lymphoma Cells capable of Proliferation *in vivo*

Till, McCulloch and Becker[1,2] have demonstrated that normal mouse hæmatopoietic tissue contains a class of cells which are characterized by their ability individually to give rise to macroscopic colonies in the spleens of heavily irradiated recipient mice. The ease with which the colonies may be enumerated has permitted quantitative investigations of the effects of irradiation[3,4] transplantation[5], and erythropoiesis[6] on the number of cells with colony-forming potentiality. These varied applications suggested to us that the spleen-colony technique might be fruitfully applied to the examination of malignant tissue. The results show that a quantitative assay of the number of murine lymphoma cells capable of proliferating *in vivo* is, indeed, possible.

Colonies macroscopically similar to those described by McCulloch and Till have been found to develop in the spleens and kidneys of normal unirradiated mice injected with murine lymphoma cells. The colonies have been demonstrated by the following procedure.

A well-dispersed suspension of lymphoma cells containing very few clumps is prepared from the thymus of a female *AKR/J* mouse 8–12 months old with spontaneous leukæmia by mincing the organ finely with scissors and washing the cells through a fine stainless steel wire mesh with *CMRL* 1066 (ref. 7) medium. The number of

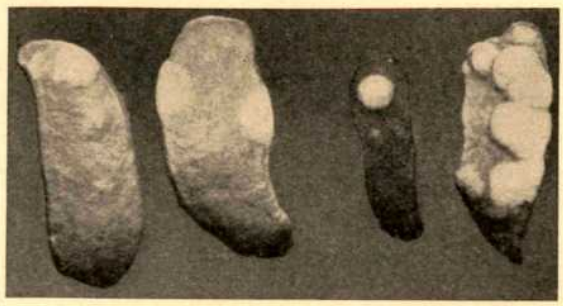


Fig. 1. The spleens of *AKR/J* mice injected 10 days previously with 10^5 lymphoma cells that display the colonies described in the text. Spleens on the left are from unirradiated animals, those on the right are from animals irradiated prior to injection to a dose of 850 rads

nucleated cells in the suspension is determined with a hæmocytometer and, after appropriate dilutions are made, 10^2–10^5 of the cells are injected intravenously into normal, unirradiated, 6–12-week-old mice of the same strain as the donor. The injected animals are killed from 8–12 days later and their spleens removed and placed in Bouin's fixative. Certain of the spleens contain discrete yellow nodules easily visible to the unaided eye (Fig 1, left). They may vary in size from 1 to 6 mm; they usually bulge very slightly from the splenic substance, and their borders may be distinct or very diffuse. The nodules first appear 7–10 days following the injection and then increase in size until they finally involve the entire spleen. The spleens of mice injected with larger numbers of cells frequently do not display any nodular appearance due to the confluence of adjacent nodules. Microscopically the discrete nodules appear as colonies of lymphoid cells quite different from the myeloblast- and erythroblast-containing colonies observed following the injection of bone marrow, spleen or fœtal liver cells into irradiated mice. While the cells in the colonies may differ considerably in appearance from one lymphoma to another, most display prominent nucleoli and many are in mitosis (Fig. 2).

Colonies have been observed in *AKR/J* mice when the cell suspensions used were obtained from *AKR/J* animals with transplanted lymphoma and *C3Hf/BiOci* and (*AKR/J* × *C3Hf/BiOci*) F_1 hybrid mice with Gross virus-induced lymphoma. No colonies have been seen following the injection of as many as 10^7 thymus cells from adult *AKR/J* mice without microscopic evidence of lymphoma.

The nature and origin of the colonies were investigated as follows: Cell suspensions were prepared from five discrete colonies dissected out of the spleen and were injected as a concentrated suspension into normal young

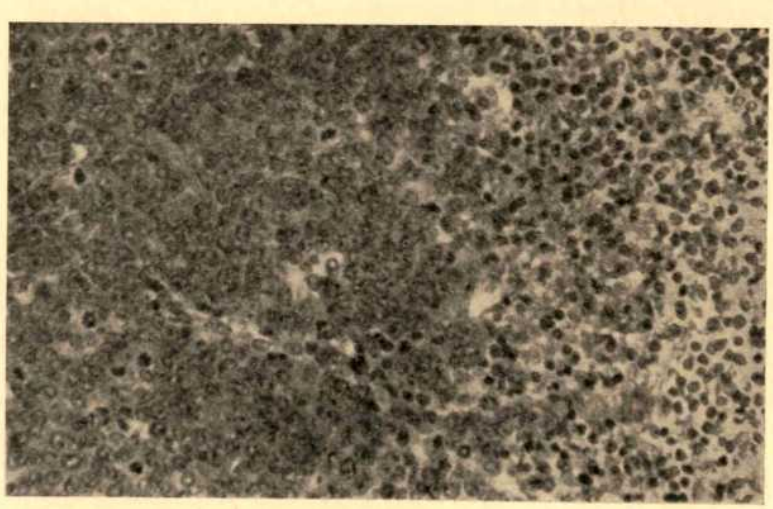


Fig. 2. The border between the colony (left) and the normal splenic tissue (right). The lymphoid cells comprising the colony display prominent nucleoli; many are in mitosis

mice. This resulted in the death of the recipients with widespread lymphomatous disease in less than 14 days, thus showing that the colonies contain malignant cells. Colonies were not observed in mice injected with heavily irradiated lymphoma cells (5,000-rads cobalt-60 γ-radiation, 250 rads/min, cells at 5° C). However, irradiation of the recipient mice (900-rads cobalt-60 γ-rays, 50 rads/min) before the injection of unirradiated cells did not alter the number of colonies observed (Fig. 1, right). Since it is well known that radiation doses of this magnitude destroy the proliferative capacity of the vast majority of mammalian cells[8], these results show that colony formation represents the multiplication of the transplanted malignant cells and not that of the host cells.

The variation in the number of colonies as a function of cell number was examined by injecting groups of 10 and 20 normal and irradiated mice with different numbers of cells. The average number of colonies per spleen obtained for each group for one typical experiment is shown in Fig. 3. The number of colonies increases with cell dose and appears to vary linearly over the range 50–400 cells with a slope corresponding to 130 ± 18 and 123 ± 12 cells injected per spleen colony observed for the normal and irradiated recipient respectively. The linearity is compatible with the assumption that each of the colonies arises from a single injected malignant cell and suggests that the experimental procedure might meaningfully be used to determine the number of colony-forming lymphoma cells in any cell suspension.

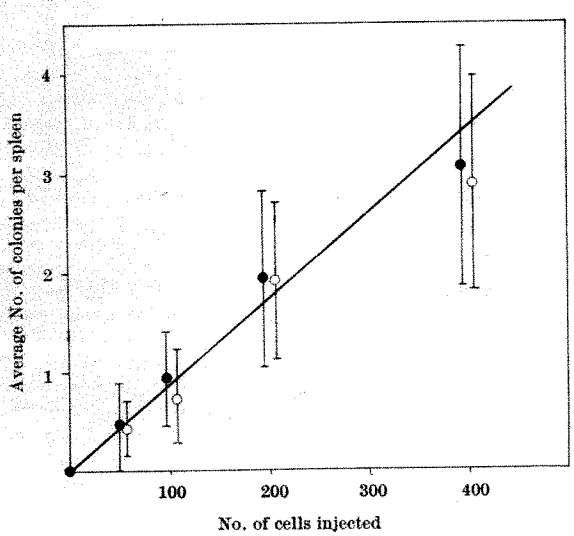


Fig. 3. The average number of colonies observed in the spleen as a function of the number of injected lymphoma cells. 95 per cent confidence limits are given for assays in both normal and heavily irradiated recipients. ○, Unirradiated recipients; ●, irradiated recipients

Thymus suspensions were prepared from 15 female *AKR/J* mice with spontaneous lymphoma and injected in appropriate numbers into groups of unirradiated recipients. The average number of cells which had to be injected in order to observe an average of one colony per spleen varied considerably. The values obtained were : 10^2, 10^2, 10^2, 10^2, 2×10^2, 2×10^2, 5×10^2, 10^3, 10^3, 10^3, 10^4, 10^4, 10^4, 3×10^4, and 10^5. Eight assays on 3 cell lines serially transplanted in *AKR/J* yielded consistently lower values of 30, 10^2, 10^2, 2×10^2, 2×10^2, 3×10^2, 3×10^2 and 3×10^2, cells injected per spleen colony observed. Since no correction has been made for the possibility that some of the colony-forming cells might come to rest in organs other than the spleen, the actual number of these cells must make up well over 1 per cent of the cells injected in some of the suspensions. Many of the spontaneous leukæmias, however, must contain only a fraction as many colony formers.

The procedure described here thus provides a method for obtaining a quantitative assay for the fraction of cells in murine lymphomas capable of proliferation *in vivo*. While it is probably less sensitive than the method of limiting dilution used by Hewitt and Wilson[9], it is certainly much more rapid. Of most importance, however, is the fact that this method permits one to obtain much more information per animal than is possible by the quantal 'alive or dead' response scored by the former method. If the clonal origin of the colonies suggested by the present results is established by further investigations, the method will, in addition, provide a rapid method for obtaining clonal lines of lymphoma cells.

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Changes in Resistance to the Proliferation of Isotransplanted Gross Virus-induced Lymphoma Cells, as measured with a Spleen Colony Assay

Klein *et al.*[1] have shown that a state of relative resistance against the isotransplantation of Gross virus-induced lymphomas could be built up in *C3H* mice by treatment with cells from lymphomas originally induced by the same virus. Sera from such resistant mice were shown by Slettenmark and Klein[2] to be cytotoxic *in vitro* for the cells of many Gross virus-induced lymphomas. These findings suggested that lymphomas induced by Gross virus possess tumour-specific antigen, and furthermore that the same antigen is present on the cells of all or most lymphomas induced by this virus.

Examination of foreign antigens in lymphoma cells would be facilitated if a simple method were available for measuring resistance to the proliferation of transplanted lymphoma cells. In this communication, a method is described which appears to be capable of measuring such resistance. Using this method, we have confirmed the findings of Klein's group, and shown that virus extracts of lymphoma cells, as well as lymphoma cells themselves, when given to adult mice, cause a reduction in the proportion of transplanted lymphoma cells which proliferate in their isologous hosts. Furthermore, we have shown that when similar extracts are given to new-born mice, they cause an increase in the proportion of lymphoma cells which can proliferate when isotransplanted into these hosts later in life.

The method is based on the following: Bruce and van der Gaag (preceding communication) found that viable lymphoma cells, injected intravenously into normal, genetically compatible mice, proliferate in spleen and kidneys to form macroscopic colonies that can be counted. Using cells from spontaneous *AK* lymphomas injected into *AK* hosts, they showed that the number of colonies produced in the spleens was proportional to the number of cells injected. The ratio of the number of spleen colonies

observed to the number of cells injected thus serves as a measure of the fraction of cells in any suspension capable of proliferation after transplantation into isologous hosts. Any change in resistance against isotransplanted lymphoma cells should be reflected in a corresponding change in the fraction of cells capable of proliferation, as estimated by the spleen colony assay. If this were so, the spleen colony assay would provide a simple means of measuring the level of resistance against the proliferation of lymphoma cells under a wide variety of conditions.

To test this, we examined the effect of repeated sub-lethal doses of lymphoma cells, isotransplanted intraperitoneally in *C3H* hosts, on the number of colonies formed from a subsequent intravenous challenge of similar cells. The lymphoma cells were obtained from 3–4-month-old female *C3Hf/BiOci* mice injected as infants with Gross passage *A* virus. Cell suspensions were prepared, from enlarged thymuses and lymph nodes, in Krebs–Ringer-phosphate medium with added gelatin and antibiotics. The hosts were *C3Hf/BiOci* adult mice of both sexes, no more than 2 inbred generations removed from the donors of the lymphoma cells. Eleven adult mice, which had not succumbed after 4½ months to the intraperitoneal injection of 10^3 viable *C3H* lymphoma cells, were re-injected with 10^5 cells from another lymphoma, and again 3 months later with 10^4 cells from another lymphoma. Six days after the last injection, these animals were challenged intravenously with 5×10^6 viable (erythrocin-unstained) cells from still another lymphoma. Thirty-six uninjected control mice of the same age received the same challenge dose of cells from the same lymphoma. Twelve days later, the animals were killed, their spleens placed in Bouin's fluid overnight, after which the colonies in the spleens were scored.

The results (Fig. 1) showed that pretreatment of adult *C3H* mice with repeated sub-lethal doses of viable lymphoma cells from isologous donors caused a 7-fold

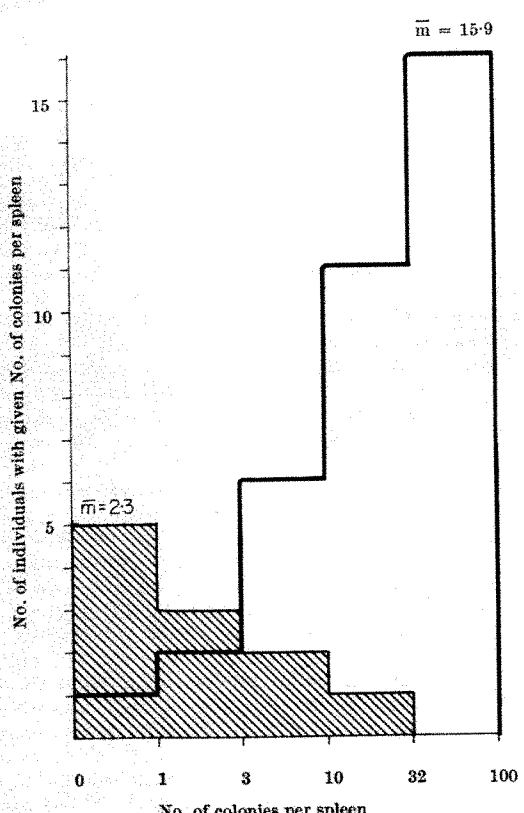


Fig. 1. Effect of pretreatment of *C3H* hosts with sub-lethal doses of isologous lymphoma cells on spleen colony-forming efficiency of *C3H* lymphoma cells. White, untreated control hosts; hatched, hosts pretreated with sub-lethal doses of lymphoma cells

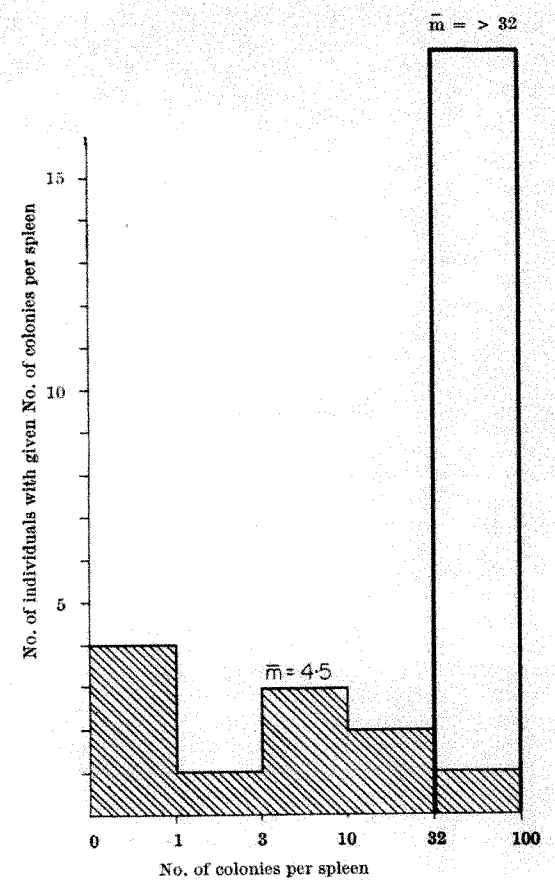


Fig. 2. Effect of pretreatment of *C3H* hosts with Gross virus extract on spleen colony-forming efficiency of *C3H* lymphoma cells. White, untreated control hosts; hatched, hosts pretreated with one dose of Gross virus extract 6 days before cell challenge

reduction in the number of colonies which formed in the spleen. Thus the system used here seems to be suitable for the quantitative investigation of resistance against the transplantation of lymphoma cells in isologous mice. The results are also consistent with those of Klein *et al.*[1] in that they demonstrate inhibition of lymphoma cell proliferation by, and cross-resistance among, the cells of different lymphomas induced by Gross virus.

Next, because Gross virus-induced lymphomas are known to carry the causative virus in infectious form, we examined the effect on the colony-forming efficiency of lymphoma cells of injecting isologous hosts with Gross virus extract before cell challenge. Virus extracts were prepared in the same way as those used for lymphoma induction[2]. Eleven *C3H* mice were given 0·2 ml. of extract intraperitoneally 6 days before challenge with lymphoma cells. Eighteen controls were uninjected before challenge. Both groups received 10^7 cells intravenously. The results (Fig. 2) show that pretreatment with a single intraperitoneal dose of virus extract 6 days before challenge caused a reduction in the number of colonies which formed in the spleen comparable with that seen after repeated injections of sub-lethal doses of lymphoma cells from isologous donors (Fig. 1).

The phenomenon of reduction in spleen colony number resulting from pretreatment with virus extract has been consistent in 9 out of 10 experiments with different lymphomas; the magnitude of the reduction in colony-forming efficiency, when extract was given 6 days before challenge, varied from 7- to 30-fold in different experiments. The effect on spleen colonies could also be detected even when virus extract was mixed with the challenge-cell inoculum. The action of virus extract was also observable on colony formation in the kidneys; the

number of kidney colonies in animals pretreated with virus extract was regularly lower than in controls.

Sachs[4] has shown, by standard methods, a similar inhibition of growth on transplantation of a Moloney virus-induced leukæmia cell line in isologous hosts pretreated with an extract of cultivated leukæmic cells containing the Moloney virus.

From these results, which show that a relative resistance to the iso-transplantation of lymphoma cells can be readily built up in *C3H* hosts by Gross virus-induced lymphoma cells or extracts of these cells, it is reasonable to assume that Gross virus extract is able to stimulate a response in the host similar to that induced by injection of lymphoma cells. This could occur, for example, if virus in the extract caused the transformation of cells in the adult host so that the cells developed a foreign antigen, the host's immune response directed against this antigen would also destroy any transplanted lymphoma cells that have the same antigen. (This hypothesis would be like that proposed by Habel[5] and by Sjögren[6] to account for the fact that infection of adult mice or hamsters with polyoma virus causes these animals to resist the growth of polyoma virus-induced tumour cells isotransplanted into them.) Alternatively, the virus particles themselves (and/or material in the extract) could share the same foreign antigen(s) with the lymphoma cells. Pretreatment with virus extract containing the antigen would stimulate the immune response of the host, which would then also destroy the challenge lymphoma cells. This could be the case, for example, if the new antigen induced by the virus in the course of lymphoma induction were at the surface of the cell. Since the Gross virus is believed to bud from the cell surface, it could carry away the antigen with it on leaving the cell. The observed cross-resistance among different lymphomas, as well as the resistance induced against different lymphomas by the same virus extracts, could thus be explained by the fact that the virus considered responsible for inducing the foreign antigen(s) (and the lymphomas) continues to multiply in the lymphoma cells.

If the resistance built up in *C3H* adult mice by exposure either to cells or to virus extract is indeed due to an antigen foreign to the host, it should be possible to induce immunological tolerance to the antigen in new-born hosts. To test this, a group of *C3H* mice was given 0·2 ml. of Gross virus extract intraperitoneally at 21 days of age, 6 days before cell challenge; a second group was left untreated; a third group was given either 1 dose of 0·08 ml. of Gross virus extract intraperitoneally within 24 h of birth or 1 dose at birth and 1 dose each week thereafter for 3 weeks (0·1, 0·1 and 0·2 ml.). All 3 groups were challenged at 4 weeks of age with 10^6 cells from one lymphoma. In addition, a group of controls and a group that received virus extract at birth, or at birth and once weekly for 3 weeks, were also given, intravenously, 3×10^4 cells from the same lymphoma. Finally, a control group was included that received virus extract at birth but no cell challenge. The results were identical in mice given 1 or 4 doses of virus extract from birth, and so these results have been pooled.

Table 1 shows again the reduction in number of colonies produced by lymphoma cells in mice that received virus extract for the first time at 6 days before cell challenge. Table 1 also illustrates rather strikingly that when virus extract was injected into new-borns which were then challenged later in life, a considerably larger number of colonies was produced from the same inoculum of lymphoma cells in these animals than in controls that had not received virus extract before the cell challenge. Not only did the injection of extract into new-borns fail to induce immunity to the cells as it did when injected into older animals, but the effect of the extract in the new-born, which persisted until the time of challenge 4 weeks later, was instead to interfere with the resistance that the normal host was apparently able to exert on the proliferation of the challenge lymphoma cells. This behaviour has now been observed in 5 out of 5 experiments, the magnitude of the increase in colony-forming efficiency ranging from 2- to 30-fold. It is as if exposure of the new-born to Gross virus extract brought about a state of actively acquired immunological tolerance to foreign antigen(s) on the lymphoma cells originally induced by Gross virus. This situation may be comparable with that described by Rubin[7] in which Rous sarcoma cells grow progressively in chickens rendered tolerant to viral antigen but regress in those that are immunologically competent.

Whatever the precise mechanisms involved, the work recorded here shows that when either Gross virus-induced lymphoma cells or virus extracts of these cells are given to adult mice which are then challenged with lymphoma cells from isologous donors, the hosts respond by causing *a reduction* in the fraction of the transplanted cells that are capable of proliferation. When, however, Gross virus extracts are given to new-born mice which are challenged with isologous lymphoma cells later in life, they respond by causing *an increase* in the fraction of these cells that are capable of proliferating in them.

Table 1. Effect of Injecting Gross Virus Extract into 3-week-old and < 1-day-old *C3H* Mice on Number of Spleen Colonies after Intravenous Challenge at 4 Weeks of Age with Lymphoma Cells from Isologous Donors

Experiment			Results					
Treatment	No. of cells injected at 4 weeks	Total No. of hosts	No. of hosts with given No. of colonies in spleen					Mean No. of colonies per spleen
			0	1–3	4–9	10–31	32+	
Virus extract at 3 weeks of age	1×10^6	9	8	1				0·11
Uninjected controls	1×10^6	10	3	2	1	1	3	3·3
Virus extract on day of birth	1×10^6	12					12	>32
Uninjected controls	3×10^4	10	8	2				0·2
Virus extract on day of birth	3×10^4	12	3	4	5			3·0
Virus extract on day of birth	0	6	6					0

It should be pointed out that great variations were found in the colony-forming efficiencies of individual lymphomas independently induced by Gross virus in different *C3H* mice and assayed under apparently identical conditions. The number of cells required to produce an average of 1 colony per spleen varied over a 10^4-fold range; most often it was around 6×10^5 cells. This fact must at present be left unexplained.

In earlier work[8,9], the suggestion was made that induction of lymphoma in *C3H* mice by Gross virus requires the presence of relatively undifferentiated cells proliferating and differentiating in the cortex of the thymus; the present observations would indicate that the animal might also have to become 'tolerant' to the foreign antigen(s) on the induced lymphoma cells. This also raises the question as to whether the cells of 'spontaneous' neoplasms possess foreign, tumour-specific antigens. In a number of experiments using systems *in vivo* that were capable of detecting tumour-specific antigens in a variety of induced neoplasms, no evidence was found for the presence of such antigens in 'spontaneous' tumours[10–12]. But the methods used in these experiments have depended on an immune reponse of the host for detecting tumour-specific antigens. If there were any impairment in the responsiveness of the host to such antigens, then it would be impossible to detect tumour-specific antigens by these means. Therefore in situations where tumour cells behave as if they do not possess foreign antigens, the possibility should be considered that such antigens may be present but not detected because the hosts may have become tolerant to antigens

on their tumour cells as a result of some event that preceded the maturation of their immune mechanisms.

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A Stochastic Model of Carcinogenesis compared with Mouse Skin Tumour Enhancement after Gastric Instillation of 9,10-Dimethyl-1,2-Benzanthracene with Subsequent Local Continuous Exposure to 'Tween 40'

A FAIRLY good agreement has been found between a stochastic model[1,2,5], and a skin tumour production experiment performed by Berenblum and Shubik[3]. In this experiment development of the skin tumour after a single local exposure of mouse skin to graded concentrations of 9,10-dimethyl-1,2-benzanthracene (DMBA) with subsequent twice-weekly exposure of the same skin area to croton oil was investigated. Since then it has been shown that systemic (oral, intravenous, intraperitoneal) initiation and subsequent local tumour enhancement provokes skin tumours[4] (for further references, see ref. 7), and a new and physicochemically well-defined group of tumour-enhancers, the dipole-type compounds, has been established[17] (for a comprehensive review, see ref. 8).

The essential importance of the so-called frequent-application technique (that is, maintaining a continuous contact between the target cells and the tumour-enhancing compound) has also been demonstrated[6]. Furthermore, it has been shown that the responses of mouse skin to treatment with carcinogens and dipole-type tumour-enhancers fundamentally differ from each other[9–12,18], at least in a skin-tumour-resistant mouse strain[13–16] (that is, a mouse strain responding by formation of no or very few skin tumours to treatment with dipole-type tumour enhancers or to mechanical wounding of the skin, unless initiated with a carcinogen). These improvements of the two-stage technique could be supposed to provide a greater consistency with the premises of the stochastic model[1,2,5], namely, a uniformly randomized distribution of the carcinogen and a constant concentration of the tumour enhancer together with the absence of an inherent tumour-susceptibility of the experimental animals.

It thus seemed interesting to compare the stochastic model with experimental results recently obtained[7] with gastric instillation of different doses of DMBA to mice and subsequent continuous local exposure of the skin to

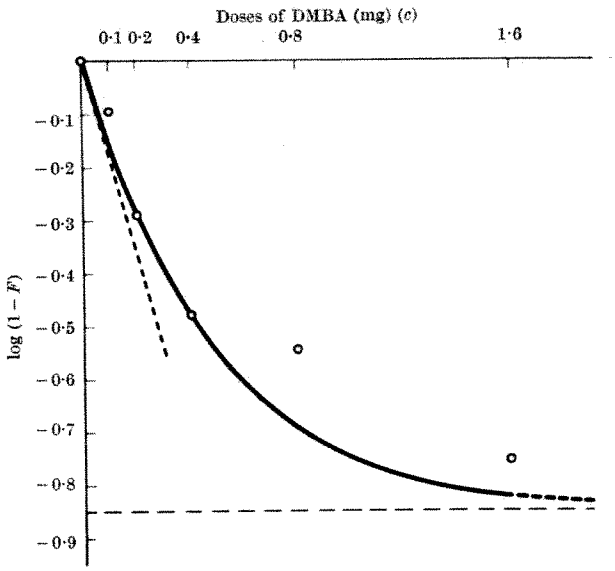


Fig. 1

'Tween 40' (polyoxyethylene sorbitan monopalmitate), a dipole-type tumour enhancer.

In each mouse of 5 groups of 50 female albino animals of the skin-tumour-resistant *RA* strain 1·6, 0·8, 0·4, 0·2, and 0·1 mg, respectively, of DMBA in 0·15 ml. of 'Carbowax 400' (polyethylene glycol 400) were injected in single intragastric dose[7]. After an interval of 30 days the hair on the back of the mice was cut, and local exposure 6 times a week to undiluted 'Tween 40' started for 38 weeks. The numbers of survivors, the numbers of tumour-bearers (n), and the numbers of tumours per tumour-bearer (M) were recorded at weekly intervals, as well as the induction time (t) for each tumour (that is, the time-interval between commencement of exposure to the tumour enhancer and the first appearance of the skin tumour). The tumour incidence in each experiment was calculated as the ratio between the number of tumour-bearers (n) and the number (n_0) of animals alive when the first tumour appeared in that experiment. From the expression $F = n/n_0$ obtained at the end of the experiments the quantities $\log(1 - F)$ were calculated and plotted versus the dose c of the carcinogen γ (Fig. 1). According to the stochastic model of carcinogenesis[2]:

$$\log(1 - \bar{F}) = -\beta(1 - \exp[-\gamma c])$$

where $\bar{F}$ is the theoretical value, that is, the mean tumour incidence, c the dose of the carcinogen, and β and γ two parameters to be estimated from the experiments. These parameters can be estimated graphically from the experimental values plotted in Fig. 1 as described in ref. 2, and yields the numerical values $\beta = 0{\cdot}85$ and $\gamma = 2{\cdot}12$ mg^{-1}. From these results can also be calculated the theoretical value $\bar{t}$ of the induction time t as described in ref. 2. The result is shown in Fig. 2 together with the experi-

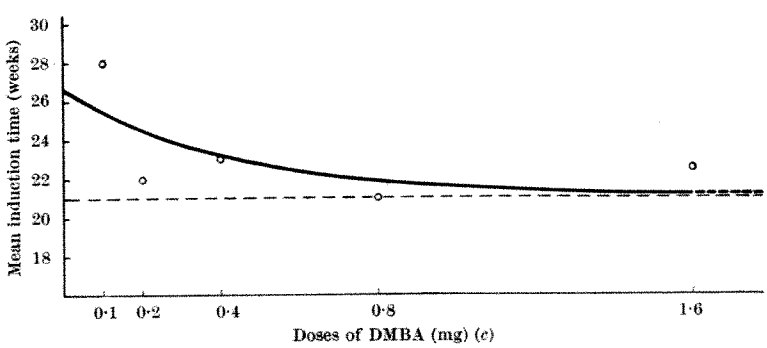


Fig. 2

mentally observed average induction times versus doses c.

As seen from the results in Figs. 1 and 2, the experimental and theoretical values of the incidences and of the induction times are in good agreement with each other. However, a closer analysis shows that the experimental values ($\bar{M}$) of the mean numbers of tumours per animal deviate somewhat from those expected from the stochastic model, calculated as described in ref. 2; but as discussed in refs. 1 and 2 there are several sources of error in the observation of M such as, for example, the fact that some tumours may have disappeared before being observable, etc. Also the individual tumours on the same animal may not have arisen independently of each other as assumed in the stochastic model. On the whole we think, therefore, that our results give new evidence in support of the somatic mutation hypothesis of carcinogenesis in its direct one-hit form discussed in ref. 1, as well as of the view previously expressed that carcinogens primarily cause a delayed maturation of the epidermal cells and that subsequent exposure to a dipole-type tumour enhancer, despite increase in the rate of mitosis, will in these circumstances not lead to the formation of an adequate amount of normally differentiating cells[7,10,18]. The ultimate result is the formation of a focal epidermal tumour.

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Skin Reaction in Children and Adults with Central Nervous System Disease after Intracutaneous Administration of Myelin Preparations derived from Human Brain

Much of the available experimental evidence[1,2] suggests that pathological demyelination in the human brain might occur as a result of a delayed-type hypersensitivity reaction against myelin[3,4], although a significant role of circulating antibody cannot be altogether ruled out[5,6]. Previously[7,8] we reported that we had been able to elicit a skin reaction against proteolipid A derived from human brain in 21 cases of multiple sclerosis and two patients with clinically diagnosed neuroses which was in agreement with the results obtained by other investigators[9,10] who had tested skin reactivity against unpurified human myelin. While the pathognomonic significance of the skin reaction thus remains doubtful we endeavoured to examine the question whether a true hypersensitivity reaction was present or not by comparing a large number of adult patients with a group of children suffering from a variety of central nervous system disorders as to their ability to react against myelin.

Table 1

Diagnosis	No. of cases	Onset of reaction (h)	Min./max. diameter of reaction (cm)
Group *A* (adult patients)			
Multiple sclerosis	22	1–7	0·5–2·1
Transverse myelitis	1	3	1·0
Neuritis	6	2–13	0·5–2·1
Pseudo bulbar paralysis	1	9	1·0–1·8
Amyotrophic lateral sclerosis	3	2–3	0·7–1·9
Syringomyelia	2	5–6	1·2–2·0
Gliosis of cervical cord	2	4	0·8–2·0
Meningioma of cervical cord	1	9	1·0–1·8
Friedreich's ataxia	2	3	0·4–1·7
Extrapyramidal motor system disease	2	1–3	1·0–1·4
Combined system disease	3	1–5	1·8–2·8
Muscular dystrophy	2	5–6	1·6–2·0
Secondary muscular atrophy	1	9	1·6–1·8
Delirium tremens	1	2	1·0
Neuroses	3	2–6	0·6–1·7
Total	52		
Group *B* (adult patients treated with steroids or salicylic acid)			
Transverse myelitis	1	negative	
Multiple sclerosis	1	1	0·5
Sydenham's chorea	1	3	0·3
Total	3		
Group *C* (children)			
Neurosis	2	negative	
Psychomotor seizures	1	negative	
Post-encephalitic syndrome	1	negative	
Sydenham's chorea	1	negative	
Meningoencephalitis	1	negative	
Behavioural disturbance	1	negative	
Psychomotor seizures	1	2	1·2
Inflammatory central nervous system disease	1	negative	
Extrapyramidal motor syndrome	1	negative	
Purulent meningitis	1	negative	
Post-commotional syndrome	1	negative	
Total	12		

Two preparations of myelin were used. The first one was obtained by extracting human white matter with chloroform/methanol following the procedure of Folch and Lees[11]. The second one was produced by ultracentrifugation using the method of Laatsch *et al.*[12]. These preparations were administered in two different concentrations, that is, 0·005 mg/ml. and 0·05 mg/ml. In some children, one half-strength of the first concentration = 0·0025 mg/ml. was used. The preparations were suspended in normal sterile saline and injected intracutaneously in a final volume of 0·2 ml. into the flexor surface of the forearm. An equal volume of saline was given simultaneously into the opposite forearm. In those cases where both myelin preparations were tested at the same time, the second test dose was injected at a distance from the site of injection of saline. At varying time intervals after injection a biopsy from the reaction site was taken under local anæsthesia.

As may be seen from Table 1 (group A) positive skin reactions were observed in 22 patients with multiple sclerosis, 1 with transverse myelitis, 6 with peripheral neuritis or polyneuritis respectively (which was verified at autopsy in one instance), 1 with bulbar paralysis, 3 with amyotrophic lateral sclerosis, 2 with syringomyelia, 2 with gliosis of the cervical spinal cord, 1 with a meningioma of the cervical portion of the spinal cord, 2 cases of Friedreich's ataxia, 2 patients with extrapyramidal motor system disease, 3 cases of combined system disease, 2 cases of muscular dystrophy, 1 case of neural (secondary) muscular atrophy and one patient with delirium tremens. Furthermore, 3 patients without organic brain disease exhibited positive skin reactions. The earliest onset of a reaction occurred after 1 h; but as a rule reddening of the skin was not seen until 2–3 h after injection. The majority of the reactions lasted up to 48 h, some longer. The diameter of the reaction site never exceeded 3·0 cm.

The second (B) group of patients consisted of one case of transverse myelitis, one case of multiple sclerosis and one case of Sydenham's chorea, all of which were under treatment with corticosteroids or salicylic acid respec-

tively. The first patient failed to show a reaction, while the remaining two reacted in the same manner like those listed in group *A*.

In group *C*, 12 children aged between 5 and 14 years were tested. This group was composed of 2 neurotic patients, 2 cases with psychomotor seizures, 1 patient with a post-encephalitic syndrome, 1 case of Sydenham's chorea, 1 patient with meningoencephalitis, 1 case of inflammatory central nervous system disease which so far has resisted diagnostic clarification, 1 patient with extrapyramidal motor system disease, 1 patient with healed purulent meningitis, 1 patient with a post-commotional syndrome and one case undergoing treatment for behavioural disturbances. Except for one of the two cases with psychomotor seizures, no positive skin reactions were observed.

In none of the three tested groups were any discernible differences noted between the two myelin preparations with respect to their ability to bring about the skin reaction. Histologically, the reaction was characterized by widespread œdema of the upper dermis, perivascular accumulation of round cells and granulocytes around the capillaries of the entire dermis and scanty infiltration of the dermis with eosinophilic granulocytes. Vascular distension and perivascular œdema persisted longest while the cellular reaction usually had disappeared after 40 h. The histological picture strongly resembled that obtained in disseminated lupus erythematosus after intracutaneous injection of vascular antigens[13].

The fact that with one exception no positive reaction could be observed in children suggests the possibility that a gradual, physiological demyelination process begins in man at puberty which results in the development of cellular hypersensitivity. While this process must be considered normal it could be imagined that in certain circumstances a modification occurs which would start off pathological demyelination. In order to test this hypothesis, persons without neurological disorders and belonging to younger age-groups are at present being examined.

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IMMUNOLOGY

Use of Oxidized Proteins in the Examination of Immunochemical Specificity in Delayed Hypersensitivity to Hapten – Protein Conjugates

Recent investigations of delayed hypersensitivity to hapten–protein conjugates have established clearly the importance of the carrier protein as one of the determinants of specificity[1–3]. Efforts to define further such determinants are hampered to some extent by the heterogeneity of the response to immunization with a hapten–protein conjugate in which many possible antigenic determinants may play a part. In previous work[4], it was shown that oxidation or reduction and alkylation of all disulphide bonds in several proteins (resulting in complete loss of tertiary structure with retention of primary structure) produced the most drastic reduction in the ability of these proteins to precipitate rabbit antibody and to sensitize guinea pigs to delayed hypersensitivity to the native proteins. It was thought that the use of such open chains to serve as hapten carrier might provide valuable information about the relative contributions of folded structure and amino-acid sequence to the specificity involved in delayed hypersensitivity.

This communication describes the results obtained with conjugates prepared by coupling excess diazotized arsanilic acid (ars) and *p*-aminobenzoic acid (benz) to guinea pig serum albumin (GSA) and oxidized guinea pig serum albumin (OxGSA) in cold alkaline solution. The coloured conjugates were dialysed exhaustively and final concentrations determined on the basis of original protein used, assuming no net loss during the chemical reactions.

Guinea pigs were immunized by injection in the 4 footpads of 1 μg N of the conjugate contained in 0·1 ml. of complete Freund's adjuvant (8·5 parts 'Arlacel', 1·5 parts mineral oil, 5 mg/ml. *Mycobacterium tuberculosis*) and skin tested two weeks later with 1 μg N of the appropriate test antigen in 0·1 ml. volume. The average diameter of induration and erythema was measured at 24 h. Desensitization was accomplished by injection of 1 mg of protein intraperitoneally in the morning and 1 mg protein intravenously in the afternoon, followed immediately by the appropriate skin tests.

Table 1. Effect of Changes in Carrier Protein on Ability to Elicit Delayed Reactions in Guinea Pigs before and after Desensitization

Immunized with	No. of guinea pigs	Delayed reactions to skin tests* with 1 μg N: Ars–GSA	Ars–Ox-GSA	Ars–RNase	Ars–Ox-RNase	Benz–GSA
Ars–GSA	6	18	12	9	6	11
	Same after desensitization with 2 mg benz–GSA	17	13	9	8	3
		Ars–GSA	Ars–BSA	Ars–Ox-BSA	Benz–GSA	
Ars–GSA	6	19	11	8	13	
	Same after desensitization with 2 mg benz–GSA	17	12	11	5	

* Induration and erythema in mm

Table 1 presents the results of an experiment to define the role of the carrier protein. When 6 guinea pigs immunized with ars–GSA were skin-tested with the same hapten conjugated to OxGSA, a substantial delayed reaction was noted. This reaction, while less than that for the homologous conjugate (ars–GSA), was in turn greater than that obtained with the conjugates of the heterologous protein ribonuclease (ars–RNase) or its oxidized form (ars–Ox–RNase). In addition, a moderately strong reaction was obtained to a conjugate of GSA prepared with a non-cross-reacting hapten, the azobenzoate group (benz–GSA). Desensitization with this material resulted in a considerable reduction in the delayed skin reaction to the homologous carrier–heterologous hapten conjugate (benz–GSA), but virtually no change with the homologous hapten and either homologous or heterologous carriers (ars–GSA, ars–RNase). A second group of guinea pigs immunized with ars–GSA and tested with the same hapten on an immunologically related carrier, bovine serum albumin (BSA), gave good reactions with conjugates of both the native and oxidized forms. Once again, desensitization with benz–GSA resulted in considerable loss of reactivity attributable to the carrier; but the reactions to the homologous hapten–heterologous carrier conjugates remained unchanged. These results suggested strongly that

one component of the large reaction observed with the immunizing antigen was attributable to a specificity revealed or produced from the carrier protein by reaction with the hapten, since the carrier (GSA) itself is non-antigenic in the guinea pig. When this contribution to the net delayed reaction was removed by desensitization with the carrier protein which had been altered in a similar fashion by conjugation with a non-cross reacting hapten, there was some slight decrease in skin reaction to the homologous material, but virtually no change in reactions to the homologous hapten–heterologous carrier combination.

To define further the relative contributions of the carrier protein and the hapten to the response resulting from immunization with conjugates, four groups of guinea pigs were immunized with 1 μg N quantities of GSA or OxGSA conjugated with either arsanilic acid or *p*-aminobenzoic acid. The results of skin tests performed with all four conjugates shown in Table 2 demonstrate again that in each case the largest skin reaction was obtained with the antigen used for immunization. When the carrier protein was altered by being oxidized to its open-chain form, conjugates prepared with it were still able to elicit a moderately large delayed reaction. Once again, a substantial reaction was obtained with the heterologous hapten on the homologous carrier. These observations strongly support the concept that the larger reaction seen with the immunizing antigen was attributable to participation by antigenic determinants revealed or produced in the protein by the chemical process of conjugation with a diazonium salt. Further evidence that this might indeed be so was seen in skin tests following desensitization with the heterologous hapten–homologous protein carrier conjugate. The almost total abolition of the skin reaction to the homologous carrier–heterologous hapten conjugate was followed in most cases by a slight reduction in skin reactions to the immunizing antigen to the point where oxidized and native forms of the carrier were virtually identical in either the azobenzoate or azobenzenearsonate systems.

Table 2. Effect of Changes in Carrier Protein and Hapten on Ability to Elicit Delayed Reactions in Guinea Pigs before and after Desensitization

Immunized with	No. of guinea pigs	Skin tests* with 1 μg N Ars–GSA	Ars–Ox-GSA	Benz–GSA	Benz–Ox-GSA
Ars–GSA	8	15	10	8	
	5 after desensitization with 2 mg benz–GSA	13	10	3	2
Ars–OxGSA	7	10	13		10
	3 after desensitization with 2 mg benz–GSA	10	10	0	3
Benz–GSA	6	9		14	12
	4 after desensitization with 2 mg ars–GSA	2	0	16	15
Benz–OxGSA	6		10	11	14
	4 after desensitization with 2 mg ars–OxGSA	2	0	9	10

* Induration and erythema in mm

One logical inference to be drawn from these observations is that a large part of the specificity involved in the delayed reaction to the hapten–protein conjugate is a consequence of the amino-acid sequence of the protein carrier, since these were common to both the oxidized and native proteins, while tertiary structure, so important to reactivity of antigens with antibody, played a secondary part. To clarify further the extent of contribution by the amino-acid sequence of the carrier to the immunological specificity of the conjugate, an experiment was performed in which guinea pigs immunized with ars–GSA or benz–GSA were tested with conjugates prepared from a wide variety of proteins in which the likelihood of common amino-acid sequences recurring seemed vanishingly small.

The results of such an experiment, given in Table 3, demonstrate what appears to be a remarkable lack of carrier specificity in evoking a delayed reaction with conjugates prepared from arsanilic acid and are in striking contrast to results obtained with conjugates of *p*-aminobenzoic acid, as has been reported previously with other haptens[3]. Thus, while the largest reactions were always seen with the immunizing antigen, significant positive reactions were seen with conjugates prepared from proteins as immunologically diverse as gelatin, ribonuclease, insulin, and elastoidin.

Table 3. Skin Reactions to Conjugates prepared with Heterologous Proteins in Guinea Pigs immunized with Ars–GSA or Benz–GSA

Immunized with	No. of guinea pigs	Skin tests* with 1 μg N Ars–GSA	Ars–RNase	Ars–gelatin	Ars–insulin	Ars–elastoidin	Ars–poly tyrosine
Ars–GSA	6	17	8				
Ars–GSA	4			11	13	13	10
		Benz–GSA		Benz-gelatin	Benz–insulin		Benz–poly-tyrosine
Benz–GSA	5	17		0		0	0

*Induration and erythema in mm

Furthermore, since conjugates prepared from carriers containing little tyrosine (gelatin) or much tyrosine (poly-tyrosine) were equivalent in eliciting reactions, it seemed most probable that a large measure of specificity is conferred by the azobenzenearsonate group alone. This is in striking contrast to results obtained by other workers[1,3] and by myself with similar haptens, such as the azobenzoate group. In the latter case, conjugates prepared with other proteins were unable to elicit positive reactions in guinea pigs sensitized with benz–GSA. Because of this striking difference between results obtained with the azobenzenearsonate and the azobenzoate radicals, the whole question of the part that size of hapten plays in determining specificity in delayed hypersensitivity is thrown into bold relief and is at present under active investigation.

A second point of interest concerns the observation that conjugates prepared with oxidized GSA are essentially equivalent to those prepared with native GSA in eliciting delayed reactions in guinea pigs which have been desensitized to those determinants arising from the GSA through the effects of chemical reactions. The implications of this observation are that the contributions made by the carrier protein to the antigenic determinant, of which the hapten is a part, are attributable to its primary rather than tertiary structure. To explain the non-reactivity of unrelated carriers conjugated with the smaller haptens, it only becomes necessary to invoke the involvement of a sufficient number of amino-acid residues to provide unique sequences available only in the homologous protein.

In the light of experiments and reports so far given, it is now possible to think of the heterogeneity of response to immunization with hapten–protein conjugates as involving three main categories of antigenic determinants: (1) those produced or revealed in the protein carrier by chemical manipulation; (2) those consisting of the hapten plus the amino-acids immediately adjoining its site of attachment; (3) perhaps in special cases (as with the azobenzenearsonate group) those consisting largely of the hapten alone. Further work should show whether the degree of complementary form involved is in all cases equivalent with different contributions being made by various haptens and amino-acids in an additive fashion until some minimal value necessary for the delayed reaction to occur is achieved in any particular hapten-carrier system.

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Duration of Antibody Response to Soluble Antigen

ANTIBODY production after immunization with particulate antigens is known to persist over long periods of time. By contrast, immunization with soluble antigens in the absence of adjuvants is generally thought to give responses of relatively brief duration. Recently, it has been reported that antibody production continued for several hundred days in rabbits given one or several intravenous injections of a number of soluble antigens[1]. Antibody produced late in the course of the response gave negative ring tests, failed to sensitize the guinea pig intestine, and could be detected only by a passive hæmagglutination technique. This communication is concerned with similar observations on the response of chickens to intravenous injections of bovine serum albumin (BSA). One major finding of this study is the persistence of precipitating antibody up to 313 days after injection.

Antisera were obtained at various times after two spaced (50 days) intravenous injections of BSA (40 mg/kg body-weight). Antibody activity was measured both by precipitin tests and by Farr's technique[2], which depends on the differential solubility of free ^{131}I-BSA and ^{131}I-BSA bound to antibody in half-saturated ammonium sulphate. The dilution effect, 'avidity', was determined by comparison of the antigen-binding capacity of serum at two concentrations of BSA. The antigen-binding capacity was determined as described by Farr: sera were diluted until 33 ± 10 per cent of a constant amount of antigen was precipitated after addition of ammonium sulphate. Correction factors were applied to normalize all values to 33 per cent binding. The following formula was used to calculate the antigen-binding capacity: (μg BSA nitrogen added/ml.) × (0·33) × (reciprocal of dilution binding 0·33 of added antigen).

The antigen-binding capacities of the three animals tested were remarkably constant over the 226-day period considered (Table 1), the only difference noted being an increase in 'avidity' between 87 and 241 days after immunization. Because all samples were tested at the same time, this avidity difference may represent an effect of storage. According to Farr[2], complexes formed at the 0·33 reference point with high avidity sera have an overall molar ratio of one antibody molecule to two antigen molecules. The weight ratio of BSA (mol. wt. 70,000) to antibody (160,000) in such complexes would be essentially one. Thus, the antigen-binding capacity of high-avidity sera approximates the actual antibody content, provided one is testing bivalent antibody. The precipitating antibody concentrations expected in the sera under investigation then would be 4–10 μg nitrogen/ml., clearly less than the resolving power of most quantitative precipitin methods.

Table 1. ANTIGEN-BINDING CAPACITY (ABC)* AT VARIOUS TIMES AFTER IMMUNIZATION

Bird No.	Days after second injection			
	87	241	297	313
28	0·93/1·6 (58)	1·4/1·8 (78)	1·5/2·0 (75)	1·2/1·7 (71)
29	1·4/2·4 (58)	1·2/1·7 (71)	0·90/1·3 (69)	1·2/1·6 (75)
30	0·54/1·1 (49)	0·72/1·1 (65)	0·93/1·4 (66)	0·74/1·1 (67)

* The figures given are: ABC at 0·16 μg BSA nitrogen per ml./ABC at 1·6 μg BSA per ml. (percentage 'avidity'). ABC values calculated for 0·25 ml. serum.

Because of the contention[1] that late antibody is non-precipitating, experiments were carried out to test rigorously for the presence of precipitating antibody. Mixtures of serum and ^{131}I-BSA of varying concentrations were made in both 0·9 and 8 per cent sodium chloride. The mixtures were incubated at 37° C for 6 h and then at 5° C for several days. The radioactivity of each tube was determined in a well-type scintillation counter both before and after centrifugation and removal of the supernatant fluid. In this manner, it was possible to detect significant precipitation of antigen in the absence of any readily visible precipitate. Control tubes containing antibody to a non-related protein retained less than 2 per cent of the added antigen, even in the absence of washing.

In all samples tested, complete antigen precipitation occurred over a wide range of concentrations (Fig. 1) and, significantly, at high enough concentrations to account completely for the antigen-binding capacity data. Because this method allows fairly accurate delineation of the region of slight antigen excess, it is also possible to estimate antibody content based on known equivalence ratios of antibody to antigen. Such estimates of antibody content were generally several times higher than the estimates based on the antigen-binding capacity. This is not entirely unexpected because the avidities of the sera were less than 100 per cent, that is, higher antigen-binding capacity values would be expected if higher concentrations of antigen were used. These results show that the late antibody response of chickens consists mainly of precipitating antibody. Because of evidence[3] indicating that non-precipitating antibody is not responsible for passive hæmagglutination reactions, it is expected that the use of more sensitive methods also would reveal the presence of precipitins in the late response rabbit antisera[1].

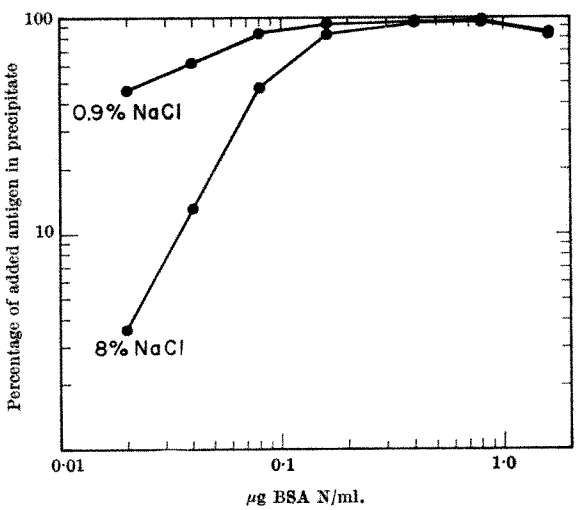


Fig. 1. Bird 29, 297 days after injection. Approximate equivalence concentration, 0·8 μg nitrogen/ml. Note marked inhibition by 8 per cent sodium chloride in antibody excess region

Although not directly related to the main point of this communication, the occurrence of a marked inhibition zone in extreme antibody excess deserves comment. As shown in Fig. 1, inhibition of antigen precipitation was greatest in the presence of 8 per cent sodium chloride. This is surprising, because it is well established that maximum precipitation occurs at this salt concentration with avian antisera. By adding ammonium sulphate to one-half saturation, all the antigen could be precipitated from the supernatants in this region. Therefore, the primary interaction between antigen and antibody was not affected.

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BIOLOGY

Electric Receptors in a Non-electric Fish (*Clarias*)

It is now well established that certain fish can detect objects electrically. These fish have weak electric organs, and set up an electric field in the surrounding water. A nearby object distorts this field, and the distortions are detected by the fish. The electric receptors are sense organs communicating with the water by means of jelly-filled canals.

In an early examination[1] of this 'electric sense' we suggested that the incoming electrical signals were 'smoothed' at the electric receptors before being coded into impulses in the afferent nerves. This hypothesis was tested in a series of experiments[2] in which a *Gymnarchus niloticus* was trained to respond to an applied electric field. The hypothesis was fully confirmed; the smoothing time-constant was found to be about 25 msec. It was further found that the fish was unexpectedly sensitive to trains of pulses of low-repetition frequency; this suggested that adaptation of the receptors was taking place with a time constant of some tens of milliseconds. Each successive pulse of the train would then give rise to a separate sensory signal. If the time taken in the central nervous system to process the sensory data and release the response (the 'perception time') were sufficiently long, several of these separate signals might be centrally summated to form a larger stimulus.

All the species of the two families of fish examined so far (Mormyridae, Gymnotidae) possess electric organs and specialized electric receptors derived from the lateralis system. On evolutionary grounds there is reason to suppose that some non-electric fish may have electric receptors[3], and that knowledge of their properties may help towards an understanding of the mechanism of electric receptors in general[4].

For our experiments an African species of *Clarias*—belonging to the very large catfish family (Siluridae)—was chosen for the following reasons: (*a*) One species of this family is known to be electric—the strong electric *Malapterurus*; no connecting link to non-electric catfish has been established through a weak electric species. (*b*) A number of members of this family (*Clarias*, *Saccobranchus*, *Malapterurus*, *Amiurus*, *Silurus*, etc.) were found to respond to a moving magnet. (*c*) Freshwater catfish possess 'small pit organs' structurally reminiscent of 'mormyromasts'; one marine species has ampullæ of Lorenzini, receptors known to be sensitive to electric currents in the Elasmobranchs[5–7]. (*d*) In life habits and ecology many catfish resemble the mormyrids and gymnotids.

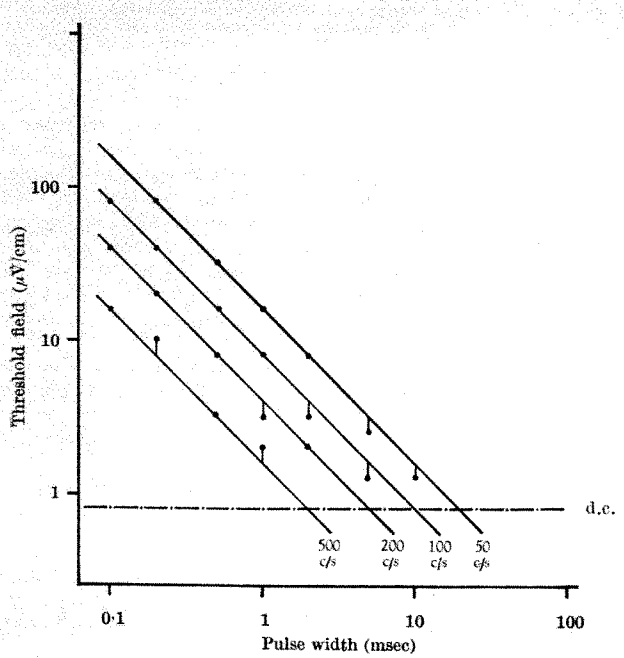


Fig. 1. Threshold field for the 'standard response' by *Clarias* to trains of pulses from 50 c/s to 500 c/s, and also to d.c.

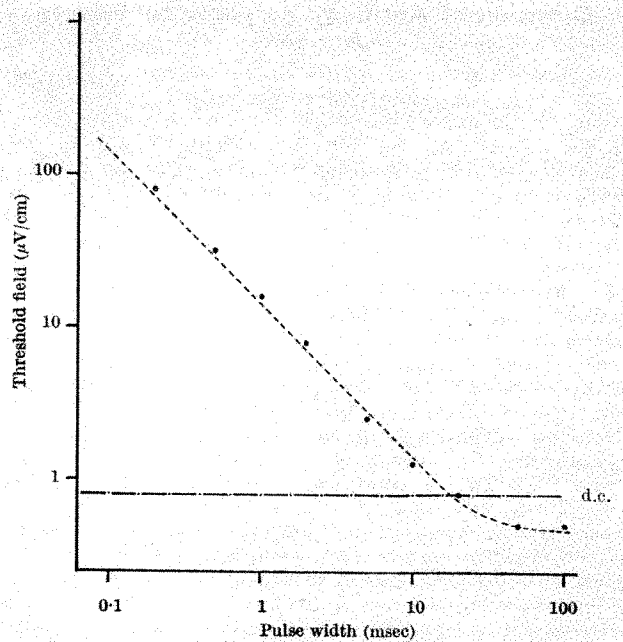


Fig. 2. Threshold field for single pulses

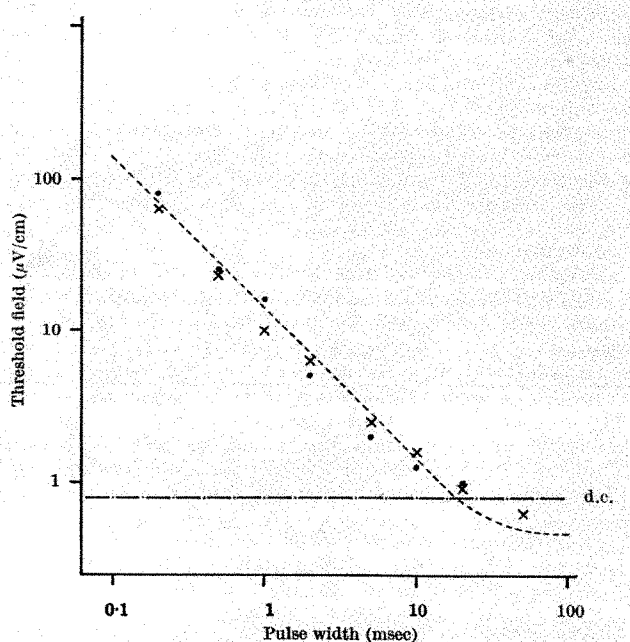


Fig. 3. Threshold field for low-frequency pulse trains. ●, 20 c/s; ×, 10 c/s; – – –, single pulses

The experimental procedure followed that used for *Gymnarchus*[2]: a specimen of *Clarias* (*anguilloides*?) was confined within a tank fitted with two electrodes between which an electric field could be established. The input to the electrodes was of controllable amplitude, and could be either d.c., single pulses or trains of pulses. The fish was trained to give 'a standard response' when the electric field was applied; the threshold field required to elicit this response was measured for the various types of stimulus.

The results for pulse trains of relatively high frequency, and for d.c., are shown in Fig. 1. As with *Gymnarchus*, the threshold is determined only by the mean value (or 'd.c.

content') of the field. Smoothing of the electrical signal is clearly taking place; from the results with single pulses (Fig. 2) it is possible to infer[2] that the time-constant is about 20 msec. The fish is more sensitive to wide single pulses than to d.c.; this suggests that a sensory signal is initiated both by the beginning and the end of the pulse. Such an effect has been demonstrated electrophysiologically in gymnotids[8].

The sensitivity of *Clarias* to low-frequency pulse trains is shown in Fig. 3, and differs little from its sensitivity to the corresponding single pulses. Apparently the perception time is sufficiently short that the fish responds on the first pulse of the train; if adaptation is taking place, it must have a time-course rather longer than the perception time. With *Clarias*, therefore, information about adaptation is not available from experiments of the present type.

Gymnarchus niloticus, an electric fish possessing an exceedingly efficient object-locating mechanism, will respond to an electric field as low at 0·15 μV/cm. The sensitivity of *Clarias*, in which no continuous electric discharge has been found, is only a factor of five worse than this. What purpose, if any, does this remarkable sensitivity serve in the normal life of the fish? Perhaps, as has been suggested[3], the fish can detect action potentials from predators or prey. Alternatively it may locate nearby moving fish by detecting electric currents set up by the movement of water in the Earth's magnetic field[1,9]. It is, in any event, almost inconceivable that this great electrical sensitivity is fortuitous, and not used by the fish in any way.

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Functional Polyovuly in the Sei Whale *Balaenoptera borealis* Lesson

On September 1, 1962, a female sei whale, 50 ft. long, was landed at Donkergat whaling station, Saldanha Bay, South Africa. It was found to be pregnant, with two fœtuses in the same cornu of the uterus but within separate fœtal membranes. There was only one corpus luteum of pregnancy in the ovaries so the twins were at first presumed to be monozygotic. However, of the two fœtuses, one proved to be male, 38 cm long, weighing 746 g, and the other female, 39·5 cm long, weighing 737 g (Fig. 1. Scale = 20 cm).

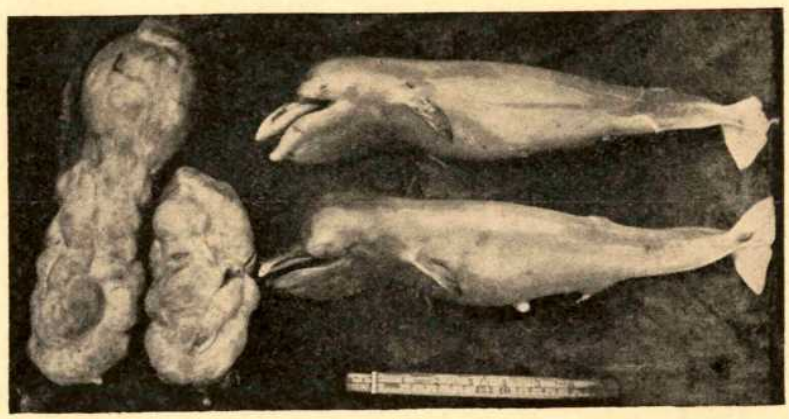


Fig. 1

When the ovaries were sectioned no evidence was found of any other recent luteal tissue representing a follicle from which a separate ovum could have originated. The only explanation therefore seems to be that the Graafian follicle concerned released at least two ova, and that fertilization occurred separately and almost simultaneously.

Polynuclear oocytes and polyovular follicles have been recorded from a number of animals, notably the opossum and dog[1], but they are usually thought to be most common in immature ovaries or among young follicles in a mature ovary. It seems that as the follicle approaches maturity the extra ova degenerate or the follicle becomes atretic, although Desaive[2] believes that in the adult rabbit, at least, resistance to atresia is greater in polyovular follicles than in monovular ones. Actual records of functional polyovuly in mammals are extremely rare. Corner[3] found in many hundreds of pregnant uteri of the sow only three or four examples where the number of embryos exceeded the number of corpora lutea, accounting for them by polyovuly and single-ovum twinning. In a sample of 2,179 rabbit litters, Allen, Brambell and Mills[4] calculated that only 0·23 per cent of the follicles ovulating could have been polyovular. On the other hand, Davis and Hall[5], who examined the ovaries of 394 wild Norway rats, suggested that the presence of polyovular follicles in at least 12 per cent of the animals could account for 20 out of 95 pregnant rats having more embryos in the uterus than corpora lutea in the ovaries.

Mackintosh and Wheeler[6] and Matsuura[7] both considered that whales might release more than one ovum at an ovulation, but Kimura[8] assumed that this possibility could be ignored. Up to the present time comparatively few sei whales have been examined by biologists, so that the significance of the present record cannot be appreciated until a larger sample has been examined. Kimura, however, showed that the sei whale seems to have a higher frequency of multiplets than any of the other large cetaceans.

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Capillary Plexuses in Gastropods stained by the Novikoff Reaction for Thiamine Pyrophosphatase

The vascular system of molluscs is often spoken of as an 'open' one, wherein the exact relations of the arterial vessels to the venous system of vessels and lacunar spaces is as yet undetermined. The absence of an endothelium makes detection of the smallest vessels difficult and capillary plexuses as such have not been described in Gastropoda.

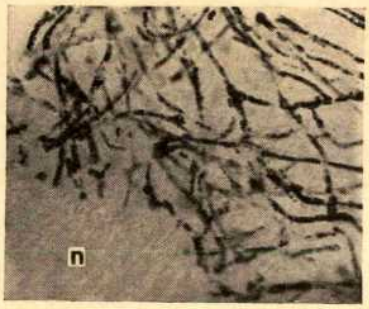


Fig. 1. Portion of the capsule of the pedal ganglion of *Helix*, containing numerous TPPase-positive granules contained within a delicate network of vessels. Neuropil *n* is TPPase-negative. Note that the plexus is coterminous with the capsule and the sharp line of demarcation with the neuropil. (× *c.* 800)

If the histochemical reaction of Novikoff *et al.*[1] for thiamine pyrophosphatase (TPPase) is applied to the pedal and cerebral ganglia of *Helix*, the periganglionic connective tissue gives a strong positive reaction. Large numbers of small darkly staining granules are seen to occupy the lumen of a system of delicate tubules which branch freely and anastomose with one another. The walls of the tubules consist of a delicate light brown coloured layer without nuclei. The appearance suggests a rich capillary plexus (Fig. 1).

Evidence supporting the concept that these vessels are in fact vascular capillaries was obtained by injecting the hæmocœle through the foot of the snail with carmine suspended in saline. The periganglionic tissue was then examined both in the fresh state and following fixation and staining with a variety of histological methods. Carmine particles were found within the tissue in positions corresponding to the TPPase positive particles; but the outlines of the vessels themselves always remained invisible.

The plexus is entirely limited to the periganglionic tissue. Vessels do not penetrate into the neural tissue (Fig. 1*n*), which in contrast is TPPase-negative. The borderline between capsule and ganglion is thus extremely sharp. Preparations impregnated by the reduced silver method of Cajal show that the capsular tissue is frequently pierced by nerve fibres, but these have a distinctive configuration and cannot be confused with the vessels revealed by the Novikoff reaction.

Why these vessels and their contained particles of TPPase-positive material should closely envelop the ganglia of *Helix* remains obscure. Their presence indicates a new significance for a tissue which has previously been regarded as merely a supporting capsule to the nervous elements of the ganglion.

Preliminary investigations have shown that similar plexuses are present in other snail organs, but they appear to be particularly well developed in the periganglionic tissues here described.

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Cellular Uptake of Chloramphenicol as revealed by Fluorescence Microscopy

As a preliminary to investigations of the effects of chloramphenicol on mammalian cells in culture, some means of measuring intracellular uptake of the drug was sought. The fluorescence of tetracycline has been used to observe its localization within cells[1,2]; an investigation was undertaken to see if similar methods could be applied to the examination of chloramphenicol.

It was noted that solutions of chloramphenicol which were originally colourless and non-fluorescing became yellow during exposure to sunlight or when irradiated with an ultra-violet lamp. The coloured solution emitted a yellow fluorescence when irradiated with ultra-violet light in the dark and a precipitate formed after prolonged irradiation.

HeLa cells were grown as monolayers on cover-slips and then were exposed to solutions of chloramphenicol in saline or Eagle's medium for a few minutes. The cover-slips were mounted on slides without washing and examined with a dark-field fluorescence microscope (peak at 360 mμ). Cells mounted in a solution of 800 μg chloramphenicol/ml. showed an overall blue-white auto-fluorescence when first viewed; after 30–60 sec of irradiation this changed to a distinct specific yellow fluorescence within the cytoplasm and nucleoli. The intensity of this fluorescence increased with continued irradiation until a diffuse glow was [illegible] throughout the cytoplasm (Fig. 1). Eventually, cytoplasmic granules were apparent, the cells became swollen, and the cell membranes had a lacy appearance.



Fig. 1. Fluorescence microscopy of HeLa cells mounted in saline containing chloramphenicol (800 μg/ml.), photographed after 5-min exposure to the ultra-violet light of the microscope (× 600)

Cells mounted in saline or Eagle's medium without chloramphenicol initially showed the non-specific blue-white auto-fluorescence after exposure to the ultra-violet light, but this soon faded. No other type of fluorescence developed and in a short time it was difficult to see the cells. The intensity of specific fluorescence was proportional to the concentration of chloramphenicol and to the length of time that the cells were irradiated. A photomultiplier was attached to the microscope tube to measure the emission of light following the irradiation of chloramphenicol in individual cells. These measurements (Table 1) revealed a gradual increase in emission during 15 min of irradiation in the presence of chloramphenicol, but a gradual decrease in emission when cells were irradiated in saline alone.

In addition to HeLa cells, HEp. No. 1 cells, *L*-cells, and human embryonic fibroblasts were tested; these developed the same type of yellow fluorescence in the presence of chloramphenicol as did the HeLa cells. Specific fluorescence could also be demonstrated when cells in suspension were exposed to chloramphenicol.

Table 1. INCREASING EMISSION OF LIGHT DURING THE IRRADIATION OF INTRACELLULAR CHLORAMPHENICOL

Time (min)	Arbitrary units of light intensity: Chloramphenicol (800 μg/ml.)	Saline
0	150	91
0·5	180	
1	210	
1·5	230	70
2	245	
3	291	61
4	335	
5	462	62
7	560	
10	700	60
15	870	59

Cell viability was not required for the reaction since cells fixed in ethanol-acetic acid and cells killed by heat or irradiation showed characteristic fluorescence when exposed to chloramphenicol and irradiated. Although specific fluorescence appeared only in the cytoplasm and nucleoli, fixed cells in which ribonucleic acid had been degraded by ribonuclease (0·05 per cent for 30 min at 37° C) developed fluorescence in these same areas when irradiated afterwards in the presence of chloramphenicol.

When cells were exposed to solutions of chloramphenicol and then washed at 4° C so that the concentration of chloramphenicol in the surrounding medium was reduced from 800 μg/ml. to less than 1 μg/ml., enough intracellular chloramphenicol was retained to produce fluorescence as intense as in unwashed cells. However, if washing was carried out at 25° C, intracellular chloramphenicol was removed and no fluorescence developed during subsequent irradiation.

Chloramphenicol itself is not fluorescent but when solutions of chloramphenicol are degraded by exposure to ultra-violet light one or more of the products formed has this property; fluorescence microscopy of these products can be used to demonstrate cellular uptake of the chloramphenicol. Cells which contain chloramphenicol become increasingly fluorescent because more fluorescent compound is being formed. Intracellular concentration or binding of chloramphenicol or its degradation products is needed for visible fluorescence since little or no fluorescence is seen in the background. The actual amount of intracellular chloramphenicol which must be present has not been determined so that the technique is, as yet, only semi-quantitative. It does, however, demonstrate that chloramphenicol enters cells in culture and is reversibly bound to a non-ribonucleic acid receptor. That this receptor is protein is suggested by the observation of Felsenheld and Carter[3] that chloramphenicol binds non-specifically with protein.

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I thank Dr. Fred Rapp for suggesting this investigation and Dr. M. Earl Balis for his advice and criticisms.

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Effects of Magnetic Fields on Tomato Ripening

ACCORDING to Duclaux[1], Pasteur explored the possibility of the effect of a magnetic field on the production of optical isomers of tartaric acid. He theorized that the Earth's magnetic field caused tartaric acid to be of one optical isomer in nature, whereas laboratory synthesis produced a mixture of both isomers. Recently, Krylov and Tarakonova[2] proposed an auxin-like effect of a magnetic field on germinating seeds. They called this effect magnetotropism. Auxins or synthetic plant growth regulators have been used to stimulate ripening of immature fruits[3].

Investigations were conducted here to determine the effect of a magnetic field on the ripening of green tomatoes (*Lycopersicon esculentum* Mill. var. V. R. Moscow). Four permanent magnets of considerable strength were utilized. Fruits of uniform maturity were placed between the magnetic poles. The experiment was conducted in a ripening chamber at 21° C and 85 per cent relative humidity under dark conditions. The ripening rates of treated fruits were compared with those of untreated controls in the same room under similar conditions. A numerical value for colour (1 = green, 2 = breakers—just starting to colour, 3 = pink, and 4 = red) was assigned for each fruit in a given sample at each observation period. The coefficient of ripening was calculated as follows:

$$\text{Ripening coefficient} = \frac{\text{total score for a sample}}{\text{No. of fruits in the sample}}$$

In all cases the treated fruits ripened faster than the controls (Fig. 1). Furthermore, the fruits nearest the magnetic south ripened faster than those nearest the magnetic north.

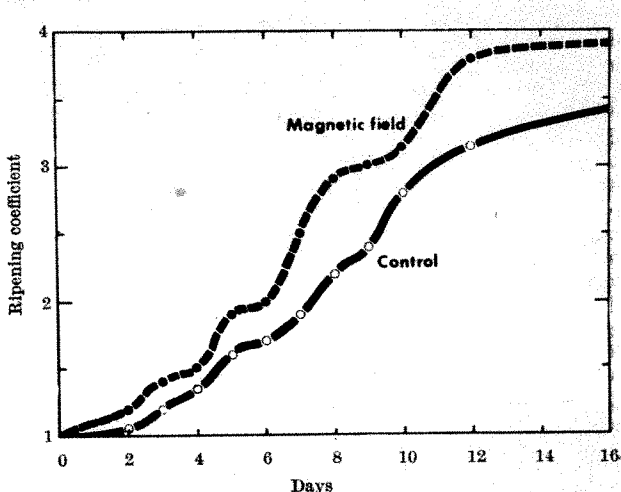


Fig. 1. Effect of magnetic fields on ripening of tomatoes (V. R. Moscow variety)

To expand this experiment further, cherry type (*Lycopersicon esculentum* Mill. Breeding lot No. 1064) tomato fruits of uniform mature-green maturity were used. The small size of these fruits made it possible to increase the number of fruit in each replication. In all cases the treated tomatoes ripened faster than the controls. The polarity effect resembled that noted in the exploratory experiment.

Speculations as to mechanisms associated with this phenomenon could be as follows: (1) an auxin-like character of the magnetic field, as proposed by Krylov and Tarakonova[2], or perhaps an auxin-activating mechanism speeds the ripening process; (2) activation and/or acceleration of the enzyme systems could enhance respiration; (3) radical formation could speed up respiration and thus accelerate ripening.

The possibility that fruit ripening is initiated by a hormone is well known. The experiments carried out by Krylov and Tarakonova[2] demonstrate the similarity of the magnetic effect and effects of plant growth regulators. Pauling and Coryell[4] have shown the magnetic characteristics of the iron porphyrin compounds. The cytochrome oxidase system, which belongs to this group of compounds,

could be functionally influenced by a magnetic field. Smith and Cook[5] have reported on the effect of magnetic field on trypsin activity. The formation of radicals is a common step in organic reactions. It is possible that a magnetic field could promote the loss or gain of protons and/or other reactive groups from compounds in the respiratory cycle.

We thank Prof. J. O. Jensen, of the Physics Department, for furnishing us with the magnets used in this work.

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Oil Spot Disease in Tea

Mulder and Shanmuganathan[1] first reported in 1959 a disease of tea referred to as 'oil-spot of tea leaves'; but they failed to ascribe any definite cause for it. From further investigations carried out here, we have now obtained evidence that the causal organism of this disease is a fungus.

The cause of the disease has for a long time remained obscure as all efforts to isolate a pathogen from the diseased shoots have proved unsuccessful, and attempts to transmit the disease by grafting and sap inoculations have also failed. A fungus has now been identified as the cause of this complex of symptoms. This organism is a weak parasite which remains for the most part in the basal portions of the tea bush, being confined mainly to the wood of the frame. When the main branch below the diseased part of a bush is cut a sector of the wood often appears discoloured, and it has been possible to isolate the pathogen from this area. The same fungus has been isolated from diseased bushes collected from several estates, and experiments indicate that it produces a toxin which acts at a distance from the site of infection, and gradually destroys the entire plant.

The fungus was grown on a modified Czapek–Dox medium containing 10–15 per cent dextrose or sucrose for 3 weeks at room temperatrue (23° C). At the end of this period, it had formed a thick mat, and the liquid was removed by filtration and rooted cuttings of tea (clone *TRI* 2049) were placed in the filtrate. Characteristic oil-spots and necrosis of the veins, typical of the disease, occurred after 4 days (Fig. 1). Cuttings placed in the original pure liquid culture remained free from symptoms. Young bean plants produced water-soaked spots on the leaves and browning of the vascular tissues when placed in the same culture filtrate, and excised tomato shoots showed rapid wilting followed by necrosis of the leaves.

Attempts were also made to extract the toxin from diseased plant material. Diseased wood was comminuted thoroughly, extracted with distilled water, and rooted cuttings were placed in the filtrate. Typical oil-spots appeared after 4–5 days; extracts from healthy wood were non-toxic.

The lesions produced by the culture filtrates are so characteristic that there can be little doubt that the toxin produced by the fungus in the host plant is the same substance as that found in the culture filtrates.

The fungus has so far remained sterile on several media, and has thus defied all attempts to identify it.



Fig. 1. Left, healthy tea leaf; right, leaf of rooted cutting which was placed in culture liquid for 5 days

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Stimulation by Citric Acid of Germination of Eastern Red Cedar (*Juniperis virginiana* L.)

I have found that pretreatment with citric acid increases both speed and total germination of eastern red cedar seed. Earlier, exploratory investigations showed that the acid also increased both speed and total germination of bald cypress seed (*Taxodium distichum* (L.) Rich) and I have reported similar effects of pretreatment of eastern white pine seed (*Pinus strobus* L.) with both citric and tartaric acids[1]. Of the several species examined, eastern red cedar showed the most striking response to pretreatment with citric acid.

A fresh lot of eastern red cedar cones was soaked in water and all pulpy material removed by hand using screens and sedimentation. The cleaned seeds were allowed to dry in air for 3 days at room temperature and then given one treatment in a factorially designed study involving 6 chemical treatments, 5 soaking periods, 4 stratification periods, and 2 stratification temperatures.

Three 100-seed samples were drawn from each treatment combination and germinated on a sand–vermiculite medium in the dark at a constant temperature of 14° C. Germination percentage was expressed as that of full seed (about 90 per cent for this work) and were tallied when the radical penetrated the medium and lifted the seed coat completely. I was primarily concerned with germination percentages occurring within 30 days because maximum germination with maximum speed is desirable.

Fig. 1 shows results for some of the better treatments. When seeds were soaked for 4 days in a 10,000 p.p.m. solution of citric acid, prior to 90-day stratification, there was 93 per cent germination at 30 days. A 4-day soak in water with 90-day stratification gave 73 per cent germination and stratification alone gave only 23 per cent germination. For the 90-day stratification period, seed soaked in citric acid and in water showed an increase in 30-day germination of 304 per cent and 217 per cent, respectively over no soaking. Seed receiving no treatments did not begin germination until 70 days and reached only 43 per cent at 120 days.

The main factor in inhibition of germination in eastern red cedar is embryo dormancy[2-4]. Its embryos exhibit both primary and secondary dormancy[2,4]; but because these were fresh seed, secondary dormancy may not be a factor. Whether citric acid creates the same response with secondary dormancy has not been determined.

The nature of the embryo dormancy of this species, therefore remains the important question. It is known that water will sometimes remove inhibitors from seed. The seed coats of eastern red cedar are impermeable to water, but

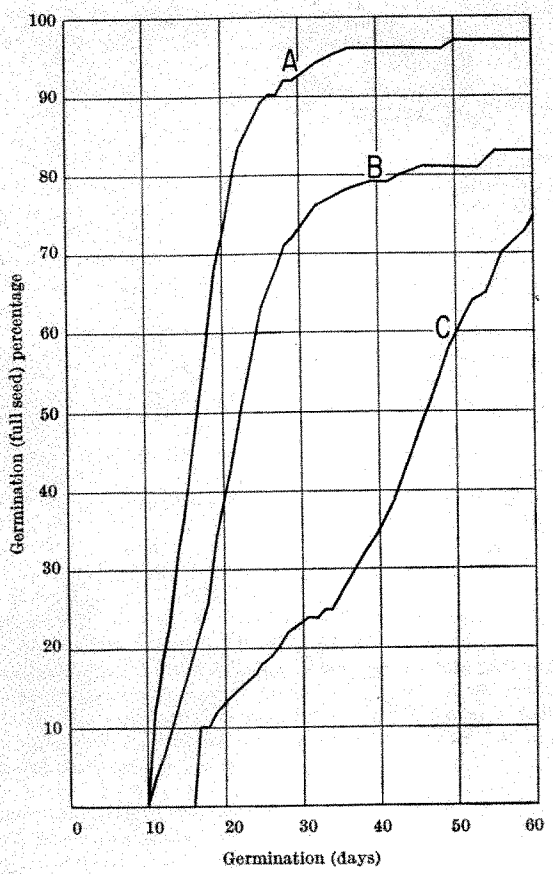


Fig. 1. Germination of eastern red cedar seed after 90-day stratification at 3° C with a prior treatment of: *A*, 4 days' soaking in a solution of 10,000 p.p.m. citric acid; *B*, 4 days' soaking in water; *C*, no treatment

can be made permeable by moist storage for 8 weeks at 25° C prior to stratification[3]. It has been shown that the dormant eastern red cedar embryo has an alkaline *p*H, but a germinating embryo has an acid *p*H (ref. 4). If this is so, What part does citric acid play? Is it one of increasing the efficiency of water as an extractant, the activation of the Krebs cycle, the production of an acid environment necessary for embryo metabolism, or is it a combination of these possibilities?

Other experiments to elucidate further the role of citric acid and similar compounds are being carried out. The response of eastern red cedar seed to a pretreatment of citric acid does not appear to be an isolated instance, as investigations now being carried out with seeds of *Pinus taeda* L., *P. strobus* L., *P. echinata* Mill., and *Taxodium distichum* (L.) Rich show that these seeds have a similar reaction to a citric acid pretreatment. Citric acid does not produce the same effect on all seed; Evanari[5] reported an inhibitory effect of this compound on some seed.

I thank Dr. A. A. Foster, of the Tennessee Valley Authority, for supplying eastern red cedar cones. This work was supported by the Georgia Forestry Commission, the Georgia Forest Research Council and the Eastern Tree Seed Laboratory.

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A Nutrient Requirement for Optimum Water Absorption by Intact Root Systems

Eaton[1] found that a tomato plant with half its root system in distilled water and the other half in nutrient solution, after an initial period of three days, absorbed more water from the nutrient solution than from the pure water. He attributed this difference to reduced root growth in distilled water. However, it has been shown that an inadequate external solute supply can lower the osmotic pressure of the xylem sap[2], and it has been postulated that the metabolism and permeability of the roots may be affected by the concentration of the external solution[3]. Part of the difference in water absorption from distilled water and nutrient solution might be caused by such effects, and this has been demonstrated during the course of experiments at this Station on the relationship between water absorption and the concentration of the external solution.

Seeds of *Zea mays* L., variety North Star, were germinated in sand and 18-day-old seedlings placed singly in jars containing 2·4 l. nutrient solution (Long Ashton solution[4], with the $NaH_2PO_4.2H_2O$ partly replaced by K_2HPO_4 to raise the *p*H). The seedlings were supported by cotton wool in small holes in the corks closing the jars. After three weeks this solution was replaced by other concentrations of the Long Ashton solution, which had osmotic pressures from zero (rain-water) to three atmospheres. There were seven replicates of each treatment. The cultures were not aerated.

Water absorption was measured every 24 h by adding demineralized water from a burette until the original level of solution in the jar was restored. The results are shown in Fig. 1. The only marked difference observed at the end of the first 24-h period was a reduction in the amount of water absorbed from the 3-atmosphere solution. During the second 24-h period there was a considerable reduction in water absorption from the rain-water. It seems unlikely that within 48 h a sufficient reduction in root growth could occur to account for the observed depression of water absorption. Plant weights were not determined at this stage, since the effects of continuing

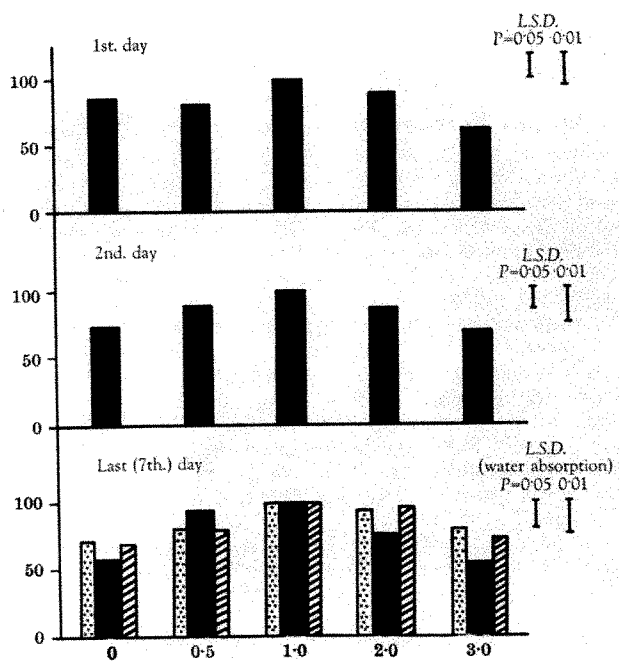


Fig. 1. Effect of concentration of the nutrient solution on water absorption and root and shoot growth of *Zea mays* L. Black, relative water absorption; stippled, relative fresh weight of roots; hatched, relative fresh weight of shoots

the treatments were being investigated. However, as can be seen from the bottom set of histograms in Fig. 1, on the seventh day the pattern of differences in fresh weights, not only of the roots but also of the shoots, was roughly similar to the pattern for water absorption.

The relationship between water absorption and root and shoot weights was clarified by calculating the regression coefficients. It was assumed that the relationships would be linear and that, when root or shoot size was zero, water absorption was also zero. The values (Table 1) show that there was a significantly lower volume of water absorbed per unit weight of root or shoot from the rain-water than from the 0·5-atmosphere solution. A similar result was obtained with *Triticum aestivum* L., variety Jufy 1, where the optimum nutrient solution had an osmotic pressure of 0·1 atmosphere.

Table 1. Effect of the Concentration of the Nutrient Solution on the Amount of Water Absorbed in 24 h per unit Fresh Weight of Root and Shoot of *Zea mays* L.

Osmotic pressure of nutrient solution (atm.)	Coefficient of regression of water absorption on: Fresh weight of roots	Fresh weight of shoots
0	1·38 ± 0·124	0·59 ± 0·052
0·5	2·03 ± 0·177	0·85 ± 0·058
1·0	1·71 ± 0·044	0·70 ± 0·034
2·0	1·38 ± 0·049	0·57 ± 0·014
3·0	1·19 ± 0·051	0·52 ± 0·018

It was concluded, therefore, that the maximum rate of water absorption by intact root systems was only achieved in the presence of nutrient ions in the external solution and that the ions affected the ability of the roots to absorb water, in addition to affecting the size of the absorbing and transpiring organs. This conclusion is supported by the results of other experiments of a different type and a full account of the methods and results is being prepared for publication elsewhere.

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ENTOMOLOGY

Queen Perception by Honey Bee Swarms

Should a honey bee (*Apis mellifera*) swarm emerge without a queen or its queen be unable to fly, the swarm soon returns to the parent colony. When the queen is taken from a swarm soon after it has settled, the bees usually become disturbed within 10–15 min[1] and soon return to the old hive. Colonies deprived of hives and combs behave like swarms[2], and were used in the present experiments. When such a colony was allowed to cluster on a branch clamped in a retort stand and its queen was taken away, the worker bees soon became restless, running about on the surface of the cluster, flying off and returning, apparently searching the immediate neighbourhood for the queen. The disturbance increased until most of the bees were taking part in it, and eventually, unless the queen was put back, all the bees (except those too young to fly) joined other colonies.

A queen in a wire screen cage stabilized the cluster, even when the cage had double walls separated by a gap across which bees could not reach. When a queen in a double-walled cage was suspended about 20 cm from a queenless cluster which had reached the disturbed stage, the worker bees that landed on the cage immediately started running agitatedly over the wire screen, vibrating their partially spread wings. Some tried to get their heads between the wires of the screen and extended their tongues towards the queen. When the queen was put in a single-walled cage, the workers tried to lick her body though usually they could not reach it; they could easily have licked her feet but were not seen to do so. With either type of cage other bees appeared to be attracted to those already there, so that the number on the cage quickly increased. Few bees were seen with their abdominal (Nassanoff) scent glands open except when the cluster of bees building up on the cage touched the queenless cluster; when this happened, bees started streaming towards the cluster with the caged queen, opening their Nassanoff glands and fanning air backwards with fully spread wings. The whole queenless cluster then soon joined that with the queen. A double-walled cage that had contained a queen stabilized the cluster for more than 12 h. The worker bees attracted to the cage congregated on the wire screening and not around the plugged hole in the wooden part through which the queen had been removed. A queen in a cage in a thin polythene bag, through which sounds she produced could be heard, did not stabilize the cluster, and the worker bees did not seem to perceive her in any way. Evidently it is the scent of a queen that stabilizes a swarm.

To investigate the origin of this scent the freshly severed head, thorax and abdomen of a laying queen were put into clean new queen mailing cages, kept with their wire screening uppermost so that bees could not touch their contents, and hung simultaneously about 20 cm from a dequeened 'swarm'. Little evidence of attractiveness was seen when the parts of the queen were whole, but there was much after they were crushed; bees that landed on the cage containing the crushed head remained on it, vibrating their wings and trying to get through the wire screen, whereas they were not attracted by the cages containing the crushed thorax and abdomen. All three cages were then held in the queenless cluster until they became covered with bees, when they were withdrawn and suspended about 20 cm away. The bees on the cage with the head remained there, showing interest in its contents; but those on the other two cages soon flew off. Within 1·5 h all the bees of the colony were on the cage with the head. The corresponding parts of a virgin queen about 3–6 days old gave similar results. The stabilizing scent was therefore mainly in the queens' heads, presumably in the mandibular glands, removal of which greatly diminishes a queen's power to attract workers in a hive[3]. Pure 9-oxodec-*trans*-2-enoic acid, the principal component of queens' mandibular gland secretion[4], presented on filter paper in a cage, did not stabilize a 'swarm', and the workers showed no response of any kind to it, even when they were allowed to touch the paper. This substance is unattractive to young worker bees[5], and queenless bees in an arena do not cluster more readily on a cage containing it than on an empty cage[6]. Evidently some other component of the secretion is responsible for its attractiveness. The unattractiveness of an intact dead queen compared with a living queen or a cage that had contained one suggests that a living queen discharges the secretion frequently and contaminates her surroundings with it.

A 'swarm' that had been left as an exposed cluster with its queen in a cage for several days suddenly dispersed and immediately flew about 120 m in a straight line, presumably towards a chosen nest site[7], halted, hovered for a time, and finally returned to the caged queen. Apparently swarming worker bees, even while flying, can detect whether or not they are accompanied by their queen. If they do this by smelling, she presumably must fly in front of the swarm. As she has not previously visited the new nest place she is unlikely to be leading the swarm to it, but she may perhaps be driven from behind. When a swarm is leaving a hive the queen appears to be driven out by the workers, which stop moving forward whenever the queen does, so that the bees coming behind pack together and prevent her from running back[8]. Possibly a travelling swarm behaves similarly with workers falling back to get behind the queen whenever they do not smell

duct is bound up near the liver. Further, about 3 cm from the duodenum, a soft rubber tube of appropriate thickness is inserted into the bile duct and fixed. The other end of the tube, provided with the silon net to prevent blocking of the tube with the killed worms, is inserted into the gall bladder. So the flow of bile is in no way interfered with, and no flukes are lost after being killed by the substance under trial. Using this method new anthelmintics have been examined on 75 sheep and the work continues.

The operation is performed in 'Thiopental^{R}' anæsthesia and usually lasts for about 35–45 min. Some hours later the anthelmintic is given. The animals are killed on the fourth or fifth day. By thorough helminthological dissection of livers the mature flukes are picked up and ascertained to what extent they became necrotic. In this way a direct comparison is made, and the percentage of worms killed or damaged is assessed exactly. By this method the action against immature stages of liver flukes could be examined, too. Apart from this the method is suitable for testing substances against *Dicrocoelium lanceolatum*.

Most of the animals lived after the operation as needed, but in the early stages of the work some animals succumbed before the fourth day. Some of the animals which were not killed lived for 3–6 months and one is still alive after more than a year.

The examination of some known anthelmintics (1,4-*bis*-trichlormethylbenzene, hexachlorophene, 'Freon 112') has shown that the method works well and enables one to perform critical test with substances acting against *Fasciola hepatica* in sheep.

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Resuscitation of Sheep Embryos and the Simulation of Intra-uterine Life

Preliminary investigations made on the short-term revival of embryos obtained from sheep at slaughter suggest that extra-uterine maintenance of mammalian embryos is a technique awaiting development and exploitation.

Embryonic sheep between 5 and 33 cm CR-length (43–113 days[1]; gestation period, approximately 150 days) were connected to a simply-constructed heart-lung machine with the roller-pump in series with the embryonic heart. An artificial amnion made of 'Perspex' was filled with 0·9 per cent sodium chloride at 39° C and through the wall of this tank entered two rubber-tubes which served as extensions for the umbilical vessels. Each tube was convoluted within the saline and then passed through the liquid surface to the operating table. The cannulæ used for connecting these tubes to a fœtus were transparent polyvinyl chloride or polythene ('Portex'). The output tubing from the pump passed to the tank and connected with the umbilical vein extension; the umbilical artery extension was taken from the tank to an oxygenator of the rotary-disk type[2,3]. Heparinized Ringer solution and acid-citrate dextrose (ACD) were used as blood substitutes. Oxygen was metered into the disk-chamber in a direction opposing the flow of the liquid; after passing the disks the liquid was recirculated by the pump.

Before an experiment the oxygenator was charged with either solution and the cannulæ mutually connected with plastic tubing. The pump was switched on and the liquid drawn from the oxygenator was passed down the umbilical vein extension through the joined cannulæ to the umbilical artery-extension and returned to the oxygenator. The pump was stopped and the cannulæ were disconnected at the operating-table level. Since this table was lower than the oxygenator there was now gravity-feed from the artery extension and this temporarily reversed flow was necessary for the insertion of that cannula. This flow was controlled by a clip and the flow from the vein extension by switching the pump.

Pregnant uteruses from the Bristol Public Abattoir were sealed in polythene bags and plunged into water at about 39° C. They were brought to the laboratory as quickly as possible, but the interval between killing the ewe and the dissection of its uterus was never less than 18 min.

A large cut was made through the uterine wall and fœtal membrane of a conceptus, the incision running between and parallel to lines of cotyledons. As the fœtus was lifted from the amniotic fluid and placed on sponge-rubber its cord was unravelled and gently stretched. An umbilical vein was entered after placing a linen-thread ready to ligate it; the pump was switched on and the stream of liquid from the cannula used to wash the site at which the vein was opened. When the vein was cannulated the pump was stopped until an artery was similarly connected, then the remaining umbilical vessels were clamped and the cord sectioned on the placental side of the connexions. The fœtus was raised to just above the level of the meniscus in the oxygenator and the heart-pump switched on. If the pumping and connexions were satisfactory, the fœtus, accompanied by cannulæ and vessel extensions, was lowered into the amnioid cavity.

Six mechanically successful transfers were effected; 3 using ACD and 3 using heparinized Ringer. In all cases serious œdema developed within 15–20 min and the embryos, still connected, were taken from the saline for examination. In those receiving ACD and in one receiving Ringer no activity was observed on exposing the heart, but in the remaining two Ringer cases strong rhythmic activity was present with ventricular as well as atrial contraction. Since, apart from several temperature measurements, the biophysical system was unmonitored and the technique empirical it was expected that unphysiological conditions would be rapidly established. Neither perfusion fluid used could be expected to sustain the embryos for long and the use of whole blood should give better results.

I thank Miss J. Honour, who helped to construct the plastic parts of the apparatus, and Mr. T. Baker, who collected the biological material.

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SOIL SCIENCE

Occurrence of a Previously Unobserved Nitrogen Gas in the Reaction Product of Nitrous Acid and Lignin

During investigations into the nature of gases produced by the reaction of nitrous acid with soil humic acids and lignins, a product was obtained from lignin which showed anomalous behaviour. There was a slow uptake of gas by permanganate solution beyond the time required for

complete adsorption of nitric oxide (formed by self-degradation of nitrous acid during the reaction). For example, with the products obtained from humic acids, there was no further uptake of gas beyond a 10-min period of contact with permanganate; with the product obtained from lignins, some gas was still being adsorbed after an 8-h period. This result suggested that the gases from lignins contained a compound which was not present in the gases recovered from humic acids. To explore this possibility, infra-red analyses were made of the gaseous products obtained by reacting nitrous acid with humic acids, lignins and some lignin-building units.

The gases were prepared for analysis by reacting a 50- to 200-mg sample of the substance in question with nitrous acid in a Van Slyke manometric apparatus, using a technique similar to that utilized for the determination of amino nitrogen by the nitrous acid method[1]. The procedure was similar to that Bremner[2] described for the reaction of nitrous acid with humic acids and lignins, with three exceptions: (1) the reaction was carried out in a dilute sulphuric acid solution instead of an acetic acid solution; (2) a Na_2HPO_4–$KMnO_4$ mixture, rather than a NaOH–$KMnO_4$ solution, was used to remove nitric oxide; (3) adsorption of nitric oxide was carried out in the reaction chamber of the manometric apparatus. Preliminary trials showed that adsorption of nitric oxide was complete within 10 min; nevertheless, the sample was allowed to remain in contact with the permanganate solution for 20–30 min before the infra-red analysis was performed.

Typical infra-red absorption patterns of the gases obtained by reacting nitrous acid with a soil humic acid and with some phenolic compounds are reproduced in Fig. 1. In every case the infra-red patterns showed only the presence of nitrous oxide[3]. In general, 0·25–0·5 of the gases consisted of nitrous oxide; the remainder was elemental nitrogen.

The infra-red patterns of the gases obtained from lignins, syringic aldehyde, and syringic acid are reproduced in Fig. 2. In these cases, absorption bands were obtained which did not correspond to nitrous oxide (Fig. 1), or to nitric oxide, nitrogen dioxide, nitrogen

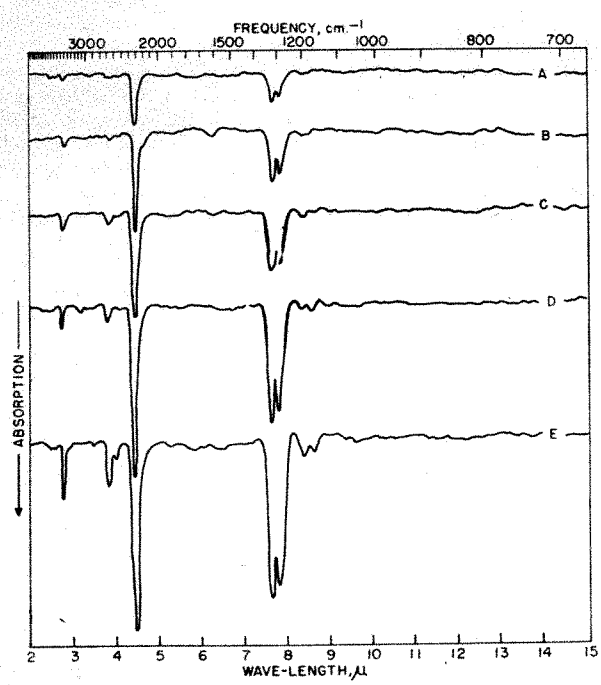


Fig. 1. Infra-red absorption spectra of gases from nitrous acid treatment of: (*A*) humic acid, 200 mg; (*B*) pyrogallol, 50 mg; (*C*) protocatechuic acid, 50 mg; (*D*) catechol, 50 mg; and (*E*) phloroglucinol, 50 mg. Nitric oxide had previously been removed by adsorption in permanganate solution

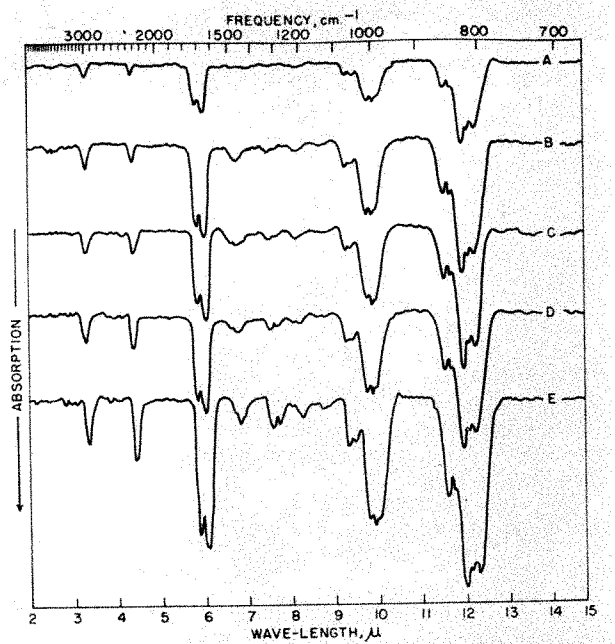


Fig. 2. Infra-red absorption spectra of gases from nitrous acid treatment of: (*A*) 'Meadol MWS' lignin, 50 mg; (*B*) methanol lignin, 100 mg; (*C*) syringic acid, 50 mg; (*D*) syringic aldehyde, 50 mg; and (*E*) 'Meadol MWS' lignin, 100 mg. Nitric oxide had previously been removed by adsorption in permanganate solution

peroxide, dinitrogen tetraoxide, nitrogen pentoxide nitromethane, hydrogen nitrate, hydrazine, ammonia hydrogen cyanide, methyl azide, cyanogen or diazo methane[3]. No report of an infra-red pattern whicl corresponded to that of the unknown was found in th scientific literature.

Other experiments showed that only compounds con taining the syringyl group produced the unknown gas indicating that this group is the structural unit in lignin responsible for the formation of the gas.

Whereas the amount of the unknown gas recovere from the gaseous mixture decreased with increasin periods of contact with permanganate solution, there wa an increase in the quantity of nitrous oxide recovered This result suggested that the unknown gas containe nitrogen. This supposition was confirmed in experiment in which sulphite was used to reduce the gas; the un known gas was completely adsorbed by aqueous sulphit and acidification of the resulting mixture resulted in th production of considerable quantities of nitrous oxide, a revealed by infra-red analysis. The unknown gas ha the properties of a polymerized nitric oxide.

Controlled experiments showed that the unknown ga was not an artefact arising from any of the reagents use for analysis, neither was it a product synthesized durin the removal of nitric oxide by permanganate. It i concluded that the gas is a true product of the reactio of nitrous acid with lignin.

This work was carried out while one of us (F. J. S was in Australia as a Fulbright Research Scholar.

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PSYCHOLOGY

Sources of Difficulty in Learning Arithmetical Facts

SEVERAL investigations have been carried out on the relative difficulties of learning the various 'arithmetical facts' or tautologies contained in the multiplication and addition tables. A variety of operational criteria of 'difficulty' have been used by different workers, one of whom[1] gives a comparative review of earlier work, with which his own results substantially agree. In particular it seems clear that the chief source of difficulty with a given combination (such as $2.7=14$ or $4+5=9$) is the size of the numbers concerned. However, on the question of which numbers, exactly, are responsible for the difficulty—the addends and factors, or the sums and products—both the evidence and the considered opinions of different investigators are nicely balanced between the two points of view.

In the case of addition, Knight and Behrens[2] concluded that the size of the sum determined the difficulty, defining the latter in terms of learning, mistakes and calculation-time. Yet they suspected that the size of the addends influenced the way in which the relative difficulties fell into groups. Wheeler[1] found similar results, but favoured the size of the addends as the primary source of difficulty. In Wheeler's experiment, after some preliminary teaching 125 children practised addition by playing an educational card-game for a fixed period each day. The different additive combinations were then ranked in order of increasing difficulty, according to the proportion of children answering correctly in a written test on the 20th day. Wheeler found correlation coefficients of 0·87 and 0·95 respectively, between difficulty-rank and size of sum, and difficulty-rank and size of addends. Since the combinations were differently grouped for the two tests it can only be inferred that 'difficulty' is closely related to both variables.

In the case of multiplication, opinion is still more narrowly divided. Wheeler[1] cites two investigations in which the size of the product, and three in which the size of the factors, were judged to be the primary source of difficulty. He himself, using a second educational card-game, found almost identical correlations; for the product, $r=0{\cdot}92$, for the factors, $r=0{\cdot}94$.

Much of the ambiguity throughout clearly stems from the fact that for both additive and multiplicative combinations (generally expressible in the forms $x_1+x_2=x_3$ and $x_1.x_2=x_3$ respectively) x_3 is heavily dependent on x_1 and x_2 by definition. Consequently the two hypotheses, linking difficulty with x_3 on one hand or with a joint measure of (x_1, x_2) on the other, are to a large extent confounded and statistically indistinguishable. Even if only for this reason, a new unifying hypothesis involving x_1, x_2 and x_3 in combination, which made it unnecessary to choose between these two alternatives by giving at least equally high correlations, would be preferable to either of them.

Some recent work on timed calculations by adults, including simple one-stage additions and multiplications of the type considered here, suggests a formula which may be the appropriate collective measure of 'calculation-size' or 'complexity' for use in the present dilemma[3]. In some circumstances at least, the average time taken to perform a simple one-stage arithmetical operation R on a pair of one-digit numbers (x_1, x_2) to give a result x_3 is strictly proportional to $\log(x_1+x_2+x_3)$. Thus for multiplications, $x_3=x_1.x_2$; for additions, $x_3=x_1+x_2$ and $\log(x_1+x_2+x_3)=\log 2(x_1+x_2)$. The correlations between 'difficulty' and the appropriate values of $\log(x_1+x_2+x_3)$, according to Wheeler's data, have therefore been examined and compared with correlations obtained on the hypothesis that either (x_1, x_2) or x_3 alone is responsible for the difficulty.

The first test was applied to the ungrouped data on multiplications. 'Double' combinations for which $x_1=x_2$ were excluded and, apart from these, a combination and its reverse were treated as distinct. All combinations with 0 or 1 as a factor were excluded. The remaining combinations were then ranked according to the percentage of children performing each one correctly in the final test. For these 64 combinations, ungrouped, the correlation coefficients (with respect to difficulty-rank number in each case) are as follows: *Product*, $(x_1.x_2)$: $r=0{\cdot}79$; *mean factor-size*, $\frac{1}{2}(x_1+x_2)$: $r=0{\cdot}80$; and for $\log(x_1+x_2+x_1.x_2)$: $r=0{\cdot}84$.

In the second test the multiplicative combinations were classed into 18 groups in the manner adopted by Wheeler, including 0 and 1 as factors but excluding 'doubles'; in each group either x_1 or x_2 is held constant. For each combination, $\frac{1}{2}(x_1+x_2)$, $(x_1.x_2)$ and $\log(x_1+x_2+x_1.x_2)$ were separately computed and the difficulty-rank taken from Wheeler's complete list. Correlations were computed for the group arithmetic means of these quantities. Correlation coefficients with respect to difficulty-rank are: *Mean product:* $r=0{\cdot}88$; *mean factor-size:* $r=0{\cdot}93$; *mean* $\log(x_1+x_2+x_1.x_2)$: $r=0{\cdot}95$.

Applying the second test to Wheeler's data for additions, excluding 0 as addend and excluding 'doubles', grouping and averaging as before, the correlations are: *Mean sum:* $r=0{\cdot}97$ (mean addend would give the same r); *Mean* $\log 2(x_1+x_2)$: $r=0{\cdot}99$.

In every case, therefore, difficulty-rank correlates at least as well with $\log(x_1+x_2+x_3)$ as with the arithmetic mean of (x_1, x_2) or with x_3.

As already observed, the argument in favour of $\log(x_1+x_2+x_3)$ as the pertinent psychophysical variable only requires this condition to be satisfied; the value of the present hypothesis is, primarily, that it removes the need to decide between two statistically indistinguishable hypotheses, each of which only takes one part of the calculation into account. However, the hypothesis gains indirect support from the fact that, unlike x_3 or $\frac{1}{2}(x_1+x_2)$, the function $\log(x_1+x_2+x_3)$—under suitable conditions—is strictly proportional to the time taken by adults to perform the corresponding calculation. It may be noted that although Wheeler ranked additions purely by 'learning-difficulty', whereas Knight and Behrens used calculation-time as one of their criteria of difficulty, the two rankings agree closely ($r=0{\cdot}91$), a fact which again suggests that one and the same measure of calculation 'size' may be a major factor in both learning-difficulty and, subsequently, calculation-time.

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Influence of Stroboscopic Illumination on the After-effect of Seen Movement

THE after-effect of seen movement (sometimes known as the waterfall effect) is a well-known illusion in which steady viewing of a moving patterned surface is followed, on transfer of one's gaze to a stationary surface, by an apparent motion in the opposite direction. Pickersgill[1] gives an exhaustive review of the literature.

We have recently investigated the effect of lighting the stationary surface stroboscopically instead of constantly. The stimulus pattern first used (by R. L. G. and S. M. A.) was a rotating Archimedes's spiral which is seen as expand-

ing or contracting, the opposite apparent motion being normally visible as an after-effect. The after-effect was viewed against a similar, stationary spiral. It was found that stroboscopic illumination of the stationary surface abolished the after-effect. If the stationary spiral is seen by dim steady illumination, the stroboscopic flashes being introduced from the same visual direction via a half-silvered mirror without illuminating the spiral, then the after-effect is not abolished.

If the stationary spiral is illuminated with both steady and stroboscopic light, the after-effect may be made just apparent with a critical balance of their intensities. The after-effect disappears when the stroboscopic illumination is just intense enough to cause apparent instability of the stationary spiral.

In later experiments (by D. M. M. and N. de M. R.) the stimulus was a rotating circular granular black-and-white pattern. In this case, the after-effect of movement was not abolished by stroboscopic illumination of the stationary granular field, but it was briefer than with constant illumination, for flash rates of 4–30/sec. A rotating Archimedes's spiral containing more turns than that used by R. L. G. and S. M. A. and producing a subjectively stronger after-effect, gave results like those obtained with the granular stimulus, except that the after-effect was more readily suppressed by the lowest flash rates (4–10 flashes/sec).

These findings may have a bearing on the insensitivity to real motion observed in stroboscopic illumination[2]. It now looks as if intermittent illumination of a moving contour not only removes motion-information from the input to the postulated velocity-abstracting system, but also may (at least partially) inhibit its action.

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ANTHROPOLOGY

Frequency of Spina Bifida Occulta in Prehistoric Human Skeletons

In a cave at Taforalt, in the Beni Znassen country of north-eastern Morocco not far from Oujda, the Abbé J. Roche excavated about 80 adult and more than 100 infantile skeletons of the Mechta-Afalou type, associated with an Ibero-Maurusian industry (Epipalæolithic). These remains give a carbon-14 date of 10,500–12,070 years according to the layers.

The adult sacra of that series are characterized by the presence of a more-or-less developed dehiscence of the crista sacralis mediana known as spina bifida. Spina bifida is a defect in the normal closure of the spinal column at any point; but its commonest site is in the lumbosacral area. In modern man, it is a rather frequent anomaly, since 14–24 per cent of the vertebræ of the lumbosacral junction are thus affected[1,2]. Some authors consider that the dehiscence of *S* I is normal. We have found this anomaly in 4 fossil sacra out of 15, that is, in 27 per cent of the cases; the two first apophyses were lacking in one of these bones.

At the present time, the dehiscence of the last processus spinosus of the sacrum is more uncommon. On 21 fossil pieces, only 5 were quite normal; 3 showed a perforation between the apophyses 3 and 4. In specimen XXVII (Fig. 1), the canal of which is open in its upper part to the top of *S* II, there is another perforation between *S* II and *S* III. In two bones, the canal is not closed at mid height of the fourth epiphysis which is divided into two in two other bones. This dehiscence goes up to the top of the fourth apophyse in three specimens and concerns the third apophysis in six sacra. Lastly, in specimen XI (Fig. 2), the whole canal is open, except at the level of the first processus spinosus.

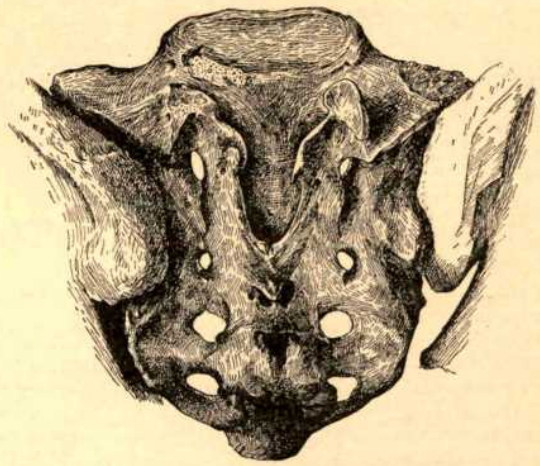


Fig. 1. Sacrum of the specimen XXVII showing perforations and a dehiscence of the upper processus spinosus

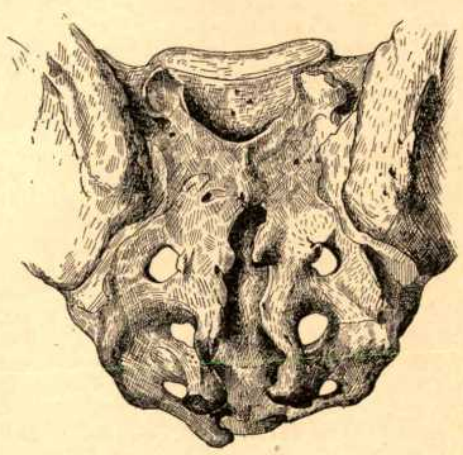


Fig. 2. Sacrum of the specimen XI, with an almost complete spina bifida occulta

If we do not take into account the pieces showing a perforation or an incomplete dehiscence of the half fourth apophysis, 10 specimens out of 21 appeared abnormal. If all the anomalies are considered, 16 specimens out of 21 should not be accepted as normal: such a percentage does not seem to be found at our time. As concerns prehistory, spina bifida occulta has been ascertained by L. Pales on a 5,000-year skeleton[3]; but we are not aware of group data.

The occurrence of spina bifida can be influenced both by environmental and genetic factors[4]. According to Shamburov[5], spina bifida occulta would be the heterozygous condition, of which spina bifida aperta, generally fatal in infancy, would be the homozygous counterpart. The first condition would therefore be due to a dominant gene, although we should remember that the mode of inheritance of spina bifida is considered by many human geneticists as least reliable[6].

The study of modern isolates has shown that a dominant gene can spread in a relatively short time. The high frequency of spina bifida in our series could signify that the inhabitants of Taforalt were highly endogamous.

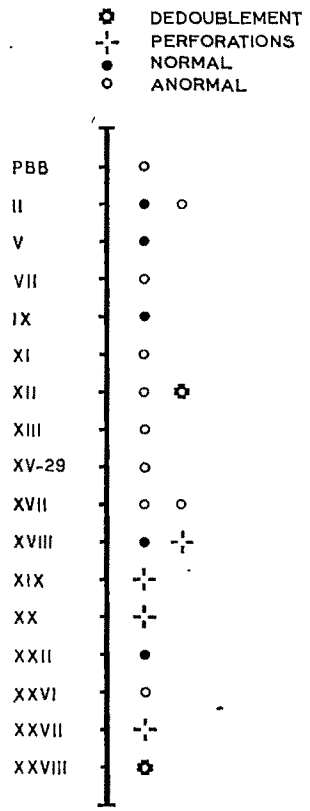


Fig. 3. Distribution of spina bifida according to the sepultures

It would also seem that there is an increase of that anomaly in time. The number of the tombs rather closely corresponds to the antiquity of the sepultures; we can see in Fig. 3 that from tomb XVIII to tomb XXVIII only one bone shows an actual dehiscence: this dehiscence is found in 9 sacra from tomb XVIII. This could suggest the diffusion of spina bifida by a dominant gene.

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Criteria for the Comparison of Skulls

THE two essentials for the satisfactory comparison of skulls are a common plane of orientation and a centring point that is universally valid, that is, common to all skulls throughout the vertebrate series. There is urgent need for agreement on these two criteria and particularly on a common centring point.

Many planes of orientation have been devised[1-3]; but it is now generally agreed that the Frankfurt Horizontal is the most satisfactory for primates, at least (but see ref. 3). There are, however, differing views on the centring point, with the basi-cranial angle as a possible favourite.

There is one point in all vertebrate skulls which can be looked on as a universally valid morphological feature—that is, the frontal extremity of the notochord. This lies just within or immediately behind the pituitary fossa in all vertebrates[4]. In man this end of the notochord is temporarily attached to the posterior lobe of the pituitary gland[5]. In the human being, and possibly in most vertebrates, the frontal extremity of the notochord is finally encased in the posterior wall of the pituitary fossa. The posterior lobe of the pituitary gland or, more readily, the pituitary fossa should, therefore, provide a good common centring point for all skulls. It has the advantage, too, that it remains constant throughout the age-range of any animal.

If skulls are centred on the pituitary fossa (in practice the floor of the fossa is the most convenient point to choose) and orientated in the Frankfurt Horizontal an interesting series of relationships emerges.

In all human beings examined, of diverse ethnic origin (European, Chinese, Australian aborigine) and of ages ranging from neonatus to adult, the Frankfurt Horizontals were virtually coincident at the level of the inferior surface of the body of the sphenoid bone. In other primates (*Gorilla*, *Pan*, *Pongo*, *Macaca*, *Papio*) and a juvenile macaque (about six months old) the Frankfurt Horizontals were also virtually coincident but at a much higher level which crossed the middle of the pituitary fossa. A quadruped skull (*Ovis aries*) was examined on the same criteria and the Frankfurt Horizontal was found to be still higher, passing just above the pituitary fossa. Moreover, it could be shown that the level of the Frankfurt Horizontal was in each case related to the plane of the foramen magnum and hence to the angle at which the skull joined the vertebral column[6]. Use of the region about the frontal extremity of the notochord has, therefore, encouraging morphological justification.

Comparison of skulls on this basis offers no difficulty if they can be sectioned in the median sagittal plane. If they must be kept intact the pituitary fossa can usually be visualized satisfactorily in orthogonal or near-orthogonal X-ray plates. Should the radiographic detail be confusing—as it is in some of the larger apes—or obscured by mineralization the pituitary fossa can be identified by inserting a thin strip of lead through the foramen magnum and hooking it over the dorsum sellæ.

Some skulls are too heavily mineralized even for this expedient and many are so filled with dense matrix as to render the manœuvre impossible. Under such circumstances an alternative is sometimes available.

The correlative of the posterior lobe of the pituitary gland is the anterior lobe which migrates from the dorsal wall of the naso-pharynx as the pouch of Rathke to reach the pituitary fossa. The point of departure from the naso-pharynx is where the posterior border of the root of the vomer joins the body of the sphenoid. This point is frequently marked by a pit on the underside of the sphenoid. It is called the 'hormion' in physical anthropology. It serves as a useful substitute for the pituitary fossa as a common cranial centre and can be used in heavily mineralized skulls and in skulls solidly filled with matrix provided the region is clean and intact. The hormion can even be used on casts if they are accurately made. The hormion is not quite so accurate as the pituitary fossa because the depth of the body of the sphenoid varies from skull to skull but it is certainly more precise than most of the centring points utilized so far.

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MISCELLANEOUS

Explosion in an Autoclave caused by Cellulose Nitrate Tubes

The practice adopted in biological laboratories when disposing of contaminated material is to sterilize by steam in an autoclave, taking care to exclude anything known or suspected to lead to combustion. A latent danger exists if certain types of laboratory ware are treated in this way, without the research worker being aware that risk exists. An example of this occurred in this Establishment when a number of celluloid centrifuge tubes, potentially contaminated and intended for destruction, were sent for autoclaving. During the process spontaneous ignition of the tubes occurred, followed by explosive burning. The autoclave was wrecked and extensive superficial damage was done to the surrounding areas.

Although it can scarcely be regarded as good practice to autoclave material based on cellulose nitrate it is not patent that an explosion hazard exists. Information issued by the suppliers of the tubes in question includes reference to the inflammability of cellulose nitrate and the statement that they "cannot be autoclaved", but the statement doubtless means that the tubes are ruined by the treatment and gives no indication that the operation is potentially dangerous; it is possible that the chance of combustion is so small that there is no record of such an occurrence.

The circumstances in which the explosion occurred are both interesting and instructive. A number of unused celluloid tubes made for use with the ultracentrifuge were found to be unserviceable, having shrunk as a result of ageing for several years. The room in which they had been stored was used for work involving toxic materials, and it was decided to autoclave them as a safety precaution before discarding. The autoclaving procedure was a standard two-cycle operation, each cycle consisting of evacuation of the sterilizing chamber followed by admission of steam until a pressure of 20 lb./in.^{2} and a corresponding temperature of 125° was achieved and maintained for a prescribed period. The explosion occurred at the beginning of the second cycle at a point where the steam pressure was approaching the operating level of 20 lb./in.^{2}.

Examination of the recording instruments showed that no malfunctioning of the equipment had occurred. The probability that spontaneous ignition of the tubes was responsible for the accident led to tests being conducted at the Explosives Research and Development Establishment, Waltham Abbey. The tests demonstrated the thermal instability of some of the samples examined, and convincing proof of the cause of the explosion was obtained when an attempt to reproduce the explosion in simulated conditions was successful. From the various tests carried out at Waltham Abbey it was possible to attribute the cause of the accident to a combination of three factors; (1) age of the tubes; (2) temperature in the autoclave; (3) presence of water vapour.

The conditions needed to produce spontaneous combustion at such a low temperature are surprising to anyone who is not an explosives expert. In addition, the probability of the event and its severity are respectively dependent on the mass of material involved and the ratio of combustible mass to free volume of the chamber. In this explosion both the mass and the mass/volume ratio were high. Nevertheless, it is clear that material made from cellulose nitrate may present an explosion hazard when autoclaved and alternative methods of decontamination and destruction should be employed.

I thank colleagues at Waltham Abbey for verifying the cause of the accident.

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Life Tables

It is a pity that in reviewing the series of life tables recently compiled by Dr. R. A. M. Case *et al.*[1], Prof. L. Hogben has ignored the historical facts and thereby implied that actuaries have not only been unaware of the principles of cohort analysis in particular but also that their methods have also been of disservice to the public.

In point of fact the use of the life table (the adjective actuarial is redundant since all such tables are actuarial) was first introduced to research workers by William Farr about a hundred years before R. R. Kuczynski, and its use was re-inforced so far as the medical officers of health were concerned by George King in 1914 (ref. 2).

The limitations of life tables based on mortality rates experienced in a particular calendar year (or period of consecutive years) have long been appreciated by actuaries and the tables derived from the mortality of life office annuitants for 1900–20 (ref. 3), and also those derived from the mortality of Government annuitants for the same period[4], were both constructed on the principle of a cohort analysis of the recorded rates of mortality. In 1927 a paper was presented to the Institute of Actuaries by V. P. A. Derrick[5], which directed attention to the advantages gained from analysing mortality rates by generations rather than by calendar year, and similar thoughts were behind a paper presented to the Faculty of Actuaries by A. R. Davidson and A. R. Reid in the same year[6]. In 1936 the official journal of the Scandinavian actuarial societies published a paper by Cramér and Wold[7] in which a series of generation mortality curves were constructed from Swedish population data; while in 1953 the Institute and Faculty of Actuaries[8] published a volume of annuity tables which contained a series of hypothetical generation tables of mortality appropriate to annuitants. It is therefore inaccurate to imply that the method of cohort analysis is foreign to actuarial thought.

On the question of the use of actuarial expertize in the construction of English Life Tables it is important not to overlook that such tables have to serve many different purposes; in particular, the allegation of actuarial gamesmanship scarcely does justice to the painstaking efforts by the Registrar General to point out the limitations of a life table based on the combination of many generations. The construction of a life table must have some regard to the purposes for which it is intended to be used and the requirements for calculations appropriate for insurance company operations (where smoothness is important) will differ from those of the medical research worker.

The implied assumption that Greville's method is the only method of constructing abridged tables is also at variance with the facts. The supplement to the 75th Annual Report of the Registrar General (Cd. 7512, 1914) shows ample support of the use of abridged methods, and a comparison of seven different methods with the relative computation times has been available for some years[9]. It could also be argued that the method adopted by the Registrar General for constructing the abridged life table which appears annually in the September *Quarterly Return* has more common sense than Greville's method, the rationale of which involves assumptions regarding the analytical form of the rate of mortality.

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[5] Derrick, V. P. A., *J. Inst. Actuaries*, **58**, 117 (1927).
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[7] Cramér, H., and Wold, H., *Skandinavisk Aktuarietidskrift*, **18**, 161 (1935).
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[9] Benjamin, B., *Elements of Vital Statistics*, 120 (George Allen and Unwin, Ltd., 1959).

FORTHCOMING EVENTS

Tuesday, July 9

JOINT HEALTH PHYSICS COMMITTEE, BRITISH OCCUPATIONAL HYGIENE SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 9.30 a.m.—Conference on "Legislation and Codes for the Safe Use of Ionising Radiation".

Tuesday, July 9—Thursday, July 11

ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N.W.1), at 10 a.m. on Tuesday and Thursday, and 9.50 a.m. on Wednesday—Symposium on "Foraging and Feeding Behaviour".

Wednesday, July 10—Friday, July 12

INSTITUTE OF PHYSICS and the PHYSICAL SOCIETY (at the University of Oxford)—Conference on "High Magnetic Fields, Their Production and Their Applications".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER IN STATISTICS—The Registrar, The University, Keele, Staffs (July 12).

LECTURER IN CHEMISTRY, primarily to assist in the teaching of physical or industrial chemistry—The Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff (July 13).

PHARMACOLOGIST, to investigate changes in the composition of body fluids in migraine—Prof. M. Weatherall, The London Hospital Medical College, Turner Street, London, E.1 (July 13).

RESEARCH ASSISTANT (with a good honours degree in physics) to Dr. J. E. Enderby, for an investigation of the structure of liquid metals and alloys using neutron methods—The Registrar, The University, Sheffield 10 (July 13).

RESEARCH ASSISTANTS (with a diploma in technology or university degree in electrical engineering, and preferably experience in industry), to work with Dr. D. Karo, Reader in Light Current Electrical Engineering, in one or more of the following fields: power generation by means of semiconductors; non-destructive testing; instrumentation and telemetering; new materials in electrical engineering; supraconductivity and cold temperature phenomena; and development of novel instruments—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref. 6/63/6 (July 13).

SCIENTIST (Lecturer or Assistant Lecturer grade) IN THE CANCER RESEARCH DEPARTMENT, Caterham, to investigate the biochemistry of tumour growth—The Secretary, Marie Curie Memorial Foundation, 124 Sloane Street, London, S.W.1 (July 13).

SENIOR LECTURER IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Leicester (July 13).

DIRECTOR OF THE CRIPPS COMPUTING CENTRE, to be responsible for the development of computing facilities throughout the University, together with the associated advisory services and appropriate research and teaching—The Registrar, The University, Nottingham (July 15).

JUNIOR LECTURER (qualified in palæontology and stratigraphy) IN GEOLOGY—The Registrar, Trinity College, Dublin 2, Republic of Ireland (July 15).

RESEARCH ASSISTANT (with experience in tissue culture and mammalian chromosome techniques) IN THE DEPARTMENT OF PATHOLOGY, for research on congenital abnormalities—The Secretary, The University, Edinburgh (July 15).

ASSISTANT LECTURER (honours graduate in physics, and preferably an interest in electronics) IN THE DEPARTMENT OF PROSTHETICS, Turner Dental School—The Registrar, The University, Manchester 13 (July 16).

ASSISTANT LECTURER (with a degree in science with chemistry as a major subject, industrial experience, and preferably teaching experience) IN CHEMISTRY at Bendigo Technical College, Bendigo, Victoria, Australia—Council for Technical Education and Training for Overseas Countries, 12 Lincoln's Inn Fields, London, W.C.2 (July 17).

EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER (with some years of experience in metallurgy or physics, and preferably experience of an electron-microscope or micro-probe analyser) IN THE DEPARTMENT OF METALLURGY, to assist in work on creep in precipitation hardening alloys, with particular reference to electron-microscopy—The Registrar, The University, Manchester 13 (July 17).

LECTURER IN PHYSICS—Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (July 17).

RESEARCH ASSISTANT (graduate in electrical engineering or physics) IN THE DEPARTMENT OF METALLURGY, to assist in the development of methods for the measurement of energy dissipation in vibrating metals—Prof. K. M. Entwistle, Department of Metallurgy, The Manchester College of Science and Technology, Manchester 1 (July 17).

RESEARCH ASSISTANT (well qualified in sociology and interested in its application to education) TO THE PROFESSOR OF EDUCATION—The Registrar, The University, Keele, Staffs (July 19).

SENIOR LECTURERS and LECTURERS IN MATHEMATICS at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, July 19).

LECTURER IN THE DEPARTMENT OF GEOLOGY AND MINERALOGY—The Secretary, The University, Aberdeen (July 20).

LECTURER (with a special interest in virology and microbial genetics) IN THE MICROBIOLOGY DEPARTMENT—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 20).

SENIOR LECTURER and/or LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Sheffield (July 20).

ASSISTANT LECTURER (preferably with an honours degree or higher degree in physiology, and a veterinary or medical qualification) IN THE DEPARTMENT OF VETERINARY PHYSIOLOGY—The Secretary, The University, Edinburgh (July 22).

ASSISTANT LECTURER (with an interest in general experimental psychology) IN PSYCHOLOGY—The Registrar, The University, Keele, Staffs (July 22).

BIOCHEMIST (with M.Sc. or Ph.D. in biochemistry or physiological chemistry or equivalent, and at least three years satisfactory experience of biochemistry or chemical pathology) for the Cork Health Authority—The Secretary, Local Appointments Commission, 45 Upper O'Connell Street, Dublin 1, Republic of Ireland (July 23).

LECTURER IN ANTHROPOLOGY; and a SENIOR TUTOR IN ANTHROPOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 26).

SUB-LIBRARIAN (with a good honours degree and preferably relevant experience and professional qualifications) IN THE FACULTY OF ENGINEERING—The Registrar, The University, Liverpool, quoting Ref. No. CV/348/N (July 26).

ASSISTANT LECTURER IN STATISTICS (in the Unit of Biometry), for work which will involve teaching, consulting and research—The Registrar (Room 22, O.R.B.), The University, Reading (July 27).

EXPERIMENTAL OFFICER (with a university degree in science, veterinary science, rural science, agricultural science or equivalent qualifications, and experience in physiology or biochemistry, for example, clinical biochemistry), AT THE CATTLE RESEARCH LABORATORY, Division of Animal Genetics, Commonwealth Scientific and Industrial Research Organization, Rockhampton, Queensland, to assist in a research programme on the physiology of cattle, which includes the study of digestive processes, gross energy metabolism, and various aspects of biochemistry and metabolism—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 682/19 (July 27).

SENIOR RESEARCH FELLOW in one of the following fields in the Faculty of Science: Civil Engineering, Computer Engineering, Electrical Engineering, Mechanical Engineering, Mechanics of Fluids, Metallurgy, Nuclear Engineering or Soil Mechanics—The Registrar, The University, Manchester 13 (July 29).

LECTURER IN MYCOLOGY at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 30).

BIOMETRICAL GENETICIST IN THE PLANT BREEDING SECTION OF THE DEPARTMENT OF AGRONOMY, Waite Agricultural Research Institute, to undertake biometrical research associated with fundamental and applied aspects of breeding self- and cross-pollinated plant species; lecturing duties will be light—The Registrar, University of Adelaide, Adelaide, South Australia (July 31).

LECTURER IN ORGANIC CHEMISTRY at the University of Adelaide—The Registrar, University of Adelaide, Adelaide, South Australia (July 31).

LECTURER or ASSISTANT LECTURER IN PARASITOLOGY (Medical Helminthology) at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 31).

LECTURER (with a good degree in electrical engineering or in solid state physics or solid state chemistry) IN THE MATERIALS SECTION OF THE DEPARTMENT OF ELECTRICAL ENGINEERING, to take part in undergraduate and postgraduate teaching and to engage actively in materials research—Dr. J. C. Anderson, Electrical Engineering Department, Imperial College, London, S.W.7 (August 1).

RESEARCH OFFICER or SENIOR RESEARCH OFFICER (Veterinary Bacteriologist) (with a degree in veterinary science, or its equivalent, together with a Ph.D. degree or postgraduate research experience of an equivalent standard and duration supported by satisfactory evidence of research ability in a relevant field of bacteriology and immunology) IN THE MCMASTER ANIMAL HEALTH LABORATORY, Commonwealth Scientific and Industrial Research Organization, Sydney, to continue and further develop the research programme on footrot and other diseases of the feet of sheep—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 202/220 (August 3).

PROFESSOR OF PSYCHOLOGICAL MEDICINE at the University of Queensland, Brisbane, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 9).

LECTURER and an ASSISTANT LECTURER IN THE MECHANICS OF FLUIDS in the Faculty of Science—The Registrar, The University, Manchester 13 (August 15).

LECTURER or SENIOR LECTURER IN PHYSICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 15).

LECTURER (preferably with interests in the field of terrestrial or freshwater ecology) IN ZOOLOGY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 15).

SENIOR LECTURER IN PHYSIOLOGY with emphasis on Histology—The Secretary of University Court, The University, Glasgow (August 15).

ANDERSON CHAIR OF PSYCHOLOGY—The Secretary, The University, Aberdeen (August 17).

CHAIR OF APPLIED MATHEMATICS at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 19).

LECTURERS (2) (with special qualifications in inorganic, structural or physical chemistry) IN CHEMISTRY at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

RESEARCH FELLOW (with experience in archæological exploration and survey and preferably at home in other of the field sciences) IN PREHISTORY at the Institute of Advanced Studies, Australian National University, to initiate, prosecute and direct archæological work in Papua/New Guinea—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

SENIOR LECTURER (qualified optometrist with postgraduate training and research interests in physiological optics and visual perception) IN OPTOMETRY IN THE DEPARTMENT OF PSYCHOLOGY, University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

READER IN MICROBIOLOGY—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (September 1).

LECTURER (well qualified in engineering science or applied mathematics with engineering experience) IN MECHANICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 2).

CHAIR OF PHYSICS; and CHAIR OF MATHEMATICS IN THE SCHOOL OF MATHEMATICAL AND PHYSICAL STUDIES—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR. 05G (September 7).

DIRECTOR OF THE UNIVERSITY OF SHEFFIELD INSTITUTE OF EDUCATION—The Registrar, The University, Sheffield (September 7).

ASSISTANT EXPERIMENTAL OFFICER (with at least G.C.E. in five subjects including two scientific subjects at advanced level or equivalent; or, at

age 22, a pass degree or equivalent), for biological work on the insecticidal control of stored-product insects—The Director, Pest Infestation Laboratory, Agricultural Research Council, London Road, Slough, Bucks.

Assistant Professor or an Associate Professor (physiologist or biochemist, M.D. and/or Ph.D.) in the Department of Physiology—Prof. M. Beznak, University of Ottawa, Ottawa, Ontario, Canada.

Demonstrator (with a good honours degree in chemistry or biochemistry, or agricultural chemistry) in Food Science in the Department of Agricultural Sciences at the School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham.

Experimental Officers and Assistant Experimental Officers (with at least two G.C.E. "A" level scientific subjects, if over 22, H.N.C. pass degree or equivalent) at the Chemical Defence Experimental Establishment, Porton, near Salisbury, for research and development work on a wide range of problems in physics, chemistry, and experimental biology and physiology—War Office C.5F, London, S.W.1.

Graduate Physicist in the Medical Physics Department, to assist with radioactive isotope studies—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

Head of the Department of Chemistry and Geology (Grade IV)—The Principal, Kingston College of Technology, Penrhyn Road, Kingston-upon-Thames, Surrey.

Junior or Student Technician in the Surgical Unit, to assist in research on organ transplants and cancer treatment—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2.

Lecturer in Physical Chemistry; a Senior Lecturer in Mathematics; and an Assistant Lecturer (Grade B) in Mathematics—Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

Physiologist (with biochemical interests), to work in the pressure chamber at the Western Infirmary, Glasgow—Sir Charles Illingworth, Department of Surgery, The Western Infirmary, Glasgow, W.1.

Postdoctoral Research Associate in Experimental Spectroscopy—Personnel Office, Harvard University, 1352 Massachusetts Avenue, Cambridge 38, Mass., U.S.A.

Research Assistant (with a good honours degree in biochemistry, together with some research experience) in Biochemistry in the Urological Surgery Division (Renal Research Unit of the Department of Surgery), to participate in research into protein metabolism in renal disease—The Registrar, The University, Leeds 2.

Research Assistant (with a good honours degree, or diploma in technology, in physics) in the Physics Department, preferably for work on gas discharges—Head of the Physics Department, Northern Polytechnic, London, N.7.

Research Officer (Entomologist) (with an honours degree in biology with appropriate postgraduate experience and a national of, and permanently resident in, Great Britain or the Republic of Ireland), to plan and carry out research on the biology of malaria-carrying mosquitoes in East Africa, and to direct field and laboratory work of an established entomological research team—The Director of Recruitment, East African Common Services Organization, 3 Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. RC318/145/02.

Scientific Officer (honours graduate, preferably in biochemistry, agricultural chemistry or chemistry), to work, both in laboratory and with experimental animals, primarily on nutritional problems in pigs—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 63/7.

Scientific Officer in the Department of Grass/Animal Ecology, to undertake, as part of a team studying the ecology of the grazing animal, research into problems of animal health and performance arising out of the application of recently developed techniques in both animal and pasture production—The Secretary, The Grassland Research Institute, Hurley, Maidenhead, Berks.

Senior Technician (Grade I or II) in Biochemistry at the Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick—The Group Secretary, West Bromwich and District Hospital Management Committee, West Bromwich and District Hospital, West Bromwich.

Technician (preferably with experience in histological techniques) at the Marine Science Laboratories, Menai Bridge—The Registrar, University College of North Wales, Bangor, North Wales.

Technician (with A.I.M.L.T. in pathological chemistry or Final Certificate (Chemistry or Biochemistry), City and Guilds of London, or A.I.S.T.), for biochemical research—The Secretary, University College Hospital Medical School, University Street, London, W.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Imperial College. Inaugural Lectures, 1960–61 and 1961–62. Pp. iv+157. (London: Imperial College, 1963.) [165

Department of Scientific and Industrial Research. Report of the National Physical Laboratory for the year 1962. Pp. ix+251+13 plates. (London: H.M. Stationery Office, 1963.) 16*s*. net. [165

The House of the Royal Institution. By A. D. R. Caroe. Pp. 40 (10 plates). (London: The Royal Institution of Great Britain, 1963.) 3*s*. 6*d*. [165

Central Electricity Generating Board: Research and Development Department. The Identification of Atmospheric Dust by Use of the Microscope. By E. M. Hamilton and W. D. Jarvis. Pp. 32. (London: Central Electricity Generating Board, 1963.) [165

Anti-Locust Research Centre. Anti-Locust Bulletin No. 39: Observations on Night Flight in the Desert Locust (*Schistocerca gregaria* Forskal). By Jeremy Roffey. Pp. ii+32. (London: Anti-Locust Research Centre, 1963.) 8*s*. 6*d*. [165

1963 Campaign for Education. The Case for Advance. By the Archbishop of Canterbury, Lady Albemarle, Alan Bullock, Sir Geoffrey Crowther, and Sir Ronald Gould. Pp. 16. (London: 1963 Campaign for Education, c/o Hamilton House, Mabledon Place, W.C.1., 1963.) 1*s*. 6*d*. [165

National Institute of Industrial Psychology. Report No. 15: After the Take Over—a Study of Reactions of Employees in a Group of Companies. By Winifred Raphael and M. W. Zimmerman. Pp. iii+35. (London: National Institute of Industrial Psychology, 1963.) [165

Admiralty Marine Science Publication No. 1: H.M.S. "Owen" Oceanographic Cruise, (North and South Atlantic), 1960–1961. Pp. 17. (H.D. 532). Admiralty Marine Science Publication No. 2: Seismic Investigations in Tremadoc Bay, July 1961. Pp. 4. (H.D. 533). (London: Hydrographic Department, Admiralty, 1963.) [165

Tube Investments, Ltd. Choosing a Career in T.I. Pp. 20. Student Apprenticeship. Pp. 8. (Birmingham: Director of Personnel, Tube Investments, Ltd., 1963.) [165

Furniture Industry Research Association. Second Annual Report, 1962. Pp. 12. (London: Furniture Industry Research Association, 1963.) [165

Medical Electronics and Biological Engineering, Vol. 1, No. 1, (January–March 1963). (Official Organ of the International Federation for Medical Electronics.) Pp. 1–158. Annual Subscription rates: (a) for libraries, government departments, or industrial establishments £7 (20 dollars); (b) for subscribers who write direct to the publisher certifying that the journal is for their personal use £3 10*s*. (10 dollars); (c) for members of the International Federation for Medical Electronics £2 2*s*. (6 dollars). (London and New York: Pergamon Press, 1963.) [165

Department of Scientific and Industrial Research. Water Pollution Research 1962: The Report of the Water Pollution Research Board, with the Report of the Director of the Water Pollution Research Laboratory. Pp. vii+126+4 plates. (London: H.M. Stationery Office, 1963.) 8*s*. 6*d*. net. [165

Petroleum Information Bureau. U.K. Petroleum Industry Statistics: Consumption and Refinery Production, 1961 and 1962. Pp. 8. (London: Petroleum Information Bureau, 1963.) [165

University of Cambridge: Department of Engineering. Report of the Head of the Department for the year 1961–62. Pp. 14. (Cambridge: Department of Engineering, The University, 1963.) [165

The British Journal Photographic Annual, 1963. Edited by Arthur J. Dalladay. Pp. 524+32 plates. (London: Henry Greenwood and Co., Ltd.; New York: Amphoto, 33 West 60th Street, 1963.) 10*s*. net. 3 dollars board covers; 12*s*. net. 3.50 dollars cloth bound. [215

Other Countries

United States Department of the Interior: Geological Survey. Water-Supply Paper 1696–A: Determination of Beta Activity in Water. By F. B. Barker and B. P. Robinson. Pp. iii+32. (Washington, D.C.: Government Printing Office, 1963.) [165

Transvaal Museum, Pretoria. Report for the year ending 31st March, 1962. Pp. 38. (Pretoria: Transvaal Museum, 1963.) [165

International Atomic Energy Agency, Vienna. Bibliographical Series, No. 9: Radioisotopes and Ionizing Radiations in Entomology. Pp. 414. (Vienna: International Atomic Energy Agency; London: H.M. Stationery Office, 1963.) 168 schillings; 48*s*.; 8 dollars; 32 NF.; 28 D.M. [165

Fonds National de la Recherche Scientifique. Trente-cinquième Rapport Annuel, 1961–1962. Pp. 349. (Bruxelles: Fonds National de la Recherche Scientifique, 1963.) [165

Smithsonian Contributions to Astrophysics. Vol. 5, No. 15: Solar Radio Bursts of Spectral Types II and IV—Their Relations to Optical Phenomena and to Geomagnetic Activity. By Barbara Fell. Pp. ii+239–257. (Washington, D.C.: Government Printing Office, 1963.) 30 cents. [165

Ceylon. Department of Fisheries: Fisheries Research Station. Bulletin No. 13: Bibliography of Pearl Oysters. By S. Sivalingam. Pp. 21. Bulletin No. 15: Some Marine Algae from Ceylon—1. By M. Durairatnam. Pp. 16. (Colombo: Fisheries Research Station, 1962.) [165

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 101: Écologie et Sociologie des Microchampignons du sol de la Cuvette Centrale Congolaise. Par Dr. J. A. Meyer. Pp. 137. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1963.) 150 francs. [165

Centro de Investigaciones Agricolas, Alberto Boerger. Memoria Anual—1962. Pp. 51. (La Estanzuela, Colonia, Uruguay: Centro de Investigaciones Agricolas, Alberto Boerger, 1963.) [165

Institutt for Atomenergi, Kjeller Research Establishment. Kjeller Report No. 42: Quarterly Progress Report: October–November–December, 1962. Pp. vii+37+14 figures. (Kjeller: Institutt for Atomenergi, Kjeller Research Establishment, 1963.) [165

University of California Publications in Geological Sciences. Vol. 43: Oligocene Biostratigraphy of the Santa Barbara Embayment, California. 1: Foraminiferal Faunas from the Gaviots and Alegria Formations. By R. M. Kleinnell and D. W. Weaver. 2: Mollusca from the Turritella Variata Zone. By D. W. Weaver and R. M. Kleinpell. 3: Systematic Catalogue and Register of Localities. Pp. vi+250+38 plates. 8 dollars. Vol. 44, No. 2: A Review of the Macropodid Genus *Protemnodon*. By R. A. Stirton. Pp. 97–162. 1.25 dollars. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1963.) [215

Council of Scientific and Industrial Research, India. Annual Report of the National Metallurgical Laboratory, 1961–1962. Pp. iv+178. (Jamshedpur: National Metallurgical Laboratory, Council of Scientific and Industrial Research, 1963.) [215

Organisation for Economic Co-operation and Development: European Nuclear Energy Agency. First Report on the Activities of the Eurochemic Company, 1959–1961. (European Company for the Chemical Processing of Irradiated Fuels). Pp. ii+378. (Paris: Organisation for Economic Co-operation and Development, European Nuclear Energy Agency, 1963.) [215

List of Publications by the Members of the European Tissue Culture Club and their Co-workers. September 1961—October 1962. Pp. 59. (Liège: Prof. M. Chevremont, Université de Liège, Institut d'Histologie, 1963.) [215

India. University Grants Commission and Ministry of Education. Vice-Chancellors' Conference, 1962. Pp. v+81. (New Delhi: University Grants Commission and Ministry of Education, 1963.) Rs. 2.00; 4*s*. 8*d*.; 0.72 dollars. [215

British Territories in Borneo: Geological Survey Department. Bulletin No. 4: Proceedings of the British Borneo Geological Conference, 1961, held at the Geological Survey Office, Kuching, Sarawak, 25 to 30 November 1961. Edited by Dr. F. H. Fitch. Pp. xx+184+13 plates. (Kuching: Government Printer, 1963.) 6 Malayan dollars. [215

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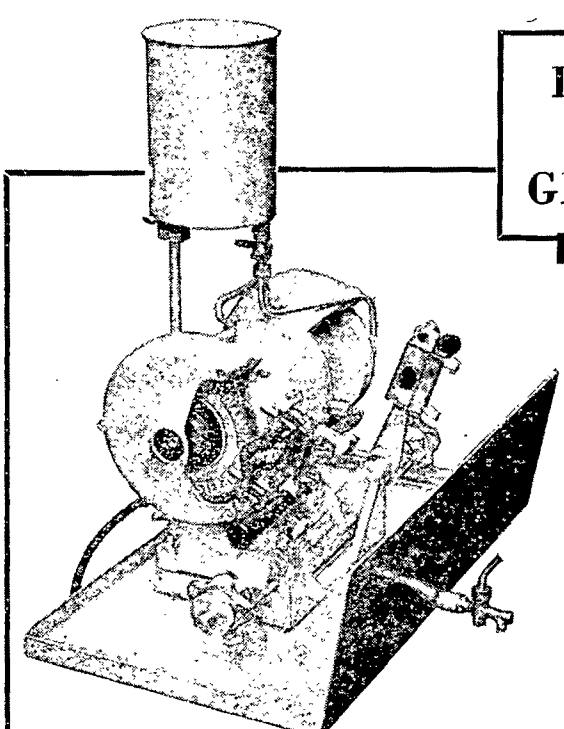
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SCIENCE AND THE INTELLECTUAL TRADITION

BY those who heard it, Dr. S. Toulmin's address to Section L, "Science and Our Intellectual Tradition", was reckoned to be one of the outstanding addresses at the 1962 meeting in Manchester of the British Association for the Advancement of Science: it has now been published in the May issue of *The Advancement of Science*—the bi-monthly journal of the Association.

Dr. Toulmin's questioning of the fragmentation of science makes stimulating reading in the light of questionings along similar lines in the United States, for example, Mr. M. K. Hubbert's presidential address to the Geological Society of America last November, in which he argued that science in the United States of America was in a state of confusion (*Science*, 139, 884; 1963). Further, some remarks of Dr. Toulmin regarding education are particularly apposite reading in the light of Prof. Kenneth Denbigh's observations in a recent book, *Science, Industry and Social Policy*, and Lord Hailsham's desiderata for education in science in *Science and Politics* (see *Nature*, 198, 1023, 1123, 1125; 1963).

Dr. Toulmin begins by pointing out that in education, as in some other spheres of human life, the direction of our thinking is deeply affected by the system of general, unexamined assumptions characteristic of our day and age and suggesting that we may now be at a turning-point, so that in the second half of the twentieth century our intellectual environment may differ widely from what it has been during the first half. In particular, he suggests that there are signs that the drive towards intense and concentrated specialization may be checked and that there is at last a real drive to re-integrate the specialist disciplines into which our educational habits have become fragmented and to set them working together in the service of a fuller understanding. This new current of thought is exerting its influence, he thinks, all over the intellectual scene, and he instances the growing impatience with class parties and rigid nationalism in politics, the attention being given in the universities to new forms of faculty structure and, within science itself, the desire to apply the technical gains of the past five or six decades to the re-integration of intellectual life.

Evidence in support of Dr. Toulmin's view is easily discerned in Lord Hailsham's writings, but when he discusses science and more particularly the conflict between the Athenian and Alexandrian elements in our scientific tradition, Dr. Toulmin goes much deeper. He suggests that the fundamental reason why Greek scientists failed to follow up thoroughly the ideas of Archimedes and Hipparchus was a failure of intellectual nerve. With the transition to Alexandria, science, from being 'natural philosophy', became a kind of intellectual technology. In the sixteenth century, scientific enquiry at a really profound level revived, not because of some supposedly novel experimental method, but because the old Ionian confidence had revived. For the next four centuries, as for those following 550 B.C., the mainspring of scientific progress has been philosophical, not technological, and the Athenian impulses at the heart of modern science have fired the imagination of literary men in both periods.

Now, however, science is becoming increasingly dominated by Alexandrian forces which threaten once again to reduce it from being regarded as natural philosophy to being considered as "an assemblage of skills—practical, technological, theoretical and mathematical". The sheer pressure of finance and patronage is siphoning off hands and brains into 'gadgeteering', most of it without serious hope of philosophical fruit. On Athenian considerations, he argues, our money would be going not into space flight or research but into international co-operation in nuclear physics, into molecular biology, embryology, psychopharmacology and ethology, apart from the other behavioural sciences. He believes there is real danger that our obsession with technique may stifle the natural philosophy from which it sprang.

If Lord Hailsham had any misgivings as to concluding his book on a philosophical note, they should be removed by Dr. Toulmin's impressive argument. Even more noteworthy, however, is the resemblance between this conclusion and that reached by Mr. Hubbert from a much more pragmatic review of the position of science in the United States. Challenging strongly the way in which the prevailing academic system of preferment, with its criterion of publication and output for research, favours the opportunist capable of producing scientific trivialities in large numbers as against the true scholar working on difficult and important problems the solution of which may demand years of concentrated effort, Mr. Hubbert is critical of the whole transition of educational into research institutions in the United States. This he regards as definitely retrogressive and threatening to science, the Government contract-grant system being a main contributory factor. In consequence of this neglect by the universities of their primary responsibilities as institutions of learning and education, scientists in the United States appear to have lost sight of their intellectual foundations and resorted to authoritarianism.

Mr. Hubbert's analysis is, however, deeper than this brief summary would suggest, and his ultimate thesis is that the present confusion and tendency to authoritarianism arise from a fundamental misconception of the nature of science and its evolution. Evolution here is not a progression from the simple to the complex but from the complex to the simple. Advance is always in the direction of reducing the initial chaos to a form within the scope of human comprehension, and without denying the value of co-operative team work he insists that thought is essentially an individual activity and that the greatest of scientific achievements have almost invariably been the work of individuals.

In his judgment, therefore, the primary condition of a renaissance in education, in scholarship and in science is to attempt to achieve an atmosphere congenial to individuals of the type of Gallileo, Kepler, Newton, Darwin or Willard Gibbs. The universities must become institutions of learning again, and be provided with more orderly support. Competent teaching must receive the respect it deserves and the curriculum so revised that it is mandatory for students to receive a working knowledge of the fundamental principles of science. The preoccupation

with trivialities that pass for research must go and with it book-keeping based on the number of papers published in a year.

Dr. Toulmin's discussion and analysis of education is more detailed, and though he prescribes with some reserve, the conclusion is not dissimilar. He, too, challenges the autonomous claims of the different academic disciplines, suggesting that instead of thinking in terms of two cultures we should think rather of the different subjects as different approaches to, and departures from, the common core of questions and ideas which originally formed the intellectual tradition of Europe, and is now shared by the whole world. Dr. Toulmin, however, suggests a picture: we should think of the different academic disciplines, not as separate entities, but as the segments of a fan, overlapping at the centre but branching off from there in different directions, or perhaps, better still, as the different branches of a tree, which have all sprung, and draw their life, from a common trunk.

The overlapping region, the common ground, or common trunk, which all the subjects share is the central core of ideas on which they all alike have a bearing. Science, for example, unites the tradition of natural philosophy with a collection of mathematical and manual skills, and, as Dr. Toulmin shows, the bodies of general ideas in all the subjects he instances are related to one another far more closely than their different skills and techniques. While we cannot afford to undervalue these techniques, since they represent the proper contribution which each subject can make to the progress of our common intellectual debate, they derive their sap, value and interest, their intellectual justification, from their connexion to a central body of general questions and ideas. They are not self-sufficient ends.

Dr. Toulmin does not question that some of the forces that have driven disciplines apart are respectable enough; however, he does suggest that it is time to think afresh not simply about our preoccupation with techniques and skills but about those administrative and pragmatic forces which in universities and elsewhere have tended to divert education away from general ideas and critical habits of mind, and towards the inculcation of skills and abilities. The importance of the experiments already being undertaken in the University of Keele, the University of Sussex and that contemplated at Norwich, or the exciting opportunities which confront these new institutions generally could scarcely be better displayed though they are not specifically mentioned. At least it is on the universities rather than the schools that he places the responsibility for the missing element in our education to-day—for the lack of the sense that the things being taught, learned and examined are living parts of a larger, developing unity, of our intellectual tradition. The schools cannot ensure that students of science no longer leave school devoid of deep understanding, even of their own professional schools, or unaware of the common traditions they share and the mutual relevance of even their own subjects unless the universities co-operate in clearing away the sandbanks built up during the first half of this century.

The problem of examinations is at the heart of Britain's practical quandary and the present fragmentation will persist just so long as the examinations for the General Certificate of Education remain geared-up to test for skills and factual knowledge rather than for understanding and a grasp of general ideas. It is not enough, Dr. Toulmin continues, for matriculation boards to exhort teachers to watch their pupils' English expression and powers of criticism: "until the Boards resolutely enforce demands for adequate intellectual grasp lucidly expressed—to the point of failing mere performers, however skilled—the general run of schools will have no sufficient motive for taking on the admittedly harder aspects of education". Despite, however, the support to be found for his views, Dr. Toulmin is not optimistic as to the chances of developing the new attitudes needed short of the establishment of some new examining body, such as a 'New Universities' Examining Board', able to create from scratch the educational organization appropriate to the need, nor is optimism warranted by the fact that something of Dr. Toulmin's approach is to be found in an address by Dorothy M. Emmet on "Science and the Unity of Thought" at a symposium in Manchester almost twenty years ago.

Nevertheless, the soil is now much better prepared for Dr. Toulmin's ideas to take root. Something of his outlook is to be found in an address by H.R.H. the Duke of Edinburgh to the Association for Science Education on April 26. Quoting Prof. J. H. Plumb's remark criticizing the reticence and ambiguities of historians and their reluctance to accept the social responsibilities that their subject imposed on them, and asserting that the prime activity of history should be to teach non-historians not to perpetrate its own practitioners, the Duke remarked that science also needed to teach non-scientists as well as to train its own practitioners, with the added responsibility of providing for a rapidly changing environment. He added that no valuable work could be done to-day in any academic subject or in industry and commerce without some basic knowledge of science, and that applied science demanded at least an understanding of social development and pure science some appreciation of its historical background. The Duke suggested that the intensity of the discussion over the relative importance of science and the humanities tended to obscure more important issues from the community at large and to overlay the common ground which a director of scientific research, a business manager or a bishop, for example, might share as administrators.

While the Duke of Edinburgh, at this point, was concerned to stress the importance of a scientific approach to management, irrespective of whether particular scientific or professional knowledge was also needed, his observation illustrates another point arising out of Dr. Toulmin's paper. It is this understanding of our common intellectual traditions, including appreciation of the scientific approach, that must form the substance of the science which the ordinary citizen requires. Beyond that, however, the appreciation of the magnitude of that common ground and tradition could help to check fragmentation in other directions that is also detrimental to modern society. In a paper, "Interaction of Technologies", also given at the British Association meeting in Manchester (see *Nature*, 197, 222; 1963) and also published in the May issue of *The Advancement of Science*, Mr. L. Holliday develops the thesis that fundamental advances in technology are more likely to accrue from searching for the fundamental principles in individual technologies, and for the relations between them than from pursuing the usual practice of studying them by industries. It follows that a more analytical approach to technology as a whole is required, and that technologists should be encouraged to look beyond their individual specialities.

Deprecating the multiplicity of technological societies, institutions and journals, Mr. Holliday, like Dr. Toulmin, to this end visualizes considerable changes in the structure of university departments of technology and of colleges of technology. Equally important, he suggests a radical reorganization of the industrial research associations into larger units in which resources for really effective fundamental research would be available. To shift in this way from a multiplicity of associations based on different industries to one based on some eight or nine technologies would present the Department of Scientific and Industrial Research with a formidable task, probably even more in respect of education and of finance than of organization and location. However, Mr. Holliday is on solid ground in making the suggestion, and Dr. Toulmin's argument gives him firm support.

This alone illustrates how much more is at stake in this question of safeguarding intellectual traditions than educational organization alone. Some of the fringe benefits, as it were, have already been indicated, as in Mr. Holliday's proposals. Another might well be an increased supply of competent technicians instead of the threatened shortage, if matriculation boards, for example, began to fail the mere performers who show insufficient intellectual grasp and directed them to the technical schools and colleges for training as technical assistants. It is, however, for its effect on the intellectual attitudes of the two new generations that a re-united and well-directed educational system is most imperative. The future of science and much else depends, as Dr. Toulmin and Mr. Hubbert both agree, on the ability of those two generations to sustain the Athenian attitudes of mind, whose critical probing and speculative freedom have been the main stimulus of scientific growth. Dr. Toulmin has provided the essential criteria by which the forthcoming proposals of the Robbins Committee should in due course be assessed.

CURRENT PROBLEMS IN VERTEBRATE PALÆONTOLOGY

Problèmes Actuels de Paléontologie (Évolution des Vertébrés)

(Colloques Internationaux du Centre National de la Recherche Scientifique, No. 104, Paris, 29 Mai–3 Juin, 1961.) Pp. 474. (Paris: Éditions du Centre National de la Recherche Scientifique, 1962.) 62 N.F.

IN June 1961 a colloquium was held in Paris on vertebrate palæontology, and the papers read, together with the discussions, are now published in book form. Two such meetings have previously been held in Paris, in 1947 and 1955, and the latest coincided with the opening of the new palæontology laboratories in the Institut de Paléontologie, Muséum National d'Histoire Naturelle. Prof. Lehman, director of the Institute, is to be congratulated on the excellence of the laboratories, the success of the colloquium which he was responsible for organizing and for the high standard of the proceedings which he has edited.

The papers in the volume rather remind one of company reports to boards of directors. It is assumed the board will know the background and wish only to hear of the year's progress. Some reports detail clear and important advances, some have little that is new to record, few introduce any startling new innovations. Evolution of the phylum Chordata is stated to be the theme of the colloquium. Of the 43 papers offered, 11 deal with fish groups, 10 with reptiles and 22 with mammals (and of these 5 deal exclusively with primates). Yet despite the weighting in favour of the higher vertebrates, papers on the lower vertebrates deal with more fundamental problems. Mammalogists are largely, when not talking monkeys, concerned with faunal problems, stratigraphy and geographic distribution. The fish and reptile enthusiasts are concerned with the origin of major groups and their affinities, phylogenies, functional anatomy and the evolution of particular organs—ear, scales, rostrooral region.

Among the papers on lower vertebrates outstanding are those of the Stockholm school, from Stensiö, Ørivg and Jarvik. Stensiö gives a concise account of his lepidomorial theory concerning the nature of the placoid scale. The work was stimulated by studies made on edestid elasmobranchs from the Upper Permian of East Greenland. All early elasmobranchs and holocephalans, up to and including Carboniferous forms, possess 'cyclomorial' scales, that is, scales that grow by marginal additions of concentric zones of growth, while all post-Permian forms to the present day possess only placoid scales. The Permian edestids have both cyclomorial and placoid scales and in them the development can be traced through partial to total reduction of growth zone. The cyclomorial scale itself is formed of a fusion of small elements—lepidomora. These studies, supported by a vast wealth of detailed observations, some of which have already appeared in print, represent a major advance. Stensiö and his colleagues are not the first to tackle the evolutionary problems of scale histology, but the first for many decades and by far the most thorough; so thorough that it is important not to lose sight of the overall patterns. Ørvig, who has collaborated closely with Stensiö on the placoid problem, contributes an interesting paper in which he argues the affinity of ptyctodont arthrodires with holocephalans. A new Devonian ptyctodont, *Ctenurella*, from Germany allows detailed comparison of cranial anatomy to be made with holocephalans. Ørvig concludes the latter are nearer arthrodires than bradyodonts, that the two groups may have a common arthrodire ancestor or share direct lineal descent. Jarvik has in earlier work championed a diphyletic origin for the amphibia, deriving anura from osteolepid crossopterygians and urodeles from porolepid crossopterygians. In the present paper he develops his ideas on descent of urodeles from porolepids, basing his thesis largely on the anatomy of snout and branchial regions. Another fish paper worth noting is Lehman's account of the double articulation in arthrodires and its importance in swimming.

Olson re-approaches the problem of reptile origins. He stresses the lack of evidence at critical points and characteristically emphasizes the importance of environmental adaptation, especially to food supplies—a theme he developed so successfully in his great paper on the Permian faunas of the United States and the U.S.S.R.[1]. Olson considers that among the first tetrapods two main adaptive trends can be distinguished, the predator fish-feeders and the small aquatic carnivorous types. He recognizes three early streams of reptile evolution, seymouriamorph—diadectid—chelonian, captorhinomorph—synapsid and captorhinomorph—milleretid—diapsid, with Sauropterygia and Ichthyopterygia more likely to originate in the middle than the latter stream. Haas gives a detailed account of the mandibular musculature of snakes and deduces from this study relationships similar to those based on other morphological data. Hoffstetter reviews critically recent work on squamate classification, but concludes that the time is not yet ripe to draw up a phylogenetic table.

Lavocat returns to the enigma of rodent origins, dwells on the conflicts between anatomical lines of evidence and geographic distribution, implies that sciurognathous and hystricognathous are more meaningful than sciuromorph and hystricomorph, and favours the evolution of similar structures in different parts of the world. Kermack

measures the degree of mammalization achieved by late Triassic times and concludes that through the Mesozoic mammalian evolution was slow and regular. Russell has examined the only two known Palæocene mammal faunas from Europe, Cernay and Walbeck. His detailed studies are keenly awaited and in a short paper he compares the composition and environmental niches of the two faunas. Cernay, with yields of about 7,000 teeth, is characterized by multituberculates, meniscotheriid condylarths, primates, insectivores and creodonts. Walbeck has yielded about 4,000 teeth, the species are more varied, insectivores and creodonts are dominant and herbivores poorly represented. Russell suggests slight age differences, and indicates that the faunal unbalance at Walbeck could be due to preservation bias as well as to ecological differences. Brink's experimental work on stereophonic hearing in dogs, while scarcely conclusive, is important in illustrating the sort of investigation that demands serious attention. Deschaseaux throws light on differing evolutionary trends in the brains of bats, brontotheres and rhinoceroses, and differentiates between fixed characters (such as the *sella turcica*) and adaptive characters (such as the optic specializations). Piveteau's continued study of Primates enables him to reaffirm that cerebralization is a major character of the class.

The selection of topics discussed is an interesting reflexion on present-day trends of thought in vertebrate palæontology. The signal generalization that can be made is that the substantive advances in understanding have all depended on the discovery of critical new field evidence—a lesson that commands reflexion.

R. J. G. Savage

[1] *Trans. Amer. Phil. Soc.*, N.S., 52: *Late Permian Terrestrial Vertebrates, U.S.A. and U.S.S.R.* By Prof. Everett C. Olson. Pp. 224 (21 plates). (Philadelphia: The American Philosophical Society, 1962.)

ADVANCES IN ENTOMOLOGY

Annual Review of Entomology

Vol. 8. Edited by Ray F. Smith, in association with Thomas E. Mittler. Pp. vii+529. (Palo Alto, Calif.: Annual Reviews, Inc., 1963.) 8.50 dollars.

THIS volume, the eighth of the series, is made up from the following papers: insect toxins and venoms (R. L. Beard); autointoxication and some stress phenomena (J. Sternburg); mode of action of carbamates (J. E. Casida); some evolutionary aspects of the insect thorax (R. Matsuda); the phylogeny of the Homoptera (J. W. Evans); insect orientation (R. Jander); soil-inhabiting arthropoda (W. Kühnelt); biological aspects of some phytophagous mites (H. B. Boudreaux); significant developments in European corn borer research (T. A. Brindley and F. F. Dicke); genetics of mosquitoes (G. Davidson and G. F. Mason); trypanosomiasis and the tsetse fly problem in Africa (K. C. Willett); development of systemic insecticides for pests of animals in the United States (R. C. Bushland, R. D. Radeleff; and R. O. Drummond); fumigation of insects (A. B. P. Page, and O. F. Lubatti); factors affecting the use of microbiological pathogens in insect control (J. W. MacBain Cameron); sericulture (T. Yokoyama); mutualism between ants and honeydew-producing Homoptera (M. J. Way); the social biology of ants (E. O. Wilson); arthropod transmission of plant viruses (K. Maramorosch); some new aspects in forest entomology (H. Francke-Grosmann); aphid feeding and nutrition (J. L. Auclair).

The editors' preface mentions two of the problems of such a series: those fields of study in which new knowledge is accumulating so rapidly that "these topics lend themselves to retreatment every several years"; and the particular difficulty of covering the field of insect systematics in reviews. The former is one consequence of the immense volume of new work, most conspicuously in the chemistry of living insects, and in experimental studies of their behaviour. Indeed, each year one puts down this *Annual Review* with a determination to read it carefully and so to become a well-informed general entomologist, and each year goes by without time even to do this. There will soon be need for an abstract of the *Annual Review*.

For various reasons details of systematics are not so palatable in a review as the details of chemistry or physiology. The systematic articles, which the editors say they try so hard to include, are either accounts of systematic methods and techniques, or occasional speculations about the phylogeny of a particular group. In the present volume J. W. Evans's views on the Homoptera may be compared with R. A. Crowson's treatment of the Coleoptera in Volume 5; I wonder if the latter author would agree with the former that Hemiptera are "the most isolated phylogenetically of all orders of insects"?

There is certainly a need for more writing about systematics as a way of looking at insects, rather than as a dry, mechanical system of classification. The editors themselves commend the evolutionary point of view, and suggest that the truest expression of systematics is to contribute to a deeper understanding of insect life. I would go further and says that a durable systematics must be an integration of evidence from all aspects of biology, morphology, physiology and behaviour alike. Reviews in these various fields tend to take their systematics for granted, using names supplied by a specialist systematist. They ought to be balanced by other reviews that use the biological evidence to question the systematics.

The present volume contains several examples of a review that can be read with pleasure for general information, and equally studied in detail as an introduction to its field.

H. Oldroyd

ASSIMILATORY CELLS AND TISSUES IN PLANTS

Das Tropische Parenchym

A: Assimilationsgewebe. Von Prof. Dr. Fritz Jürgen Meyer. (Handbuch der Pflanzenanatomie, Bd. IV, Teil 7 A.) Pp. x+188. (Berlin–Nikolassee: Gebrüder Borntraeger, 1962.) 88 D.M.

ALL students of botany are familiar with the structure of assimilatory tissues and cells in at least a few species of plants. There are, however, many variations in the structure both of the assimilatory tissues and of the cells of which they are composed that are less generally known. Because of this, plant anatomists and those who are concerned with the physiology of photosynthesis will welcome the appearance of a new edition of F. J. Meyer's treatise on the subject. The first edition of Dr. Meyer's book was published in 1923 and constituted a slim volume of 87 pages that formed part of Linsbauer's great work of reference entitled *Handbuch der Pflanzenanatomie*. Since the Second World War the publication of the *Handbuch* has been resumed, and a series of new volumes on various aspects of plant anatomy are being prepared by specialists and edited by Prof. W. Zimmerman (Tübingen) and Prof. P. G. Ozenda (Grenoble). In its new guise the *Handbuch* has become an international encyclopædia of plant anatomy and will include contributions in English and French as well as in German.

In order to bring the subject up to date, Dr. Meyer's volume on assimilatory tissue has now been expanded to 188 pages. In it, after a brief discussion of the meaning of the terms assimilation and assimilatory tissue, the author describes and classifies the various forms assumed by assimilatory cells. This classification is based not only on the shapes of the cells but also on any peculiarities which they may exhibit, such as infoldings or invaginations

of the cell walls. Here it seems strange to find that the well-known assimilatory cells of the Coniferae with finely invaginated walls are not classified as arm-cells, which are described separately. This, however, is only a minor point of criticism, for the classification of cell types seems otherwise to be generally acceptable. After discussing the assimilatory cells themselves the author goes on to describe their organization into tissues and the distribution of the assimilatory tissues within the plant body, and we are also told about the mode of development of assimilatory cells and the secondary changes that they undergo. Part 3 of the book is concerned with the walls of assimilatory cells and in Part 4 we pass on to the cell protoplasts and their inclusions. In Part 5, which concludes the main body of the text, the structure of assimilatory tissues is considered in relation to its physiological function and to the strains and stresses which are imposed on it by the environment. The book concludes with a bibliography occupying 10 pages, the compilation of which was closed in August 1961, and with separate indexes referring respectively to subjects, to the families and genera of plants and to authors.

The book is one for which a place should be found in all botanical reference libraries and in all laboratories where the investigation of plant structure is being actively pursued.

C. R. METCALFE

OXYGENASES

Oxygenases

Edited by Osamu Hayaishi. Pp. xii + 588. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962). 125*s*.

FIFTY years have elapsed since Sir Frederick Gowland Hopkins, in his address to the British Association at Birmingham, described the life of the cell as "the expression of a particular dynamic equilibrium which obtains in a polyphasic system". As the science of biochemistry has developed since 1913, Hopkins's central idea of a dynamic state of chemical compounds in the living cell has been accepted universally. This conception in turn raised a problem: How may these unceasing biochemical transformations be reconciled with the constancy of pattern which we discern both in the chemical events themselves and in the cellular architecture, complex yet persistent in finest detail? The mechanism, as envisaged by Watson and Crick, by which the intricate molecular designs of proteins are replicated has been the inspiration of a great and increasing sector of biochemical research; and indeed we may need to remind ourselves that there still remain to be investigated many of the enzymes by which, as Hopkins also foresaw in his Birmingham address, all these metabolic reactions are catalysed. This excellent book reminds us that much of one important area of enzymology remains unexplored.

As Osamu Hayaishi remarks in a chapter which surveys the history and scope of the subject, the investigation of biological oxidation was begun by Lavoisier about two hundred years ago. Since that time, it became a firmly accepted principle of biochemistry that oxidations proceed by the removal of hydrogen atoms or electrons which may be transferred from primary substrates through carriers to molecular oxygen: in some instances, oxygen might serve as their direct acceptor, but in all cases the oxygen was converted into water or hydrogen peroxide. However, eight years ago investigations by two groups of workers associated with H. S. Mason and O. Hayaishi respectively proved, by the use of $^{18}O_2$ and $H_2{}^{18}O$, that, in the course of certain oxidations, oxygen was incorporated directly into the substrate molecule and did not act simply as an ultimate electron acceptor. These reactions are catalysed by enzymes to which the name 'oxygenase' has been given. A 'true' oxygenase incorporates two atoms of atmospheric oxygen into one molecule of substrate, as in the conversion of catechol into *cis–cis*-muconic acid; whereas a 'mixed function' oxygenase, or hydroxylase, incorporates one atom of the oxygen molecule into the substrate and requires an electron donor despite the fact that the substrate is oxidized: the second atom of oxygen is reduced to water. Mixed function oxidases that hydroxylate the benzene nucleus are reviewed in an authoritative chapter by Seymour Kaufman.

The work described in this book establishes the significance of various oxygenases in general metabolism. Hayaishi quotes a striking experiment in which he grew *Pseudomonas* with various aromatic compounds as sources of carbon and showed that 4–9 per cent of the oxygen of cell constituents was derived from atmospheric oxygen. One is reminded of the situation concerning carbon dioxide fixation in 1935 when, on the basis of very careful chemical analysis, Werkman and Wood showed that this gas could be fixed by heterotrophic bacteria, whereas it was then generally believed that this type of assimilation was confined to plants and autotrophic bacteria. The principle of heterotrophic utilization of carbon dioxide was not accepted by many biochemists at first, but by the use of isotopes of carbon it has since been proved that ability to fix carbon dioxide is possessed by practically all forms of life.

The methodology of oxygen isotopes is fully described in a chapter by D. Samuel; and it is because of findings made with this valuable technique that we must accept the fact that oxygen can be fixed biologically, despite a reluctance that might stem from our knowledge of biological oxidations built up from investigations spanning a quarter of a century. P. Nicholls considers "peroxidase as an oxygenase" which, apparently, it is not; however, this chapter takes us skilfully through the maze of contradictory findings and theories about peroxidases. One who has not worked in this field is left with the impression that this indeed is the pursuit of truth for its own sake: much has been learned about the pitfalls of enzymology, the interpretation of enzyme kinetic data and the devising of elegant techniques; but it is difficult to assess the physiological significance of peroxidases and the investigation of dihydroxyfumarate oxidase appears to be, in the words of Nicholls "the study of a chemical accident". On the other hand, there is no doubt, when one considers the carbon economy of this planet, about the significance of phenolytic oxygenases discussed by A. Mehler.

Plants lock up carbon atoms in cyclic structures and soil bacteria release them by the action of these enzymes: they are therefore essential for the continuance of life. Until four years ago it was thought that bacteria invariably open the benzene nucleus between adjacent carbon atoms bearing hydroxyl groups. It is now known that this does not always occur and that ring-fission may be catalysed by '2:3-oxygenases' which, while attacking dihydric phenols, open the benzene ring between a carbon atom bearing a hydroxyl group and one that does not. Very recent work at Bangor (Prof. W. C. Evans, private communication) has shown that such enzymes attack condensed aromatic ring compounds, which are discussed by J. W. Foster in a chapter on the bacterial oxidation of hydrocarbons; accordingly it is probable that some of the metabolic schemes presented in this chapter will shortly require modification and extension. Other chapters deal with oxygenases in lipid and steroid metabolism, phenolase, model oxygenase systems, cytochrome oxidase, hæmoglobin and myoglobin, and hæmerythrin and hæmocyanin; they are all written by acknowledged experts in the respective fields and the book is edited by Osamu Hayaishi, whose researches on the oxygenases have led to so much fruitful activity.

This book is a notable contribution to enzymology.

S. DAGLEY

DISTRIBUTIONS OF *n*-PARAFFINS IN CRUDE OILS AND THEIR IMPLICATIONS TO ORIGIN OF PETROLEUM

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PETROLEUM is now generally accepted to have been derived from organic remains of plant and animal life deposited with sediments. The types of organic compounds which served as precursors, however, are open to conjecture. Among materials prominently mentioned are fatty acids, alcohols, free hydrocarbons, steroids, isoprenoids of several types, and amino-acids.

Fatty acids have probably received the most support as petroleum precursors[1–3]. This is because they are widely distributed in plants and animals and have structures similar to *n*-paraffins, which are abundant in crude oils. The fatty acids—saturated as well as unsaturated—exist as parts of both fats and waxes. In fats, they occur as triglycerides and generally are unbranched with an even number of carbon atoms between C_{12} and C_{22} (ref. 4). In waxes, they usually exist as simple esters and again are generally unbranched with an even number of carbon atoms; although some have chains under C_{22}, most are generally between C_{24} and C_{36} (ref. 4).

The waxes also contain long-chain alcohols and free hydrocarbons, both of which also are possible petroleum precursors. The alcohols occur both in the free state and as esters with the fatty acids; they generally have straight chains and are monohydric. Alcohols between C_{10} and C_{36} have been identified; the more abundant ones[5] are the even-numbered members of C_{14}–C_{18} and C_{26}–C_{34}. Free hydrocarbons of each carbon number between C_{22} and C_{36} have been found in certain waxes[6]; the members with an odd number of carbon atoms are in the majority.

A likely reaction for fatty acid source materials, among many possible, would have been decarboxylation to give hydrocarbons with an odd number of carbon atoms. If this reaction occurred, it could have left petroleum with an excess of straight-chained compounds with an odd number of carbon atoms. An excess of *n*-paraffins with odd numbers of carbon atoms in the C_{23}–C_{35} range has been found in the organic matter of recent sediments[7], but until now has not been found in crude oils. Previous failures to find any such excesses in crude oils have cast doubts on whether fatty acids acted as petroleum precursors. Theories have been proposed[3], however, to explain how fatty acids could have been source materials without leaving an excess of odd-numbered remnants.

As part of an extensive programme on the analysis of crude oils[8], we began a gas-chromatographic investigation of *n*-paraffin distributions in crude oils. The *n*-paraffin amounts were sought for the clues they might give on petroleum precursors.

Forty crude oils, many selected on the basis of a high *n*-paraffin content, have now been surveyed for *n*-paraffin distributions by gas chromatography. Eight of the more interesting ones have been analysed intensively to obtain accurate values for each *n*-paraffin to C_{35}. Some crude oils show 'predominances' of certain odd-numbered members; that is, they contain at least several per cent more of these than would be predicted from the amounts of the neighbouring even-numbered *n*-paraffins. The major such predominances are between C_{11} and C_{19}. Those between C_{21} and C_{35}, where much of the previous searching has been concentrated[7], are smaller and less frequent.

Several different analytical techniques were used for determining distributions of *n*-paraffins. Initially, the 40 crudes were run on a packed gas-chromatographic column with temperature programming. Eight of the more interesting of these were then examined by one or both of two other techniques to obtain a more precise determination of the *n*-paraffins. In the first technique, paraffins were concentrated by removing sulphonatable components (for example, aromatics) by reacting the whole crude oil with sulphuric acid; the paraffin concentrates were then run on a temperature-programmed capillary column of high efficiency. This approach gave a better resolution of *n*-paraffins from the background of other peaks and was especially useful between C_8 and C_{18}. In the second technique, *n*-paraffins above C_{15} were removed essentially quantitatively from the crudes by adduction with urea[9]; the *n*-paraffin concentrates, which contained only small amounts of interfering compounds, were then analysed by gas chromatography on the packed column with temperature programming. Analyses for *n*-paraffins to C_{35} were obtained by comparing results from these three types of runs. Uncertainties in the analyses should be no greater than 7 per cent of the amounts found or 0·02 per cent, whichever is larger.

In the programmed-temperature runs on whole crude, about 90 per cent of the samples gave *n*-paraffin peaks which clearly projected above the background of other peaks and were measurable semi-quantitatively. In these, the range and maximum of the distributions of *n*-paraffins could be determined, and any significant predominance of odd-numbered members could be detected.

The packed-column temperature-programmed runs were made on a column, 6 m × 3 mm, containing 10 per cent '*SE*-30' silicone rubber (General Electric Co.) on 'Chromosorb-*P*. The temperature was started at 60° C and programmed at 4°/min to 400°. Detection was by thermal conductivity or hydrogen flame ionization.

Distributions of *n*-paraffins in the 40 crude oils varied considerably. Typically, the *n*-paraffins comprised 15–20 per cent of the total crude. In four crude oils, however, *n*-paraffins above C_7 were barely detectable. In four others, the members through C_{35} comprised more than 30 per cent of the total crude. When *n*-paraffins were measurable, they were present throughout the entire range to C_{35}. In many crudes, however, they were nearing extinction at C_{35}. The maximum concentrations usually were at C_6 or C_7, but sometimes occurred as low as C_4, and in one case as high as C_{27}.

A predominance of certain odd-numbered *n*-paraffins was detectable in 14 of the 40 crude oils. It was clearly evident in seven of the fourteen. It was only slightly above the detectable limit in the other seven. A predominance was detectable when an *n*-paraffin was 3–7 per cent higher than would be predicted from the sizes of the neighbouring *n*-paraffins.

Table 1. Normal Paraffins in Eight Crude Oils
(% by volume)

No. of Carbons	John Creek	Kawkawlin	Pine Unit	Ponca City	Uinta Basin	Darius	North Smyer	State Line
4	0·21	0·77	1·67	0·60	0·36	0·33	2·33	0·14
5	0·47	1·27	2·13	1·38	0·40	0·84	2·16	0·27
6	0·81	2·07	2·18	1·97	0·40	1·37	1·55	0·45
7	1·12	2·46	2·53	2·21	0·44	1·57	1·34	0·54
8	1·37	3·08	2·67	2·09	0·44	1·50	1·11	0·65
9	1·55	3·20	2·89	2·02	0·46	1·35	0·98	0·76
10	1·71	3·14	2·95	1·83	0·44	1·14	0·82	0·90
11	2·28	3·65	3·65	1·65	0·45	1·09	0·70	1·18
12	1·50	2·48	2·29	1·61	0·43	0·94	0·63	1·37
13	2·07	2·57	3·08	1·48	0·45	0·81	0·55	1·47
14	1·54	2·06	2·09	1·17	0·52	0·77	0·50	1·54
15	1·80	2·09	1·83	1·09	0·61	0·62	0·47	1·62
16	1·09	1·14	1·36	0·79	0·68	0·57	0·44	1·68
17	1·72	1·40	1·63	0·83	0·75	0·50	0·41	1·66
18	0·33	0·36	0·29	0·55	0·73	0·46	0·36	1·68
19	0·79	0·48	0·65	0·55	0·76	0·40	0·34	1·71
20	0·18	0·18	0·21	0·41	0·79	0·35	0·30	1·64
21	0·14	0·16	0·16	0·38	0·87	0·30	0·23	1·56
22	0·11	0·14	0·15	0·33	0·93	0·25	0·18	1·46
23	0·10	0·12	0·12	0·29	1·00	0·22	0·13	1·44
24	0·08	0·10	0·11	0·26	0·97	0·20	0·11	1·36
25	0·06	0·08	0·08	0·23	1·04	0·18	0·09	1·28
26	0·05	0·07	0·06	0·22	0·99	0·15	0·07	1·10
27	0·04	0·06	0·06	0·17	1·07	0·12	0·06	1·01
28	0·03	0·05	0·05	0·15	0·95	0·10	0·04	0·72
29	0·02	0·04	0·04	0·11	0·98	0·09	0·04	0·65
30	0·02	0·03	0·03	0·09	0·72	0·07	0·03	0·45
31	0·01	0·02	0·02	0·07	0·69	0·06	0·02	0·36
32	0·01	0·02	0·02	0·06	0·50	0·05	0·02	0·18
33	0·01	0·01	0·02	0·05	0·43	0·04	0·01	0·16
34	0·01	0·01	0·02	0·04	0·28	0·03	0·01	0·13
35	0·00	0·01	0·01	0·03	0·21	0·02	0·01	0·10
	21·23	33·32	35·05	24·71	20·74	16·49	16·04	31·22

Table 2. Description of Crude Oils

Field	Location	Gravity (°API)	Producing formation		
			Name	Era	Age
John Creek	Morris Co., Kansas	26·6	Viola	Palæozoic	Ordovician
Kawkawlin	Bay Co., Michigan	35·0	Dundee	Palæozoic	Devonian
Pine Unit	Wibaux Co., Montana	31·9	Red River	Palæozoic	Ordovician
Ponca City	Kay Co., Oklahoma	42·0	Simpson	Palæozoic	Ordovician
Uinta Basin (field unnamed)	Uintah Co., Utah	30·6	Green River	Cainozoic	Tertiary (Eocene)
Darius	Persian Gulf	29·0	Yamama	Mesozoic	Cretaceous
North Smyer	Hockley Co., Texas	43·2	Strawn	Palæozoic	Pennsylvanian
State Line	Sweetwater Co., Wyoming	39·4	Fort Union	Cainozoic	Tertiary (Palæocene)

Distributions of *n*-paraffins for the eight intensively analysed crudes are given in Table 1. Five of them—John Creek, Kawkawlin, Pine Unit, Ponca City, and Uinta Basin—show predominances of certain odd-numbered *n*-paraffins. The three others with little or no predominances—Darius, North Smyer, and State Line—are included for comparison. Table 2 gives field location, age and name of producing formation, and American Petroleum Institute (API) gravity for the eight crude oils.

The *n*-paraffin distributions in four of the crude oils are shown in Fig. 1; three different types of distributions are illustrated. John Creek and Pine Unit show large predominances of odd-numbered members between C_{11} and C_{19}. Uinta Basin shows small odd-numbered predominances and a high proportion of *n*-paraffins in the C_{20}'s and C_{30}'s. Darius shows a 'typical' distribution.

Three crudes—John Creek and Pine Unit (Fig. 1), and Kawkawlin—have similar and strikingly large odd-numbered predominances. All have significantly more of the C_{11}, C_{13}, C_{15}, C_{17}, and C_{19} members than would be predicted from the amounts of the neighbouring even-numbered members. Also, the *n*-paraffins in each show a maximum at C_{11} and a sharp drop around C_{18}. Small differences exist, however, in the degree of the predominances at the different carbon numbers. Unlike the other two, Pine Unit shows only a very slight predominance at C_{15}. Pine Unit and Kawkawlin show their largest predominances at C_{11}; John Creek has its largest at C_{17}.

Ponca City also shows some similarities to John Creek, Kawkawlin, and Pine Unit—with predominances at C_{13}, C_{15}, C_{17}, and C_{19} and a drop around C_{18}. The odd-numbered predominances, however, are smaller and the drop around C_{18} is less sharp.

Strikingly, all four of these crudes are produced from early Palæozoic formations, which are the oldest normally yielding petroleum. Moreover, of the crude oils surveyed, essentially all of this age show detectable predominances of this sort, whereas none of younger age shows more than a suggestion of such predominances. Examples of other crude oils with predominances between C_{11} and C_{19}, are: Davis Ranch (Wabaunsee Co., Kansas), Eola McLish and Eola Oil Creek (Garvin Co., Oklahoma), Glendive (Dawson Co., Montana), Redwater (Alberta, Canada), and Teas (Garza Co., Texas). All these are from Ordovician formations except Redwater, which is from Devonian. Predominances in Davis Ranch and Glendive are large and somewhat similar to those in John Creek, Kawkawlin, and Pine Unit. No crudes from Cambrian or Silurian formations were analysed.

The distribution of *n*-paraffins in the Eocene-Age Uinta Basin crude is much different from that in the Ordovician and Devonian crudes. The proportion of *n*-paraffins in the C_{20}'s and C_{30}'s is significantly greater than in any other crude analysed; the largest member is C_{27}. Except for C_{17}, there is little or no predominance of any *n*-paraffin below C_{21}; however, from C_{23} to at least C_{33}, there is a small predominance of the odd-numbered members, each of which is a few hundredths of a per cent higher than would be indicated from the even-numbered members. *n*-Paraffins in Uinta Basin appear to be in two groups, one with a maximum at about C_9 and the other with a maximum at about C_{27}.

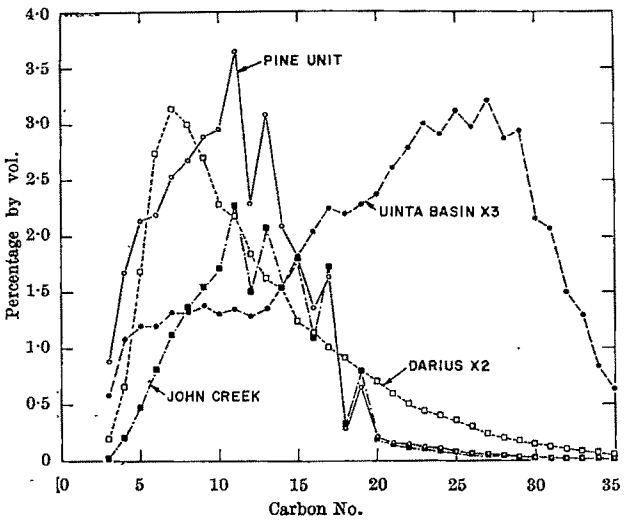


Fig. 1. Distributions of *n*-paraffins in four crude oils

Although Darius, North Smyer, and State Line possess little or no predominances, they again illustrate different *n*-paraffin distributions. The *n*-paraffins in Darius illustrate a 'typical' distribution—no significant predominances, a maximum at C_7, and no sharp drop around C_{18}. North Smyer shows a steady decrease of *n*-paraffins from C_4 to C_{35}. State Line, on the other hand, has an unusually high proportion of *n*-paraffins in the higher carbon numbers, with a maximum at about C_{17}; it gives indications of possessing small odd-numbered predominances in the C_{20}'s.

Two of the crude oils in Fig. 1—Uinta Basin and Pine Unit—show indications of slight odd-numbered predominances between C_5 and C_9. Unfortunately, these predominances, if real, are too small to be identified with certainty.

Implications of Results to Origin of Petroleum

The strikingly high proportions of *n*-paraffins in crude oils, typically 15–20 per cent and sometimes more than 30 per cent, suggest straight-chain compounds as an important type of petroleum source material. Such facts, among others, have led other investigators[1–3] to postulate fatty acids as principal petroleum precursors. Our findings of predominances of odd-numbered *n*-paraffins between C_{11} and C_{19} support this idea.

John Creek, Kawkawlin, Pine Unit, and Ponca City were probably derived from source materials rich in fatty acids from naturally occurring fats. The C_{11}–C_{19} predominances match well with the usual carbon range of the fatty acids in fats, which is C_{12}–C_{22}. Simple decarboxylation, by one means or another, would yield compounds of the correct chain-length. Alcohols—some of which exist naturally with chains of C_{12}–C_{20}—possibly could have caused the C_{11}–C_{19} predominances, but they would have had to lose a carbon atom along with the hydroxyl group. Waxes, which generally contain chains of C_{24}–C_{36}, are not indicated as principal source materials for these four crudes.

In contrast, Uinta Basin crude, with odd-numbered predominances and a large proportion of *n*-paraffins between C_{23} and C_{33}, could well have been derived from source materials rich in waxes. The odd-numbered predominances approximate the range of carbon chain-lengths in naturally occurring waxes. This proposed wax derivation agrees with its supposed non-marine origin; waxes in the C_{22}–C_{36} range are more prevalent in terrestrial life than in marine life[10]. The State Line crude also could have been derived from a source material rich in waxes. It also is believed to be of non-marine origin.

Most of the crudes observed to have high proportions of *n*-paraffins in the C_{20}'s and C_{30}'s, like Uinta Basin and State Line, have been of Cainozoic age. This could be a result of a larger proportion of plants having high wax contents in this era than in the earlier eras[10]. Waxes are normally most abundant in the cuticles of leaves, petals, and fruit coatings; they would probably be supplied in greater quantity by the flowering plants dominating the Cainozoic Era than by the fern and related plants prominent during the late Palæozoic and early Mesozoic Eras.

The odd-numbered predominances between C_{23} and C_{33} in Uinta Basin crude may indicate derivation from fatty acids, alcohols, or free hydrocarbons—all of which occur in waxes. Fatty acids might be the most logical guess. The alcohols, most of which are even numbered as they exist naturally, would have had to lose a carbon atom along with a hydroxyl group. The naturally occurring free hydrocarbons are mainly odd numbered, and, although present only in very small amounts, also are a possibility[11].

Even if C_{12}–C_{20} fatty acids were accepted as important source materials for the Ordovician and Devonian crudes, several puzzling questions remain:

(1) Why are the predominances at C_{11} and C_{13} so large, when the C_{12} and C_{14} acids typically comprise only 5–10 per cent of the fatty acids in naturally occurring fats? Most of the fatty acids are generally C_{18} and C_{16}.

(2) Why do most crude oils not possess odd-numbered predominances, and not show a sharp drop in *n*-paraffins around C_{18}?

(3) Why do the crudes showing odd-numbered predominances between C_{11} and C_{19} come only from early Palæozoic formations?

The strong odd-numbered predominances at C_{11} and C_{13} seem explainable, if all the predominances are assumed to have resulted entirely from *saturated* fatty acids. Naturally occurring saturated acids, unlike unsaturated ones, are not concentrated so heavily at C_{18} and C_{16}. For example, the overall proportions of saturated acids among total fatty acids in world-wide vegetable fats[4] are: C_{12} (7 per cent), C_{14} (3 per cent), C_{16} (11 per cent), C_{18} (3 per cent), and C_{20} (< 3 per cent). Although the fats in the simple animals and plants of the Palæozoic Era may not have been identical to present-day vegetable fats, the example illustrates that naturally occurring saturated acids can exist relatively evenly dispersed between C_{12} and C_{20}. It is unlikely that unsaturated acids could have been dispersed in this manner, because the C_{12} and C_{14} unsaturated acids are not major constituents of natural fats[4].

Most of the crude oils examined did not show odd-numbered predominances or a sharp drop around C_{18}; a possible explanation follows from the assumption that the predominances where present came from saturated acids. *Unsaturated* acids, it seems to us, could have been the principal source materials for most crudes, especially those younger than Devonian. The unsaturated acids, or perhaps their olefinic remnants, would have been more prone to other reactions[1], including polymerization, than the saturated acids. Extensive polymerization followed by random cracking[2] could have yielded a smooth distribution of *n*-paraffins with no sharp drop after C_{18}, even if the source acids were mainly C_{18} and C_{16}.

The sharp drops after C_{18} with John Creek, Kawkawlin, and Pine Unit suggest, of course, that very little polymerization occurred with these crudes. Cracking reactions also must have been relatively small, because the maxima of the *n*-paraffin distributions are at C_{11}, rather than at the more usual C_6 or C_7. With only slight polymerization, less cracking would be expected, because the smaller molecules would be thermodynamically more difficult to crack.

The usual lack of both odd-numbered predominances and a sharp drop after C_{18} also could be explained by

source materials with carbon chains above C_{18}. For example, extensive cracking of source materials principally of C_{24}–C_{36} could have yielded the observed distributions. Such an explanation, however, would require an abrupt change in principal source material after the Devonian age, where the source material appears to have been principally in the C_{12}–C_{20} range.

The explanation for finding C_{11}–C_{19} predominances only in crude oils from early Palæozoic formations is even more conjectural. Some of the arguments have already been introduced. The first, of course, would be that higher proportions of saturated acids were produced by the plants and animals in that age. This is a possibility, because uniformly warm climates are believed to have prevailed during most of the early Palæozoic Era[12], and living forms in warm climates tend to produce a higher proportion of saturated acids[4]. For example, in those plants which thrive in both hot and cold climates, higher proportions of saturated acids are produced in the hot climates[4]. As further support for saturated acids, Breger[1] has proposed the theory that the paraffinic character of old oils may be a consequence of a higher proportion of saturated lipids in their source materials.

The fact that C_{11}–C_{19} predominances were found only in early Palæozoic crude oils also could be a consequence of simpler forms of life present during that time[10]. The proportion of animal life possibly was higher then. Plant life apparently increased both in amount and complexity during the Devonian age[10]. Crude oils younger than Devonian, then, could have had higher proportions of source materials other than fatty acids; these other materials might have yielded a majority of the *n*-paraffins. Other evidence for a greater proportion of fatty acids in the source material for Palæozoic crude oils comes from carbon-isotope ratios. Lower values of $^{13}C/^{12}C$ are found in early Palæozoic crude oils than in most younger crudes[13,14]. This has been interpreted to indicate a relatively greater contribution from the lipid fraction, which has a lower value for $^{13}C/^{12}C$ than that from the whole plant or animal[13].

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DUPLEX ORIGIN OF PETROLEUM

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RECENT contributions bearing on theories of the origin of petroleum have considered the problems from the geological point of view, naturally including some of the geochemical aspects associated with questions of distribution. The organic chemical arguments derived from an investigation of the composition of petroleum (also natural gas, waxes, and bitumens) have been neglected or slurred over, the serious difficulties being set aside for later resolution.

An examination of the available evidence convinced me that petroleum hydrocarbons are both biogenic and abiogenic, the latter portion being very much the older, probably by a factor of 4 or 5 times the maximum hitherto contemplated for biogenic petroleum. These views have been expounded in lectures in many centres from 1961 to the present year—Delhi, Milan, Glasgow, Manchester, Bonn, Oxford, Aerojet Research Laboratory (California), Johannesburg, Westchester Chemical Society (Tarrytown, New York), Dublin and again at Milan. An abstract of the paper to the symposium at Bonn was published[2].

The chief points are the following:

(*a*) The evidence for the biogenesis of petroleum is incontrovertible; but this may only apply to a part of the material. The indications of biogenesis are clear in young oils, less in evidence in those of middle age, and all but absent in the older crudes[3]. They do not completely disappear in any of the crudes yet examined with sufficient care.

(*b*) The adherents of 100 per cent biogenesis assume that the organic matter laid down in the sediments could be modified at quite moderate temperatures and pressures in the course of ages until it became the ancient petroleum which we describe as a 'crude' oil. The apology for the improbabilities that have to be accepted is the large time-scale involved, perhaps 400–500 million years. It is the inverse of the 'housemaid's baby' plea and equally unconvincing. Actually it cannot be too strongly emphasized that petroleum does not present the composition picture expected of modified biogenic products and all the arguments from the constituents of ancient oils fit equally well, or better, with the conception of a primordial hydrocarbon mixture to which bio-products have been added.

(*c*) Improvements in chromatographic analysis and other new resources, such as separation of normal hydrocarbons as complexes with urea, have given us a much more complete knowledge of the constituents of petroleum in the lower ranges[4]. It emerges that the straight-chain hydrocarbons always predominate over any one of the branch-chain isomers, though the latter in total in each carbon-number exceed 50 per cent of the hydrocarbons having a carbon number of 6 or more. In the ancient petroleums (including natural gas) the percentage of the whole drops steeply from methane to pentane and then gradually through all the carbon numbers examined. There are a few weak peaks of occurrence at odd carbon numbers, but the alternation in the occurrence of hydrocarbons as we pass up the series is almost absent. Such alternation is perhaps the most characteristic proof of biogenesis in oils from recent sediments.

(*d*) An examination of the nature of branch-chain hydrocarbons in the smaller carbon numbers (C_5–C_8) and of their quantitative relation to the corresponding *n*-alkane suggests that the branch-chain hydrocarbons could have been derived by isomerization processes. These have apparently been accompanied by *cyclo*dehydrogenation, for example, *n*-hexane to 2-methylpentane and methyl*cyclo*pentane, *n*-heptane to 2- and 3-methylhexane, methyl*cyclo*hexane and then to dimethyl*cyclo*pentane.

(*e*) Certain minor constituents are best regarded as the outcome of synthesis. For example, adamantane is most plausibly postulated as arising from *cyclo*pentadiene by dimerization, reduction and then pyrolysis[5].

(*f*) The absence of oxygenated compounds and of olefines is indicative of a hydroforming type of operation. Aromatic hydrocarbons, thiophenes, and pyridines survive because of their relative high degree of resistance to hydrogenation.

(*g*) Crude petroleum is similar to the mixture which could be expected as the result of hydroforming of Fischer–Tropsch hydrocarbons. As an alternative to the Fischer–Tropsch synthesis of the primordial oil, the action of radiation on methane (with formation of carbene) may be considered. This would furnish straight-chain hydrocarbons, but the ingenious theory of A. T. Wilson[1] using methyl radicals and terminal attack of crowded chains is rather improbable, and could be submitted to test by experiment.

(*h*) It is supposed that the primordial oil (perhaps ± 2,000 million years ago, and later up to the deposition of the sediments) was used as a source of carbon by primitive organisms. These contributed components to the oil and, later, phytoplankton and land plants did likewise. It can easily be understood that the picture is blurred, and that its original component parts are difficult to recognize in even semi-quantitative detail at the present time.

We start with a synthetic mixture of hydrocarbons which may be assumed to be essentially a series of *n*-alkanes. This was submitted to thermal and possibly catalytic reforming under reducing conditions. Such processes may have been effective more than once, for example, both before and after the incorporation of biological material. At certain stages cracking to lower hydrocarbons (perhaps olefines) and synthesis appear to have occurred and it is noteworthy that the postulated constituents from bio-sources must also have encountered strong reducing conditions. After the association with bio-activities the material was distributed and may well have been separated in various ways, two of which are solution and deposition in water, and clathrate filtration through sediments or porous rocks.

The branched and *n*-alkanes in the C_5, C_6 and C_7 groups (beyond C_7 our information is still insufficient) are not present in the proportions required by attainment of full thermal equilibrium under conditions so far examined. However, we are not dealing with a pure substance, and cross-reactions between the molecules not only might, but probably would, affect the outcome.

As an example of the type of difficulty encountered in attempting an analysis of the data it may be mentioned that the content of 2-methylpentane is greater than that of 3-methylpentane[4]. This is the anticipated result of isomerization of *n*-hexane. On the other hand, the content of 3-methylhexane is usually a little greater than that of 2-methylhexane, and there must be some special reason for this apparent anomaly. It is not at all difficult to devise explanations for these and other similar findings, but it is scarcely worth while to discuss the alternatives, especially as this aspect of the problem is not beyond the reach of experimental investigation.

Addendum. Since submitting this article, I have been afforded the opportunity to read the preceding article by R. H. Martin, J. C. Winters and J. A. Williams in advance of publication. It is a contribution of outstanding value and, as the authors indicate, the new information raises fresh problems, the solution of which cannot be obtained on inspection.

Full comment must be reserved; but some general remarks may be made.

The authors start by noting that the biogenic theory is now generally accepted. They proceed to attempt to explain their results in terms of this (100 per cent) hypothesis. Actually, however, the striking and novel observation of odd predominance in crude oils from palæozoic formations refers to the minority of the petroleum examined.

The authors' puzzling question (2) should perhaps be re-phrased so as to ask for an explanation of these exceptional cases.

The remarkable differences found for the first time in the *n*-alkane distribution in ancient crude oils are, it seems, to be related to changes in the source organisms or their biochemistry. They are at last equally consistent with the duplex origin of petroleum which, indeed, could be expected to lead to such divergencies. Finally, the authors' suggestion of a decarboxylation mechanism is not strongly supported by what is known of the metabolism of fatty acids. If it occurred as a major process in the formation of *n*-alkanes in the remote past there should surely be some evidence of its importance at the present time. For example, *n*-pentadecane and *n*-heptadecane should be found in quantity in some plant or animal.

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CHEMICAL ALTERATION OF URINE

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RE-USE and reclamation of water have been widely practised in industry and irrigation. The need is a result of a limited number of easily accessible and suitable sources. Water recovery systems for manned-space flights have received considerable attention. This type of need also exists in community fall-out shelters. Re-use and reclamation of water in the latter two cases need not be restricted to recovery of potable water. Uses of water necessary for maintaining proper hygienic conditions include bathing and laundry, and as a waste disposal medium.

The quality requirements of a water supply depend on the usage or function for which the supply is intended. High-pressure boiler water has a low tolerance for inorganic scale-forming impurities; characteristics of potable water may vary over a considerable range, but rigid standards for specific chemicals or pathogenic organisms are desirable (it is to be noted that distilled water is not the optimal potable water); water used for agricultural purposes may impose unique limitations, for example, boron.

One possible method of purification of urine which requires no external source of power is the alteration of urine by purely chemical means. Most of the research in purification of urine has aimed at producing a potable water and has utilized techniques such as distillation and vacuum distillation followed by vapour pyrolysis, zone refining, freeze-drying, electrodialysis, etc.[1–4]. Physico-chemical treatments have been utilized only as secondary processes following an initial step such as distillation. The relative advantage of a given process depends on the characteristics of the feed, the desired quality of the effluent, the amount of power available, and the absence of nuisance conditions. The reclamation of water from

urine by distillation or any process requiring heat causes the liberation of volatile compounds not originally present and which are highly odoriferous. In certain cases, such as community fall-out shelters, it may therefore be undesirable to produce a water from human urine by processes requiring heat.

The chemical and physical characteristics of urine vary with the diet of the individual and the length of time elapsed between excretion and analysis. For example, the excretion of urea ranges from 4·7 g/day for persons on a low-protein diet to 31·5 g/day for persons on a high-protein diet[5]; ammonia may be present in large amounts as a result of the decomposition of urea. A number of authors present urine analyses[6]. Any listing of the constituents is necessarily incomplete because of the presence of many trace compounds and the difficulties associated with microanalysis for these compounds. The two gross constituents are urea and sodium chloride. Other materials which are excreted in amounts greater than 1 g/day are creatinine, potassium, phosphates and sulphates.

Craig[1] reports on operationally distilling urine for a drinking water supply. Investigations were also made on the distillation of urine by Ingram *et al.*[7]. Konecci and Wood[2] outlined a urine-distillation unit capable of distilling 20 lb. of water a day and requiring 300 W of power; the urine is centrifuged and heated; the vapour then passes through an activated charcoal filter and condenses on a 40° F condenser panel; the condensed water is then passed through another charcoal filter; a second distillation may be desirable. Golueke *et al.*[8], in discussing distillation, point out that a large amount of heat is required, and toxic fumes, particularly ammonia, are present.

Sendroy and Collison[3] and Bambenek and Zeff[9] mention freeze-drying as another means of purification. This consists of freezing urine, subliming the ice at 0·11–0·15 mm pressure, and then collecting the vapour.

The two processes of distillation and freeze-drying have proved workable; however, they do have some shortcomings. One of the main problems associated with distillation is the thermal breakdown of the compounds in urine which produces toxic or obnoxious fumes. Ingram[7] points out that the distillate from a distillation process will require additional treatment to remove carry-over taste and odour-producing compounds. Konecci and Wood[2] also allude to this possibility. Both the freeze-drying and distillation processes need fairly complex equipment. This is especially true of the former method because of the high vacuum needed. The power requirements of both processes may be disadvantageous in certain cases.

One of the possible methods of urine purification which requires no external source of power is the alteration of urine by purely chemical means. Chemical oxidation followed by ion exchange has the potential of producing a usable water from urine. Urea is the major component present in urine, and the successful removal of the former would provide the essential initial step in any scheme for chemical alteration. Two possible oxidizing agents would be nitrous acid and calcium hypochlorite. The nitrous acid scheme is attractive since the reaction products would be carbon dioxide, nitrogen, and water; thus, apparently no additional ions would have been added to the treated urine. Precipitation of urea as urea nitrate and hydrolysis of urea by urease are other possible methods of removal of urea from urine. For the removal of objectionable inorganic and certain ionizable organic molecules, the technique of ion exchange demineralization could be used. The sequence of a base exchange resin followed by an acid exchange resin would be advantageous from the point of view of utilization of the maximum exchange capacities of the resins.

This investigation attempted to explore the feasibility of removal of urea followed by ion exchange, using chemical and physical tests as criteria of water quality. Oxidation of urea by nitrous acid and calcium hypochlorite, precipitation of urea by nitric acid, and hydrolysis of urea by urease were examined under ambient conditions (24° C and 760 mm mercury). The exchange capacities of two commercial resins operating on an acid-base cycle were determined using raw urine as the feed. Analyses were made on the feed and effluents from the exchange columns for total solids, for *p*H, for colour, and for the ionic concentrations of sodium, potassium, calcium, chloride, and sulphate.

Reaction of Urea. The schemes for urea removal were suggested by available methods for the analytical determination of urea in urine. The oxidation of urea by nitrous acid giving water, nitrogen, and carbon dioxide as end-products is attractive if the urine is to be treated afterwards by ion exchange. Unfortunately, nitrous acid is known only in solution, and must be prepared as needed by the reaction of ammonia and hydrogen peroxide, sodium nitrite and sulphuric acid, or similar schemes. Urea concentrations in aqueous solution and in urine were determined with the Mclean ureometer[10]. As with many reactions involving organic compounds, the oxidation of urea does not proceed quantitatively as outlined by the stoichiometric equation. Approximately twice the stoichiometric amount of nitrous acid (63 g/l.) was necessary for the oxidation of urea in aqueous solution (24 g/l.) and in urine.

The result of using calcium hypochlorite, a stronger oxidizing agent than nitrous acid, is shown in Fig. 1.

$$3\,Ca(OCl)_2 + 2CO(NH_2)_2 = 3CaCl_2 + 2CO_2 + 2N_2 + 4H_2O \quad (1)$$

Approximately 120 per cent of the stoichiometric amount of calcium hypochlorite (144 g HTH, 65 per cent available chlorine) is required for the oxidation of urea in aqueous solution (20 g/l.).

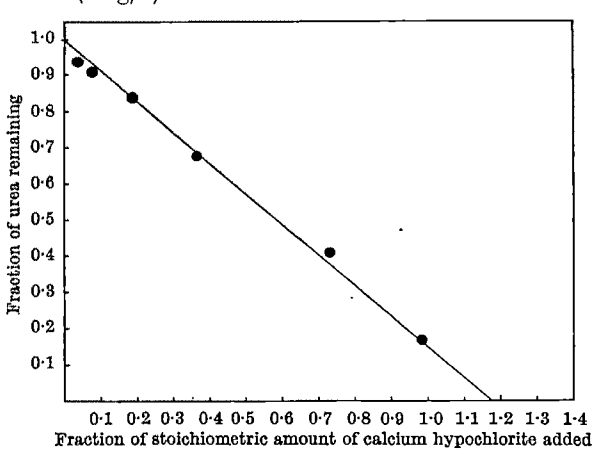


Fig. 1. Oxidation of urea solution by calcium hypochlorite. Initial urea concentration, 20·0 g/l.

The possibility of precipitating urea as urea nitrate may be dismissed because of its relatively high solubility in aqueous solution.

The hydrolysis of urea by the enzyme urease is most rapid when the solution is buffered at a *p*H of 7 and the concentration of urea is in the order of magnitude of 1–2 g/l.[11]. The dependence of hydrolysis of a high concentration of urea (20 g/l.) as a function of urease concentration, under ambient temperature and with the solution not buffered with respect to *p*H, is shown in Table 1. A 90 per cent hydrolysis of urea in 24 h was observed for urease concentrations ⩾ 2 g/l.

Table 1. HYDROLYSIS OF UREA BY UREASE UREA CONCENTRATION: 20 GM./L.

Urease concentration (g/l.)	% Urea hydrolysed
1·0	72
2·0	92
6·0	88
10·0	90

The most promising method of urea removal appears to be the use of calcium hypochlorite. The reaction products are nitrogen, carbon dioxide, water and calcium chloride. However, precipitation of calcium and chloride ions in solution resulting from the oxidation step can be effected if ion exchange is to follow. Approximately 24 per cent of the calcium content of the calcium hypochlorite added was precipitated accompanying the oxidation reaction; the introduction of silver and carbonate ions into a solution containing calcium and chloride ions seemed likely to precipitate silver chloride and calcium carbonate, since the solubility products of these compounds are quite low. Four precipitation methods were evaluated: (1) use of a saturated solution of silver carbonate in ammonium hydroxide; (2) use of dry silver carbonate; (3) use of silver carbonate followed by silver oxide; (4) use of dry silver oxide.

The complete removal of both calcium and chloride ions was effected by the procedures of methods 1 and 2, using the stoichiometric quantity of silver carbonate based on chloride content. Analysis showed that addition of silver carbonate beyond the requirement for complete precipitation of calcium continued to precipitate chloride, but introduced undesirable carbonate ions into solution. A two-stage precipitation was attempted (method 3) using the stoichiometric quantity of silver carbonate based on the calcium content and 130 per cent of the stoichiometric quantity of silver oxide based on the remaining chloride content. Complete removal of both calcium and chloride ions was effected by the precipitation and filtration steps. Silver oxide increases the alkalinity of an aqueous solution through hydrolysis, and reaction of dissolved carbon dioxide with the alkalinity produces carbonate ions. Approximately 130 per cent of the stoichiometric quantity of silver oxide (based on chloride content) was contacted with a urea solution (method 4) following the oxidation step. Again, complete precipitation of both calcium and chloride ions was effected. For a 24 g/l. urea solution method 3 required 160 g/l. of calcium hypochlorite, 180 g/l. of silver carbonate, and 82 g/l. of silver oxide; method 4 required 160 g/l. of calcium hypochlorite and 306 g/l. of silver oxide.

Ion Exchange. The utilization of ion exchange as a method of treating urine has been explored to a very limited extent. Bonting[12] made use of ion exchange resins in a laboratory analysis of urine. He established the total cation content of urine by removing all the cations with a small ion exchange column and by determining the quantity of hydrogen ions in the effluent. He reported that better than 99 per cent removal was achieved from contacting 0·7 mequiv. of cations with the exchange resin; he did not consider the capacity of the resin. McNeil[13] attempted to develop a method of waste disposal in aircraft by passing urine through a combined anion-cation exchanger and then by permitting the effluent to be picked up by a large wick for evaporation into a warm air stream. It was found that the resins were exhausted after the passage of 400 ml. of urine. A few additional references have been made in the literature to the use of ion exchange as a final purification technique following distillation or freeze-drying.

Although ion exchange would be best utilized as a secondary treatment process, following initial urea removal and precipitation, it is not unreasonable to assume that ion exchange could be the initial step in a urine purification process, particularly if some urea could be exchanged. 'Amberlite *IRA* 402', a quaternized chloromethylated polystyrene (strongly basic), was chosen as the anion exchanger, and 'Amberlite *IRC* 50', a poly (methacrylic acid) (weakly acidic) was chosen as the cation exchanger.

Two cylindrical glass columns, 24 in. long with an inside diameter of 1⅞ in. and containing 330 g dry resin, were operated in series. Untreated urine was passed through the column containing '*IRA* 402' (anion exchanger) and the effluent from the anion exchanger was passed through

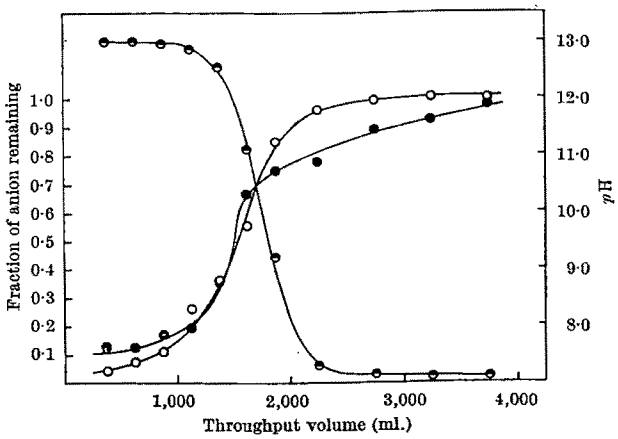


Fig. 2. Variation in chloride, sulphate, and *p*H of effluent from anion exchanger. ○, chloride; ●, sulphate; ◓, *p*H

'*IRC* 50' (cation exchanger). This order was adopted so as to provide an influent to the '*IRC* 50' with a high *p*H. The urine was collected from six male adults over a period of several days and was stored at a temperature of 1°–4° C. During the storage periods there were no signs of degradation; the colour, odour, and *p*H remained unchanged. The effluents were generally collected in 250 ml. batches and were kept refrigerated. Analyses were carried out to determine the average characteristics of the influent to the anion exchanger and of the influent to the cation exchanger. Similar tests were applied to a series of effluent samples from each column. Analyses were made for hydrogen ion concentration, absorptivity at 430 mμ, chloride, sulphate, calcium, sodium, potassium, total solids, and urea.

The zero point of the colorimeter was set with distilled water, and the absorptivity measurements were converted to colour units by comparison with standard solutions containing potassium chloroplatinate and cobaltous chloride[14]. Total solids were determined by evaporation of the samples to dryness at 103°–105° C. Sulphate ion was analysed by precipitation with barium chloride, followed by ignition of the filter residue at 800° C. Chloride was determined volumetrically using silver nitrate and potassium dichromate, and sodium and potassium ion concentrations were measured with a flame photometer. Calcium was analysed by a modification of the standard permanganate test and by the ethylene-diamine tetraacetic acid titration method[14]. An analysis of untreated urine is presented in Table 2. This represents

Table 2. Analysis of Untreated Urine

Test	Results
*p*H	6·6
Colour	1,143 units
Chloride	6·8 g/l.
Sulphate	2·5 g/l.
Calcium	0·15 g/l.
Sodium	3·7 g/l.
Potassium	3·3 g/l.
Total solids	37·6 g/l.

Table 3. Analysis of Influent to Cation Exchanger

Test	Results
*p*H	12·6
Colour	90·0 units
Chloride	1·0 g/l.
Sulphate	0·5 g/l.
Calcium	0·0 g/l.
Sodium	3·7 g/l.
Potassium	3·3 g/l.
Total solids	28·4 g/l.

Table 4. Analysis for *p*H, Sodium, and Potassium of Effluent from Cation Exchanger

Throughput volume (ml.)	*p*H	Sodium (g/l.)	Potassium (g/l.)
Influent	12·6	3·7	3·3
125	3·4	1·0	0·95
625	3·4	0·8	0·7
2,125	4·0	0·55	0·8
4,125	5·0	0·55	0·8
4,625	10·4	0·75	1·8
4,700	11·9	1·2	2·1

the average composition of the influent to the anion exchange column. Analysis of the influent to the cation exchanger is shown in Table 3. The total absence of calcium ion should be noted. It is likely that the calcium was precipitated out in the anion exchanger as calcium phosphate; this was enhanced by the elevated *p*H at which the anion exchanger operated. Although no quantitative analysis was made for magnesium, it seems probable that this cation was also precipitated out as magnesium hydroxide; this was qualitatively determined from the permanganate calcium analysis.

The results for the exchangers are presented in Fig. 2 and Table 4. A break occurred in the effectiveness of the anion exchange resin corresponding to 1,700 ml. of throughput volume, as may be observed from the *p*H and chloride values. Sulphate was still being removed until 2,500 ml. of effluent had been collected. Only the effluent from the anion exchanger prior to the break was fed to the cation exchanger. 5,100 ml. of effluent were collected from three anion columns and were passed through the cation column. It may be observed that the cation column was operating effectively up to 4,700 ml. of throughput volume.

The anion exchanger removed approximately 260 m.equiv. of chloride and 80 m.equiv. of sulphate before the resin was exhausted. This corresponds to 1·03 m.equiv. per gram of dry resin. Four ml. of effluent per cm^3 of resin were collected before exhaustion. In addition, significant quantities of calcium and magnesium were removed, apparently by precipitation; this may have influenced the rapid exhaustion of the anion exchanger by forming a film to block available exchange sites. The cation exchanger removed approximately 500 m.equiv. of sodium and 250 m.equiv. of potassium before the resin was exhausted. This corresponds to 2·27 m.equiv. per gram of dry resin. Eleven ml. of effluent per cm^3 of resin were collected before exhaustion. The combination of both columns reduced the total solids content by 73 per cent. The total solids removal was greater than would be expected from the sulphate, chloride, sodium, and potassium analyses. This may be attributed to exchange of other ions and the filtering action of the exchange beds. Colour reduction by the anion exchanger is shown in Fig. 3; the colour of the effluent was 40 units compared with 1,143 units for the influent. Removal of urea was negligible.

Some success was achieved in the ion exchange studies, from the point of view of producing a usable water. Obviously the greatest disadvantage of such a process is the large quantity of ion exchange resins needed to treat a given quantity of water. Chemical pretreatment of the urine to reduce the ionic load greatly increases the volume of urine treated per cm^3 resin. Better results may conceivably be obtained by using other exchange resins, by reversing the anion and cation columns, or by the use of a mixed ion exchange bed.

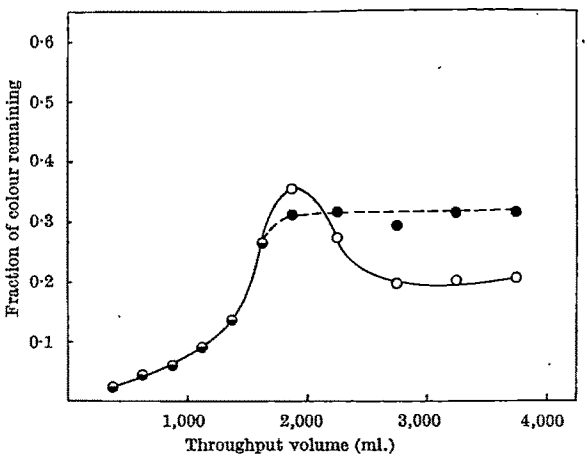


Fig. 3. Variation in colour of effluent from anion exchanger. —○—, First day test; - - - ● - - -, Third day test. Colour of untreated urine, 1,143 units

We thank the Office of the Surgeon General and Headquarters, U.S. Army Medical Research and Development Command, and the National Institutes of Health, for supporting this work under contract *DA*-49-193-*MD*-2316 and 2*G*-529(*R*2) respectively.

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TEACHING BIOLOGY

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THE various branches of science are perpetually changing their character and content. We are well aware of this general property of evolution for we regard it as the natural result of our own activities. Of the rules that may be said to govern it, however, we are less clearly aware. Indeed, we observe confusion rather than rule, paradox rather than principle. Sciences sometimes split; and they sometimes fuse. Continually they become more connected in their foundations. But continually their newest developments become more remote from these foundations. Their methods are transformed from the descriptive to the experimental, from the individual to the statistical, and from the analytical to the synthetic. The physical apparatus that they use is correspondingly transformed. What is more serious is that the kind of training and the type of intelligence and temperament needed by the man or woman who wants to pursue any branch of science by teaching or research is also transformed: and what happened formerly from generation to generation happens nowadays—to use our academic unit of time—from quinquennium to quinquennium.

This property of science is emphasized by the acceleration of discovery which has been one of the consequences of its regular teaching in universities. Regular teaching, however, has carried with it other quite different consequences: the need for a neat division into departments; for a hierarchy of professional teachers often permanently attached to the same department, and housed in great buildings; for routines of laboratory work served by convenient text-tooks which become stereotyped; for a system

of examinations administered by committees and regulated by statutes. All these needs have been met in our older universities: and they have created an establishment, massive and privileged, and in many respects fitted to resist the changes demanded by the processes of discovery and the growth of science which they are supposed to be encouraging.

The obstacles to change have been most forbidding in the sciences dealing with life. In the nineteenth century, when biological teaching developed in the universities, there was no single clear purpose in it. It might be designed as propaganda for a belief either in creation or in evolution. It might be intended as a preparation for medical training or as an introduction to the practices of cultivation. But whatever reasons were entertained the organization that came into being nearly always entailed a separation of botany and zoology. They were put in separate departments no doubt because, at the descriptive level of the time, the study of plants and animals appealed to different kinds of people: and for the same reason the study of man, with his unique theoretical and practical interest, was regularly and utterly excluded from both.

All this is quite recent. Lawrence's *Natural History of Man*, which was the climax of an age of amateurs, put man in the centre of the picture of life. That was in 1819 [1]. But when Huxley wrote his version of the same theme in 1863 and called it *Man's Place in Nature* he ushered in a professional age in which, by a *tour de force*, man was left out of his one obvious place in Nature; he was omitted from the study of life.

Thus for a hundred years, and quite systematically, man and all his activities have been cut out of biology in the universities. The separate parts of plant and animal studies have accordingly been presented as though they were without application to man and therefore to a large extent as though they were useless. In due course, this absence of application, like the separation into departments, has gradually come to be an end in itself. When agricultural teaching developed, universities found they had to create new departments or institutions which were not ashamed to discuss plants and animals in a useful way. Thus the study of life was broken up into smaller and smaller fragments, less and less connected in theory and less and less connected in practice.

In the present century the teaching of biology has for these reasons gone to pieces; and by a paradox the teaching has gone to pieces at the very time when research on living things and living processes was putting the pieces together. For the investigations of the cell and its heredity, the individual and its development, the community and its relations, the species and its evolution, have all the time been revealing internal and external connexions. All the time they have been bringing unity into the study of life and making it useful to man: and all the time they have been exposing the inadequacy of the methods of teaching the subject and of the institutions available for it.

This contradiction between our institutions and what we want from them has long been realized [2] and frequently [3] asserted. Many methods also have been used to resolve it. Above all, our predecessors arranged elementary courses which combined the teaching of botany and zoology with chemistry, physics and geology. Sixty years or more of experience of such courses has, however, proved disappointing. What they have produced, as Catcheside has put it in a valuable discussion [4], are obvious chimeras—at once monstrous and obsolete. The reason for this failure is now clear. Elementary courses have always dealt with the older parts of a science. The older parts of botany and zoology are based on description, and so far as possible naked-eye description. The union of life sciences occurs, however, through their newest growth: it is through the newest parts that we need to graft our living tissues. Our mistake has been that what is new we have held to be too advanced and too abstruse. To be sure what is new requires more refined chemistry, higher powers of the microscope, and a little more mathematics than are generally available in schools. But might these requirements not be met in universities?

To put the matter in another way: what we need in university biology is not a continuation of school teaching or adding together of old-fashioned parts of university subjects. For fundamental biology is not old botany plus old zoology; it is something new, created in the present century, not in the previous century. What we need therefore is a new approach to teaching which strides over barriers between old subjects and old departments, an approach to teaching which seizes on and exploits the newest discoveries. In this way we shall get the connected, integrative and useful character we need in the study of life.

Eminent scholars and scientists who manage universities, no less than those who do the humdrum work, have perhaps been more inclined to look back to their own achievements rather than forward to those of their pupils. The greatest effort to set a new course has been made in the United States. But even there only in a few universities has decisive progress been made. In most the innovations have not been possible, and the staff has not been available for carrying out any profound and up-to-date reorganization of teaching. In Great Britain also we shall have to surmount great obstacles before a provision for biological teaching, based on present understanding, will be possible.

In Oxford, following six years of laborious discussion, we have drawn up plans for a beginning of integrated teaching. We now feel able to undertake our programme at once. Beginning in October 1963 we shall have an obligatory one-year course as an introduction to biology [5]. It will be intended for pupils with a school training in biology, chemistry and physics to the Advanced-level of the General Certificate of Education. But it will not, we believe, be beyond those who are new to the subject. It will be divided into three biological sections: (1) genetics, including the chromosomes; (2) physiology with biochemistry; (3) ecology and taxonomy.

In addition, there will be special courses (the functions of which have been described by Catcheside) in chemistry, physics, geology and mathematics, each of them designed for biologists. The biological courses will make use in due proportion, as convenience dictates, of plant and animal, human and microbial, material. Each will have its experimental foundations. Each will have its analytical and also its integrative aspects. The result we hope will show the connectedness and organization which distinguish biology from the physical sciences; and it will also show the physical foundations on which this organization rests. All the teaching we intend to accommodate ultimately in a single building.

The problems of teaching, and of learning, involved in this programme are far from being simple, and indeed far from being solved. The work involved for the teacher and for the pupil will be great; and the expense of equipment will be heavy. But we believe we shall succeed because we know ourselves that biology makes sense, and we believe that the teacher and pupil will find it useful to show and to see the sense emerging from the very beginning. When the advantages of working together make themselves felt, we believe the boundaries between traditional departments will, at least at the elementary level, gradually dissolve.

Following the first-year course the undergraduate will decide whether to continue to an honours course in botany or zoology, agriculture or forestry. Which it is will indeed depend on what kind of sense he has made out of the first year, whether it was the practical or the theoretical sense; and what kind of material excites his interest—plant, animal or microbial.

That is how the matter stands at present. But it is clear that new possibilities of honours courses will arise

and new kinds of connexions will establish themselves from the reformed first year. The obstacles to these later steps will be very great. But they can scarcely be so serious as were those to this first step. A new world will be opened to the teacher, the pupil, and the university. How large a world it will be we may leave the future to discover. But it will certainly be a world which includes something of man and mankind: and it will probably be a world in which the teaching of biology has a place not far from the centre of university education.

[1] Darlington, C. D., *Darwin's Place in History* (1959).
[2] Ashby, E., Crew, F. A. E., Darlington, C. D., Ford, E. B., Haldane, J. B. S., Salisbury, E. J., Turrill, W. B., and Waddington, C. H., *Nature*, **138**, 972 (1936).
[3] Fifteenth Ann. Rep. Adv. Counc. Sci. Policy, Cmnd. 1920 (H.M.S.O., 1961–62).
[4] Catcheside, D. G., *Nature*, **197**, 427 (1963).
[5] Pringle, J. W. S., *School. Sci. Rev.* (in the press).

DR. MARGARET MURRAY

TRIBUTE TO A CENTENARIAN

MARGARET ALICE MURRAY was born on July 13, 1863, in Calcutta; her parents' generation had witnessed the Indian Mutiny, but she grew up in the Golden Age when Britain's prestige and prosperity were at their height. The child's first journey home was by way of the Cape, for the Suez Canal was not open. She remembers rolling bandages for the wounded of the Franco-Prussian War, and her compassion was early aroused by her mother's devotion to the education of the women of India. Her own was that of a young girl of her time; but, not content with a social life, she worked in a local hospital and was soon 'in charge' of a ward.

She left India for good before she was twenty-five years old, her prodigious and accurate memory stored with impressions of the life and religion of many races and creeds, which were to stand her in good stead in her chosen career. Miss Murray was fortunate to become a student at University College in the newly formed Department of Egyptology when Sir Flinders Petrie was evolving a new science. They had to write their own text-books from direct observation, and a measure of Dr. Murray's contribution is seen in about a hundred books and articles. But more valuable still, two distinguished generations of archæologists are in her debt as a wise and witty teacher, who never forgets that her subject is the study of man, mentally and spiritually, from the time that he emerged from the animal.

Dr. Murray's excavations were carried out mainly in Egypt, Malta, Petra, Palestine and Britain. She retired from the assistant professorship of Egyptology in University College, London, in 1935. She is also well known for her studies in folk-lore and witchcraft.

Dr. Murray follows the discoveries of the space age with as much zest as she gave to the invention of the bicycle; her delight in her particular fields of research is undimmed and distilled in her latest book *The Genesis of Religion*. Her autobiography, *My First Hundred Years*, follows this month, and if she sometimes regrets that she has led an uneventful life, this is surely an understatement for someone who has lived gallantly through the adventure of one century and looks forward to the next.



By courtesy of Prof. W. B. Emery

M. A. Murray 1961

OLGA TUFNELL

NEWS and VIEWS

The Royal Society of Edinburgh: Foreign Honorary Fellows

THE following have been elected Foreign Honorary Fellows of the Royal Society of Edinburgh: Prof. M. Calvin, professor of chemistry, University of California, Berkeley; Dr. V. A. Engelhardt, director, Institute of Physico-Chemical and Radiation Biology, Moscow; Prof. A. Frey-Wyssling, professor of botany, Eidgenössische Technische Hochschule, Zurich; Prof. P. E. M. J. Pruvost, professor of geology, Paris; Prof. S. L. Sobolev, professor of mathematics, University of Moscow; Prof. W. Szafer, professor of botany, Cracow; Prof. V. F. Weisskopf, director-general, European Organization for Nuclear Research, Geneva.

Awards

DR. E. M. PATTERSON, Department of Mathematics, University of Aberdeen, has been awarded the Makdougall-Brisbane Prize for the period 1960–62 for his papers on linear algebras published in the *Proceedings* of the Society during the period of the award and earlier papers on cognate subjects also published by the Society. Mr. K. V. Blaiklock, Kenton, Exeter, has been awarded the Dr. W. S. Bruce Memorial Prize for 1962 commemorating Dr. W. S. Bruce, the Scottish explorer and scientific investigator in polar regions, in recognition of his contributions by way of navigation to the exploration of the Antarctic Continent and for his mapping of lands bordering the Weddell Sea.

The Royal Society of South Africa : New Members

At a joint meeting of the Society and the Astronomical Society of South Africa, held on April 17, at the South African Museum, Queen Victoria Street, Cape Town, the following were elected to membership: Mr. L. R. Alexander, Prof. S. Biesheuvel, Mr. M. Jehu and Dr. O. Williams.

Geometry at Oxford: Prof. M. F. Atiyah, F.R.S.

Prof. M. F. Atiyah, who has been appointed to succeed the late Prof. E. C. Titchmarsh (see *Nature*, 198, 1039; 1963) to the Savilian professorship of geometry at Oxford, is one of the leading experts in algebraic geometry, topology and differential geometry. He has been a reader in mathematics at Oxford since 1961 and has already made a considerable contribution to the development of pure mathematics there. He was born in April 1929 and was educated first in the Victoria College in Egypt and then at Manchester Grammar School. He discharged his military service with the Royal Electrical and Mechanical Engineers in the years 1947–49, and in the latter year he was elected a Scholar of Trinity College, Cambridge, where he proceeded to take the Mathematical Tripos as a wrangler in 1951, together with a distinction in Part III in 1952. He was awarded the first Smith's Prize in 1954 and obtained his Ph.D. in 1955. In the years 1954–58 he was a Research Fellow of Trinity College, Cambridge, during which period (1955–57) he also studied as a Commonwealth Fund Fellow at the Institute for Advanced Study, Princeton. He was an assistant lecturer and afterwards a lecturer and Staff Fellow at Cambridge during the years 1957–61, and also a member of the Institute for Advanced Study at Princeton from September 1959 until January 1960. He was elected a Fellow of the Royal Society in 1962. Prof. Atiyah is distinguished for his researches in algebraic topology, but his interest and influence extend over a wide field of pure mathematics.

United Kingdom Scientific Mission in Washington : Dr. J. A. Saxton

Dr. John A. Saxton has been appointed director of the United Kingdom Scientific Mission in Washington, D.C., and scientific attaché at the British Embassy there. He will take up his duties early in 1964, replacing Dr. Harry Hookway, who will be returning to the United Kingdom on completion of a three-year tour of duty. Dr. Saxton, who is forty-nine, has been deputy director of the Radio Research Station (Department of Scientific and Industrial Research) since 1960. He joined the Department in 1938, working in the Radio Division of the National Physical Laboratory until the Division developed into the Radio Research Station in the early 1950's. He is well known for work on the dielectric properties of the atmosphere and on investigations of radiowave propagation at very high frequencies.

The Cold Spring Harbor Laboratory of Quantitative Biology

The Carnegie Institution of Washington has transferred its two laboratory buildings at Cold Spring Harbor, Long Island, New York, to the Cold Spring Harbor Laboratory of Quantitative Biology. The Carnegie Institution will continue its present Genetics Research Unit on the premises. The Cold Spring Harbor Laboratory of Quantitative Biology has been organized by a group of eight universities and research institutes. Its director is Dr. John Cairns, formerly of the Australian National University at Canberra. The chairman of the board of directors is Dr. Edward Tatum, professor at the Rockefeller Institute of New York. The sponsoring institutions are the Albert Einstein College of Medicine, Brooklyn College, Duke University, the New York University School of Medicine, Princeton University, the Public Health Research Institute of the City of New York, the Rockefeller Institute of New York, and the Sloan-Kettering Institute. The Carnegie Institution established its biological research at Cold Spring Harbor in 1904, as the Station for Experimental Evolution, with Dr. C. B. Davenport as director. It became the Institution's Department of Genetics in 1921. The present name was adopted in 1962. It was there that, in the early 1900's, Dr. George H. Shull carried on the research that led to the development of hybrid corn, which enormously increased corn yields and brought economic gain to the United States alone of more than 10 million dollars. Other research at Cold Spring Harbor has included methods of changing the hereditary characteristics of plants by means of radiation and chemicals, leading to much new theoretical knowledge as well as to the development of new and more productive types of food plants and flowers. Fundamental work in genetics continues to-day at the Carnegie Institution's Genetics Research Unit at Cold Spring Harbor, of which Dr. Alfred D. Hershey is director. Also at the Unit is Dr. Barbara McClintock, well known for her work on the genetics of maize (Indian corn); she is a member of the board of directors of the new Cold Spring Harbor Laboratory of Quantitative Biology. The Carnegie Institution will provide an immediate grant of 10,000 dollars to the Laboratory.

The British Industrial Biological Research Association, Ltd.

Work has started on the erection of the laboratories of the British Industrial Biological Research Association on a site of 16½ acres at Woodmansterne Road, Carshalton, Surrey. The site is adjacent to the Medical Research Council Laboratories at Carshalton, where the Toxicology Research Unit, Neuropsychiatric Research Unit, Virus Research Unit and Laboratory Animals Centre are accommodated, and overlooks the Oaks Park. The buildings provide accommodation for laboratories, rodent rooms, dog kennels, animal administration, administration and information services, etc., totalling 18,900 sq. ft. The whole project is expected to cost £190,000 and equipment will cost an additional £50,000. Building operations commenced at the beginning of May and the estimated date of completion is May 30, 1964. The laboratories will house a staff of approximately 60 in the initial stages. The buildings will provide up-to-date and well-equipped accommodation for carrying out all modern techniques of toxicity testing, and provision is made for workers in the scientific disciplines of toxicology, experimental pathology, pharmacology, biochemistry and organic chemistry. They will enable the British Industrial Biological Research Association to provide first-class services for its member companies in its field of toxicology of food additives, cosmetics and toiletries.

Business Management Studies at Liverpool

The University of Liverpool has recently received grants totalling £107,000 towards the further development of Management Studies within the University. £84,000 of this sum has been contributed by Littlewoods Mail Order Stores, Ltd. The balance of £23,000 is being contributed jointly by the Government (through the University Grants Committee) and by the Foundation for Management Education. The University intends to extend the provision of management education for practising business men in the Department of Economics. Under this scheme a Management Development Programme has been provided for directors and senior managers operating in companies over a wide area of the North of England—approximately 300 have participated in this Programme. The emphasis throughout has been on directing business men's attentions to operational problems and the ways and means of raising operational performance. It is anticipated that the future organization of management studies within the University will be based on a number of

Faculties or Departments and will be inter-disciplinary. It may also involve the establishment of a special institute, governed by a committee or board representing the various interests both within and without the University. Further attention will be directed to analysing the implications of international management application and the forms of management organization and techniques applied to international business operations, while the study of the forces making for successful marketing management will also figure importantly in the programme.

Contributions of Science to the Resolution of International Conflict

A STUDY conference on "The Contributions of Science to the Resolution of International Conflict" was held at Windsor during June 28–July 1. The sponsors included Lord Boyd-Orr, Rear-Admiral Sir A. W. Buzzard, R. G. Cooke, Sir Charles Goodeve, Prof. A. Haddow, Dame Kathleen Lonsdale, Prof. G. Schwarzenberger and Prof. C. H. Waddington. The conference had two aims: to show how much scientific work has been done already towards the resolution of international conflict, and to develop ways of broadening the scope, accelerating the progress, and ensuring the relevance of future scientific research in this field. So far as the first aim was concerned, Prof. K. Boulding, Ann Arbor, Michigan, gave a brief historical summary of work done, or in progress. Concerning the second, it was felt that there are two tasks that must be tackled before it can be said that scientific work on international conflict is on a sound footing. First, research techniques and working arrangements are required that will enable different kinds of scientists to work fruitfully together. Secondly, a common language is needed or, at least, a body of translators between the scientists who do the research and the people who face the problems and want to apply the findings. Among those presenting papers were Dr. F. Emery, Stafford Beer and Dr. John Burton. Others participating included Prof. J. Meade, Prof. L. Penrose, Sir Charles Goodeve and Prof. G. Schwarzenberger.

Animal Health

THE Animal Health Trust has added to its campaigns by the introduction of a new popular magazine, *Animal Health* (Vol. 1, No. 1; January–March 1963. Journal of the Animal Health Trust. Pp. 1–76. London and New York: Pergamon Press, 1963. 5*s*.). With an impressive editorial board, it aims at providing interesting information about animal health subjects designed to appeal to a wide range of readers. The first number has a dozen articles: two are of a historical nature, the history of dogs, by Dorothy Hodgman, and the first outbreak of foot-and-mouth disease in Britain, by J. W. Barber-Lomax. Sir John Hammond provides a thoughtful article on nutrition during pregnancy, and Dr. W. R. Wooldridge discusses man and his animals. W. C. Miller has written a brief tribute to 'Hyperion' and also the first part of an article on the place of the horse in modern society in Great Britain. Ralph Wightman has an article on animal husbandry, and poultry interests are represented by articles on Newcastle disease vaccination, by J. S. Garside, and by the influence of the changing structure of the poultry industry on avian disease in the United Kingdom, by R. F. Gordon. With items on new products of interest to animal owners, notices about particular film-strips available, and some book reviews this is a very bright and useful journal.

Diseases of Free-Living Wild Animals

DR. A. MCDIARMID, of the staff of the Field Station of the Agricultural Research Council, at Compton, Berkshire, has written a monograph, *Diseases of Free-Living Wild Animals* (FAO Agricultural Studies No. 57. Rome: Food and Agriculture Organization, 1962). This is a much neglected subject; only in the past couple of decades has much attention been directed to the value as well as the necessity of bringing wild animal and bird life into the epidemiological environment of both human and animal disease research. Previously, certain 'peculiarities' had been recognized, such as the finding of an antelope with tuberculosis in a game reserve, without proper recognition that an important item in epidemiology was concerned. Decades ago a few enlightened workers realized the value of linking veterinary and medical epidemiology, but the opportunity to bring this about did not come easily. To younger workers these statements about former neglect might require justification, and the story of rabies emphasizes the role of wildlife. Earlier in the century it was believed that, if dogs were to cease to exist, rabies would cease to be important. We now know that there are few diseases with such a capacity to exist in the absence of any human or canine agency. It also shows the infinite capacity of the disease to take advantage of certain changes in environmental condition; with the political and military changes in Eastern Europe at the end of the Second World War possession of firearms by civilians was not possible, and, as a result, the balance of predatory animals changed; foxes and other biting animals increased in numbers, rabies became heavily prevalent and the disease spread steadily across continental Europe and became a serious problem, dogs being the victims rather than the main source of spread. The information provided in this monograph, covering scores of different infective conditions and at least one thousand references, will be of great value.

The Museum of Comparative Zoology, Cambridge, Massachusetts

DR. ERNST MAYR, in submitting his first report as director of the Museum of Comparative Zoology at Harvard College, feels that at the present time it is not desirable to make fundamental changes in the policy of the institution (Annual Report of the Museum of Comparative Zoology, 1961–62. Pp. 32 + 2 plates. Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1963). The first task of a university museum is to serve as a combined centre of instruction and research. With six professors and twelve additional research zoologists on the curatorial staff the Museum is indeed well qualified to carry out this task. Active research is an inseparable part of instruction in the sciences. The Museum of Comparative Zoology has stressed research since the days of its founding by Louis Agassiz, yet in the whole history of the institution there has been no period of greater scientific activity and productivity than the present.

A Clay Fertility Figurine from Lower Austria

THE latest volume of the *Mitteilungen der Prähistorischen Kommission der Österreichischen Akademie der Wissenschaften* (Band 10. Pp. iv + 162. Wien: Hermann Böhlaus NACHF, 1960–62) consists of a detailed study of a clay fertility figurine from Lower Austria (Alois Gulder, Die urnenfelderzeitliche "Frauenkröte" von Maissau in Niederösterreich und ihr geistesgeschichtlicher Hintergrund). The figure was found during excavations of an early Hallstatt settlement between Haselbach and Maissau before the Second World War, and unfortunately its exact find-spot and associations are somewhat obscure. The author, however, has made a successful attempt to establish its contemporaneity with traces of an Urnfield hut of rectangular form. The pottery from the site, and the few metal objects, generally belong to the earlier Urnfields, Hallstatt A in this area, with a suggested date in the eleventh century B.C. The figurine is of clay, 75 mm tall and almost half as wide, with outstretched arms, rather gross facial features and small breasts. A large 'bun' over the head has probably influenced the author

into considering that this figurine has its closest analogues in the toad-like figures represented in many areas and belonging generally to the latter first millenium B.C. and after (from sites such as Dernovo, Bornholm and Vetulonia), although earlier examples are known (Vinča, Eberstadt). Such forms are generally believed to be closely linked with fertility cults, often associated with the idea of the embryo and womb, and the author produces many examples of this from Europe and Asia. It is, however, also possible that the Maissau figurine should lie not so much with this toad-like group as with the mother goddess idea, which is so well represented in the Mediterranean and the continental Neolithic, with perhaps a basis back, again in Austria, in the Upper Palæolithic Venus figurines of more than 20,000 years ago.

Constituents of Tobacco Smoke

The Tobacco Research Council (formerly the Tobacco Manufacturers' Standing Committee) have published a second supplement to their Research Paper No. 3, *The Constituents of Tobacco Smoke: An Annotated Bibliography*, which appeared in 1958 (Pp. ii+24. London: Tobacco Research Council. 1963). The second supplement, which is edited by E. G. N. Berry, refers to publications appearing during the period 1960–62, and more than one hundred references to papers on the estimation or identification of constituents of tobacco smoke are given. As in the earlier volumes, references are listed in sections based on classes of compound, and the bibliography provides a handy reading list in which publications on any particular compound or group of compounds in tobacco smoke can be easily located.

Antioxidants in Food : Detection and Determination

The coming into force of the Antioxidant in Food Regulations, 1958, has posed new problems for public analysts and other chemists as it has meant that certain foods have to be examined regularly for an additional number of compounds. The recent publication of a booklet by the Association of Public Analysts, entitled *The Detection and Determination of Antioxidants in Food*, will therefore be welcomed by many food chemists (Pp. ii+25. London: The Association of Public Analysts, 1963. 10*s*.). A scheme of analysis is presented in which the permitted antioxidants can be separately detected and determined on the same methanol extract. Gallates and butylated hydroxyanisole are determined by the blue colours produced with ferrous ammonium sulphate and Gibbs reagent respectively. The recommended methods for butylated hydroxytoluene make use of the new colorimetric test of Szalkowski and Garber (*J. Agric. Food Chem.*, **10** (6), 490; 1962), in which a purplish-red colour is produced with *o*-dianisidine and sodium nitrite. For a screening test the figure obtained is doubled to allow for the incomplete extraction of the antioxidant by the methanol. For more accurate results, however, it is necessary to separate the butylated hydroxytoluene first by steam distillation. Methods are also described for the identification of the permitted and various non-permitted antioxidants by reverse-phase paper chromatography and the corresponding R_F values are tabulated. Numerous practical tips, the effect of possible interfering substances and the results of collaborative trials are also included, together with summaries of the British and U.S. Regulations relating to antioxidants.

The Industrial Pest Control Association

The following have been elected officers and executive committee of the Industrial Pest Control Association for the year 1963–64: *President*, Dr. E. R. Goodliffe (Ratsouris, Ltd.); *Vice-President*, Mr. D. Boocock (S.D.C. Pesticides, Ltd.); *Honorary Treasurer*, Mr. D. M. Simpson (Cooper McDougall and Robertson, Ltd.); *Executive Committee*, Mr. S. Egleton (Verminex, Ltd.), Mr. S. Farrow (London Fumigation Co., Ltd.), Mr. B. Kesterton (British Insecticides, Ltd.), Mr. A. G. Stutely (Sorex (London), Ltd.) and Mr. E. L. Williams (Shell Chemical Co., Ltd.); *Secretary*, Mr. W. A. Williams, Cecil Chambers, 86 Strand, London, W.C.2.

Committee on Scientific Library Services

The Library Association has appointed a Committee on Scientific Library Services to review the existing provision of specialist library services for scientists, technologists and other specialist workers, and to make recommendations on the services needed and the staff required to provide them. Major-General C. Lloyd has accepted the chairmanship, and representatives of the Library Association include the chairmen of the Council and of the Executive, Mr. D. J. Foskett and Mr. F. M. Gardner. Representatives will be invited from universities, colleges, research establishments and industry.

Announcements

Dr. A. D. Booth has been appointed to a specially created inter-disciplinary research professorship in autonetics at Western Reserve University, Cleveland. The appointment will be held in parallel with the post of dean of the College of Engineering at Saskatchewan which Dr. Booth holds (see *Nature*, **195**, 946; 1962).

Dr. John O'Connor has been appointed research associate at the Institute for Scientific Information, Philadelphia. Dr. O'Connor, a specialist in information retrieval, is at present carrying out research on high-quality subject indexing by electronic computer. He was formerly associated with the University of Pennsylvania and Remington Rand Univac.

The fifth international conference on "Medical Electronics" will be held in the University of Liège during July 22–26. Further information can be obtained from Dr. F. Bostem, 23 Boulevard Frère Orban, Liège.

The third European Mycological Congress will be held in Glasgow during August 31–September 7. Further information can be obtained from Dr. J. G. Manners, Botany Department, the University, Southampton.

An international symposium on "Pollen Physiology and Fertilization" will be held at the University of Nijmegen during August 29–31. Further information can be obtained from Prof. H. F. Linskens, Department of Botany, Driehuizerweg 200, Nijmegen, The Netherlands.

The sixteenth International Congress of Zoology, organized by the National Academy of Sciences–National Research Council and the American Institute of Biological Sciences, will be held in Washington during August 20–27. Further information can be obtained from Dr. G. B. Moment, c/o National Academy of Sciences–National Research Council, 2101 Constitution Avenue, N.W., Washington 25.

A joint international conference on "Creep and Fracture", organized by the American Society of Mechanical Engineers, the Institution of Mechanical Engineers and the American Society for Testing Materials, will be held in New York during August 25–29 and in London during September 30–October 4. Further information can be obtained from the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

Erratum. In the communication by W. L. Templeton and V. M. Brown, entitled "The Accumulation of Calcium and Strontium by Brown Trout in Waters in the United Kingdom", published in the April 13 issue of *Nature*, on p. 199, column 2, line 51 should read: "1/2,000th of the M.P.C. as calculated here", not "1/2,000th of the $(M.P.C.)_w$ as calculated here", and line 54 should read "one-twentieth of the calculated M.P.C", not "one-twentieth of the calculated $(M.P.C.)_w$".

THE ASSOCIATED ELECTRICAL INDUSTRIES LEVITRON

By Dr. N. L. ALLEN, Dr. D. BALFOUR, V. C. CLOKE, R. F. HEMMINGS, T. P. HUGHES, B. JORDAN, B. S. LILEY, H. B. MUNDY, Dr. R. M. PAYNE and Dr. A. A. WARE

Research Laboratory, Associated Electrical Industries, Ltd., Aldermaston Court, Berkshire

THE levitron, first proposed by Colgate and Furth[1], is a toroidal version of the linear hardcore or inverse pinch discharge[2,3]. The object of such machines is to produce hydromagnetically stable plasma, where the term 'hydromagnetic' refers to those theories which assume infinite plasma conductivity. In cylindrical geometry a sufficient condition for this type of stability is that the inequality:

$$j_z B_\theta < 0 \qquad (1)$$

should be satisfied throughout the plasma. j_z is the axial current density and B_θ the azimuthal magnetic field. In the linear experiments this condition is automatically satisfied. In toroidal geometry the condition[4] corresponding to equation (1) is more complicated, and it is neither obvious nor certain that hydromagnetically stable configurations exist. On the other hand, a toroidal system has the advantage of eliminating end effects and enabling plasma to be produced over a wider range of initial gas pressures.

In addition to hydromagnetic instabilities, others may occur. Of these, current[5] and resistive[6] types are proving to be of importance. The object of the investigation reported here is to determine the nature of the instabilities that can occur in a levitron machine, and estimate the importance of these in energy- and particle-loss processes. So far few results of a conclusive nature have been obtained; but many interesting and encouraging observations have been made. The purpose of this article is to report on these, and give a brief description of the design and operation of the machine.

The vacuum vessel is a torus with a major diameter of 92 cm and a minor diameter of 30 cm. It consists of eight aluminium pressings with walls 1·25 cm thick. It is constructed in such a manner that gaps requiring vacuum seals run in both the toroidal (φ) and poloidal (θ) directions. These seals are of 'Viton' rubber and are protected from the plasma by silica tape. The tape is held in position by copper strips riveted to the aluminium (Fig. 1). The hardcore is also of aluminium, being a toroidal tube of the same major diameter as the vacuum vessel. The minor diameter of this tube is 11·4 cm, the wall thickness 0·63 cm and the weight 14 kg. For the duration of an experiment ($\sim$ 1 msec) the hardcore is unsupported and co-axial with the vacuum vessel.

On the outside of the torus are two sets of windings which run in the θ and φ directions respectively. These are connected via switches to the condenser banks referred to below. A transformer iron core links the torus.

The operation of the machine is best understood by reference to Figs. 1 and 2. Initially the hardcore is at the bottom of the torus resting on four aluminium pads, 8 mm high and 2·5 cm wide. The levitation condenser bank is discharged through the axial or φ windings. Interaction between the current in the hardcore and the eddy currents induced in the walls of the torus gives the hardcore an impulse. This impulse is sufficient to raise the core to the centre of the torus, where it interrupts a light beam. This generates a pulse which triggers the main φ condenser bank (the nomenclature refers to the direction of the induced electric field in the torus). The resulting current in the hardcore is clamped at its peak value.

Subsequent to clamping, a radio-frequency preionizing pulse (100 μsec duration) is applied, and at the end of this the θ bank is fired. This breaks the gas down and drives the plasma towards the hardcore. The azimuthal magnetic field, B_θ, is trapped in the process. The position of the discharge at any instant of time is determined by the value of I_θ and the initial value of the current in the hardcore. The θ bank is also clamped. As the core returns to its original position at the bottom of the torus it is 'caught' by the catching bank. In a different mode of operation the sequence of firing the main φ and θ banks is reversed. In this way a pinch-type[7] discharge is produced with, however, a hardcore present.

In normal levitron operation trapping of the B_θ field ensures that:

$$j_\varphi B_\theta < 0$$

where j_φ is the axial current density in the plasma. As already pointed out, this is not a sufficient condition for hydromagnetic stability in a torus; but it is a basic requirement for this condition to be satisfied.

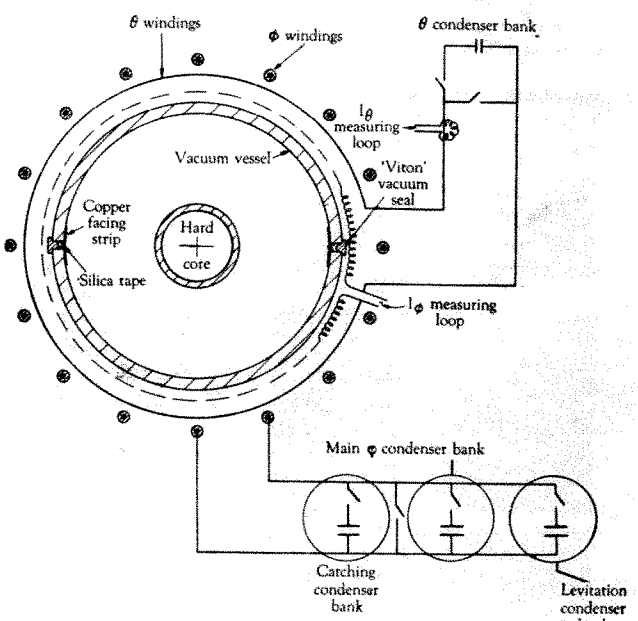


Fig. 1. Schematic diagram of levitron

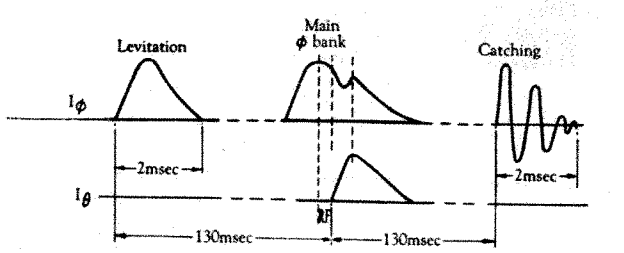


Fig. 2. Levitron trigger sequence. Upper, I_φ currents; lower, I_θ

The energy required for levitation is about 3,600 joules and that for 'catching' of the same order. The turns ratio of both the φ and θ windings can be 8 : 1, 16 : 1 or 32 : 1. The characteristics of the main φ and θ banks are given in Table 1.

Table 1

Main φ bank	Charging voltage, V_φ	20 kV max.
	Capacitance	94 μF
	Peak hardcore current (in absence of plasma)	~12 kamp/kV
	Current rise time at 32 : 1 turns ratio	400 μsec
	1/e clamp time at 32 : 1 turns ratio	2·5 msec
θ bank	Charging voltage, V_θ	20 kV max.
	Capacitance	423 μF
	Peak magnetic field, B_φ (in absence of plasma)	~350 gauss/kV
	Current rise time at 16 : 1 turns ratio	110 μsec
	1/e clamp time at 16 : 1 turns ratio	430 μsec
	Initial azimuthal electric field, E_θ, at walls of torus for 16 : 1 turns ratio	~1/3 V/cm/kV

The maximum repetition rate of the machine is one discharge per minute. This is limited by the rate at which the hardcore can cool. The normal working pressures are 2 millitorr and 4 millitorr. However, discharges have been obtained at pressures as low as 6×10^{-2} millitorr and as high as 16 millitorr. No attempts have been made to operate at a higher pressure. Most work has been done in hydrogen; but several experiments have also been carried out in helium and argon.

On three occasions faults and modifications have necessitated the dismantling and re-assembly of the machine. In the first assembly (September–October 1961) the only observations made were those required to test the engineering performance. During the period February–May 1962, a successful series of experiments were carried out in the second assembly. In the third assembly (August–October 1962) levitation was unreliable, the fault being traced to distortions in the hardcore. Even so, many interesting results were still obtained from experiments in this assembly. In the fourth assembly a new copper hardcore (weight 23 kg, minor diameter 8·9 cm) will be used. In addition, extra facilities for probe and spectroscopic observations will be included and the θ and φ turns ratios will be increased to 64 : 1. This assembly is at present in the process of construction.

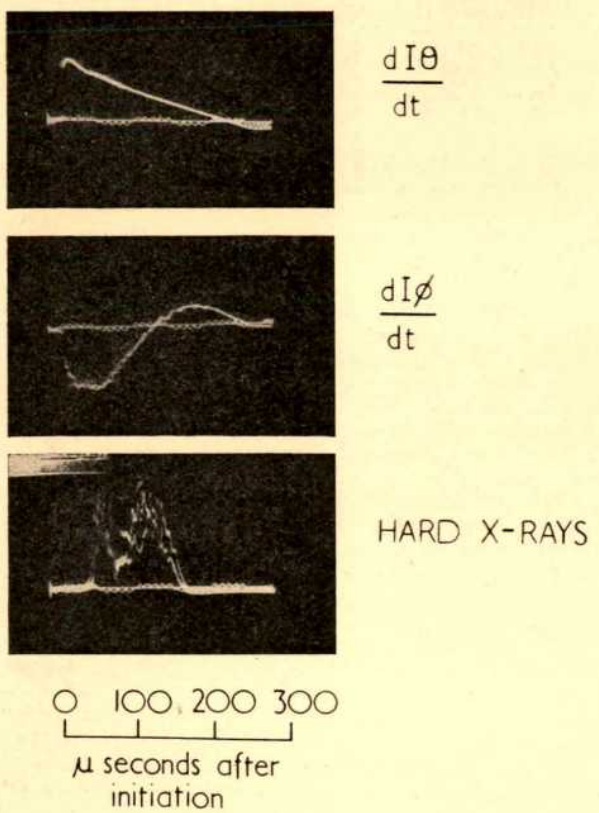


Fig. 3. $\dot{I}_\theta$, $\dot{I}_\varphi$ and hard X-rays as a function of time in hydrogen. V_θ = 10 kV; V_φ = 12 kV; press = 4 millitorr. θ turns ratio = 32 : 1 overlay of three discharges

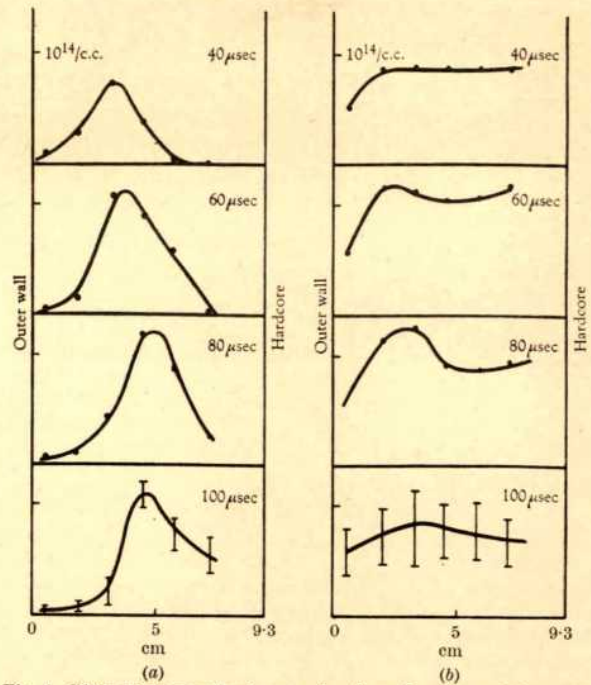


Fig. 4. Plots of plasma density as a function of radius and time: (*a*) for normal levitron operation; (*b*) for *Sceptre* type operation in the levitron. Condition for (*a*): $V_\theta = V_\varphi = 10$ kV; $p = 4$ millitorr. θ turns ratio = 32 : 1

Measurements have been made with double Langmuir probes, magnetic probes, a Hilger medium quartz spectrograph and two monochromators. In the third assembly observations were also made on X-rays, and streak photographs were taken with a rotating mirror camera. Measurements of I_φ and I_θ (Fig. 1) and the volts per turn in both the φ and θ directions have been made under all conditions.

I_φ is the sum of the axial gas and hardcore currents. The decrease in this quantity on the application of the θ bank, as shown in Fig. 2, is a measure of the gas current. This current is a function of $V_{\dot{\theta}}$ and V_φ and is of the same order as that in the hardcore. The gas currents cease to be measurable soon after clamping of the main θ bank (see Fig. 3), but plasma persists for an appreciably longer time (~500 μsec).

As measured by the Langmuir probe, the maximum value of electron temperature, T_e, so far recorded is $2{\cdot}3 \times 10^5$ °K. This was obtained for a θ turns ratio of 16 : 1 and V_θ = 13·5 kV, V_φ = 12 kV. For higher values of V_θ there is little or no increase in T_e. For very low values of V_θ the discharge is relatively clean, but for V_θ greater than about 12 kV large quantities of silicon and oxygen, among other impurities, are present. Ov was observed in the second assembly, but it was not sufficiently intense to allow an estimate of ion temperature to be made.

With low energies on the θ bank, the Langmuir probes give ample evidence of the plasma leaving the walls of the torus (Fig. 4*a*). This is confirmed by the streak photographs. In Fig. 5 part of the hardcore and plasma are obscured by the walls of the torus. Comparison of the ion density plots with H_β oscillograms indicate that high degrees of ionization (~ 100 per cent) are achieved by the time the gas current reaches peak value.

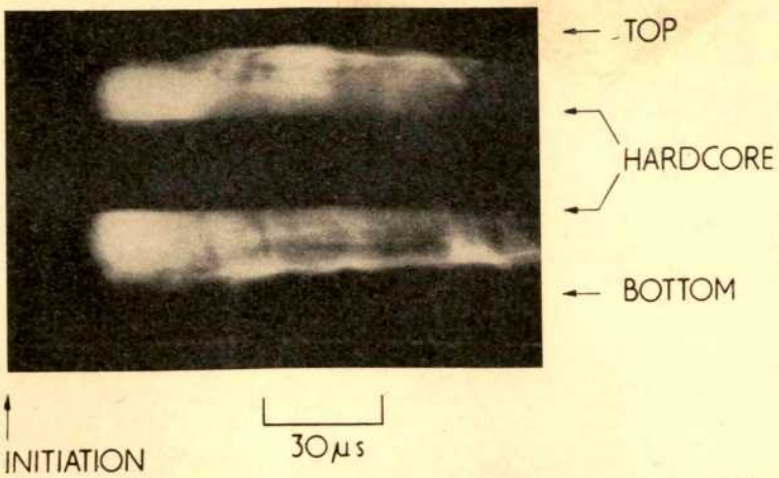


Fig. 5. Streak photograph of hydrogen discharge in levitron. $V_\theta = 10$ kV; $V_\varphi = 12$kV; press. = 2 millitorr; θ turns ratio = 32:1

Magnetic probe measurements have been made as a function of radius and time. As shown in ref. 8, subject to certain assumptions, current densities, pressure distributions, electric fields, mass velocities and conductivities may be deduced from these. Using these results energy containment times may be obtained. The details of this analysis, as applied to the levitron, will be given in a subsequent paper. The estimates of the energy containment times, although at present crude, are not greater than 10–20 μsec.

The perturbing effects of a probe have yet to be fully investigated, but a probe is known to have a marked effect on the production of X-rays. In the absence of a probe the X-rays begin almost immediately the discharge leaves the walls of the torus. However, with a probe, or to be more exact a probe envelope present, comparison between the streak photographs and hard X-ray (> 30 keV) oscillograms shows that the X-ray intensity is almost negligible until the plasma has moved beyond the end of the envelope. In one experiment in which the hardcore was supported on insulated rods, no hard X-rays at all were observed. In general, such rods appear to have undesirable effects and they have been utilized in only a few experiments. The origin and spectra of the X-rays have yet to be fully investigated.

All types of measurements show fluctuations. In most cases, however, these are small and under certain conditions of operation the good reproducibility of the $\frac{dI}{dt}$ and X-ray oscillograms is remarkable (Fig. 3). Likewise, fluctuations in the magnetic field are small, being only a few per cent of the mean value.

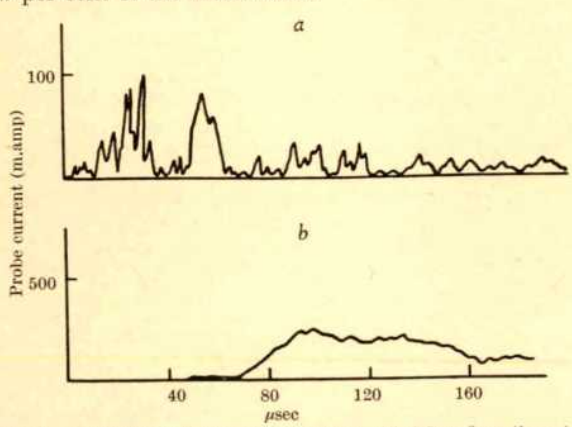


Fig. 6. Langmuir probe traces in hydrogen. (*a*), 0·5 cm from the outer wall; (*b*), 7 cm from the outer wall. $V_\theta = V_\varphi = 10$ kV; $p_h = 4$ millitorr; θ turns ratio = 32 : 1

On the other hand, the fluctuations in ion density can be large. Expressed as a percentage they are small near the hardcore but large near the torus wall (Fig. 6). The reproducibility of these measurements at $t = 100$ μsec is indicated in Fig. 4. The density plots shown in this figure were obtained by using a double-electrode Langmuir probe, and not a multiple set. Overlays of three oscillograms, of the type displayed in Fig. 6, at six different radii were used. It has been confirmed that these fluctuations are due to changes in ion density and not electric fields.

To obtain a better understanding of the significance of these fluctuations the following experiment was carried out. A pinch-type discharge, produced in the manner already described, was compared with a normal levitron discharge. In an attempt to make the comparison meaningful, the following parameters were kept the same in the two experiments: the initial gas pressure, the rise time of the gas currents, the initial electric fields at the walls of the torus, the pinch parameters $\left(\text{determined by the ratios } \frac{V_\theta}{V_\varphi} \text{ and } \frac{V_\varphi}{V_\theta}\right)$ and the energies on those condenser banks that initiated the plasma.

The electron temperature proved to be the same in both types of discharge, while even in the pinch-type the magnetic field fluctuations were small (due no doubt to the presence of the hardcore). However, in the case of pinch operation the plasma density was relatively high near the walls of the torus and fluctuations were large (Fig. 4*b*). Furthermore, arc damage, which in normal levitron operation was confined to regions near the gaps, was distributed at random over the walls of the torus. Again, from magnetic probe measurements made during pinch-type operation it was possible to show that Suydam's criterion[9] was violated at all radii for all times. The significance of these results will be discussed in the next section.

One feature of this comparative experiment was that in pinch-type operation the axial gas current was appreciably larger than that in the corresponding levitron case. This was not, nevertheless, believed to be a basic fault with the experiment. The stability behaviour of the levitron-type plasma is not markedly dependent on the axial gas current.

The rapid decay of the gas currents, subsequent to clamping of the θ bank, and the low estimates of the energy containment times are quite consistent with a relatively high level of impurity and an associated low electron temperature. Although these results are significant a more important measure of the effectiveness of the levitron configuration is the particle containment time. This can be estimated in the following manner.

On the assumption of cylindrical symmetry, and extrapolating in the vicinity of the hardcore, density plots of the type shown in Fig. 4*a* may be integrated to give the number of trapped ions as a function of time. For a 32 : 1 θ turns ratio this number goes through a maximum after about 70 μsec and then begins to decay. At a 16 : 1 θ turns ratio the time to reach a maximum is 40 μsec. During the decay the H_β signal is small and the decay time may be taken as a measure of particle containment.

In the second assembly these decay times, prior to clamping of the θ bank, were 50 μsec for a 16 : 1 θ turns ratio and 150 μsec for a 32 : 1 θ turns ratio. In the third assembly they were somewhat shorter, 70 μsec at 32 : 1, but this may have been associated with the erratic behaviour of the hardcore. Following clamping, the times for a 32 : 1 turns ratio are about twice as long. Insufficient

information was obtained to estimate the equivalent result for a 16 : 1 turns ratio.

The maximum number of trapped ions in hydrogen does not appear ever to exceed 30 per cent of the initial filling. Although charged particles are undoubtedly being lost it is also possible that during the early stages, when the H^β is still large, the escape of 'fast' neutral atoms (~ 2 eV) produced in the dissociation of H_2 may also be contributing to the loss processes.

The particle containment times are short, but there is no evidence as yet that they are limited by an instability. In fact it is probable that they are merely representative of the time taken for a particle to be driven into the hardcore by the applied $E \wedge B$ forces and induced pressure gradients. The streak photographs imply mass velocities of the order of 10^5 cm/sec, directed towards the hardcore, while the analysis so far carried out on the magnetic probe results and general electrical measurements suggest similar values. The exact values of these velocities are, of course, among other variables, functions of time, radius and θ turns ratio.

The behaviour of the hard X-ray intensity, as the plasma moves beyond the end of a probe envelope, implies good containment of the magnetic lines of force.

The large ion density fluctuations are not incompatible with the small magnetic field fluctuations. It has been pointed out by Wesson[10] that it is most likely that $\delta p \sim \delta\left(\frac{B^2}{2\mu_0}\right)$ (M.K.S. units). B is the magnetic field, p the kinetic pressure. That is, $\frac{\delta p}{p} \sim \frac{2B^2}{2\mu_0 p}\frac{\delta B}{B}$. Therefore, for a value of $\frac{B^2}{2\mu_0 p} = \frac{1}{\beta} = 5$; a 5 per cent fluctuation in B could correspond to a 50 per cent fluctuation in p. The magnetic probe results indicate that β is relatively small and values of this parameter even less than 1/5 are not unreasonable.

Finally, a brief comment must be made on the comparison experiment. In the case of the pinch, the fact that Suydam's criterion was violated accounts for the observed instability. In the case of the inverse pinch, however, the fact that $j_z B_\theta < 0$ automatically ensures that Suydam's criterion is satisfied, and this criterion is of no help in assessing the stability of such plasmas. As already pointed out, for normal levitron operation, the condition corresponding to equation (1) is much more complicated and so far the measurements necessary to test this criterion have not been performed. Therefore it is possible that the fluctuations observed in normal operation are also of hydromagnetic origin. However, whether or not this is the case there is little doubt that the containment properties of the normal levitron type discharge are superior to those of the pinch.

The levitron discharge is more stable than a toroidal pinch discharge and shows better isolation of the plasma from the walls of the torus. However, there is still an instability, the nature of which is unknown at present. There is no evidence as yet to show that this instability limits the particle containment time or the electron temperature. In fact, it is probable that particles are lost by normal collisional processes and that the electron temperature is limited by the presence of impurities. It is believed that these impurities are associated with arcing at the gaps. In an attempt to avoid this trouble both the θ and φ turns ratios are to be increased to 64 : 1 in the next assembly.

This work is being done under contract to the Culham Laboratory of the U.K. Atomic Energy Authority and we thank the members of that Laboratory for their interest. We also thank the many members of this Research Laboratory who have contributed to this project. In particular we acknowledge the interest and encouragement of Dr. T. E. Allibone and Dr. D. R. Chick; Mr. P. Tanner for the design and construction of much of the electronics; Mr. J. B. Hansell for assistance in many of the engineering problems, particularly levitation, and Mr. J. A. McKinnon for the daily maintenance and operation of the machine. Finally, we thank Sir George Thomson for his interest in this work and Dr. T. E. Allibone, director of the laboratory, for permission to publish this paper.

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POST-TERTIARY SPECIATION IN EUROPE

IF the present distribution of species and sub-species in animals and plants is drawn on a map, the boundaries represent geographical discontinuities. In the past these boundaries have fluctuated as migration has proceeded. Phylogenetic patterns are produced by the interaction of such fluctuating boundaries with evolutionary changes arising from a variable rate of morphological modification. The Systematics Association attempted to explore the nature of such patterns during a symposium held at the University of Leicester in April 1962. The symposium, which was confined to the discussion of the speciation process during post-Tertiary time in Europe, was arranged in three sessions under the chairmanship of Dr. J. P. Harding (president), Prof. T. G. Tutin and Prof. P. C. Sylvester-Bradley respectively, and culminated in a vigorous discussion led by Prof. F. E. Zeuner.

Prof. F. E. Zeuner (Institute of Archæology, University of London) directed attention to varying rates of speciation. Palæontological evidence for the rate of speciation is best assessed on the assumption that taxonomists in each of the groups in question apply the same species concept to both Recent and fossil material. Usually, and for obvious reasons, the time-rates of morphological change are studied in fast-evolving groups. In some groups, however, species survive for a long time as, for example, in the Mollusca (Table 1). That species should maintain their stability for such long periods is possible only if either their rate of genetic mutation is very slow, or if all or most mutations are weeded out by natural selection as fast as they appear.

Table 1. Java—Marine Mollusca

Age in million years		Species indistinguishable from Recent forms (per cent)
30	Miocene	10– 30
10	Pliocene	50– 70
1	Pleistocene	80– 90
0	Holocene	100

Mammals and insects present a different picture. In mammals, no Recent species are known that are older than the Pleistocene, and only a few from the beginning of the Pleistocene—among them *Cervus elaphus* L. Even in the late Tertiary, there is an abundance of genera which are

now extinct. Insects are somewhat more conservative; there are even a few species in the Tertiary which have been regarded as identical with Recent ones. Many Recent insect genera, however, existed in the late Tertiary, though their number decreases with increasing geological age. Three groups may be used to illustrate this. In Tables 2–5, genera and sub-genera, and species and sub-species, respectively, are combined.

Table 2. Percentage Analysis of 92 Species of Fossil *Apoidea* (Bees)

	Extinct genera with extinct species (per cent)	Recent genera with extinct species (per cent)	Recent genera with recent species (per cent)
Eocene	94	6	0
Oligocene	50	50	0
Lower Miocene	38	60	2
Upper Miocene	24	76	0
Pleistocene	0	0	100

The two 'Recent' species recorded from the Lower Miocene are probably spurious. The honey bee, however, presents a case of exceptionally slow evolution. During 30 million years, since the Oligocene, it has scarcely changed at all. Palæontological and cytological evidence suggests that evolution came virtually to a standstill when polyploidy appeared and when the social structure of the hive had isolated the queen from the external environment[1].

Table 3. Percentage Analysis of 75 Species of Fossil *Lepidoptera* (Butterflies and Moths) (Ref. 5)

	Extinct genera with extinct species (per cent)	Recent genera with extinct species (per cent)	Recent genera with recent species (per cent)
Eocene	96	4	0
Oligocene and Lower Miocene	85	15	0
Upper Miocene	50	50	0
Pleistocene	0	29	71

Table 4. Percentage Analysis of 46 Species of Fossil *Saltatoria* (Grasshoppers S.L).

	Extinct genera with extinct species (per cent)	Recent genera with extinct species (per cent)	Recent genera with recent species (per cent)
Eocene	72	28	0
Oligocene	30	70	0
Lower Miocene	55	45	0
Upper Miocene	80	20	0
Pleistocene	0	54	46

The number of species included in these tables is too small to show consistent rates of change, but the trend is clear, especially when the three groups are combined (Table 5) and plotted on the time scale (Fig. 1).

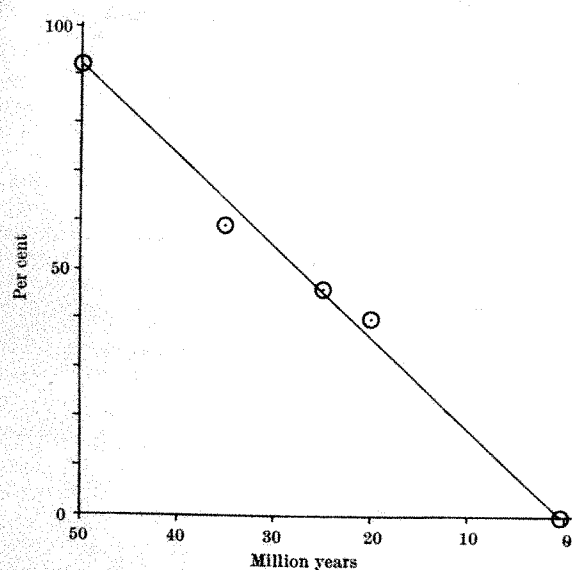


Fig. 1. Percentage of insect species now extinct plotted against geological age. Data from Table 5

Table 5. Percentage Analysis of 212 Species of Fossil *Apoidea*, *Lepidoptera* and *Saltatoria*

	Extinct genera with extinct species (per cent)	Recent genera with extinct species (per cent)	Recent genera with recent species (per cent)
Eocene	92	8	0
Oligocene	59	41	0
Lower Miocene	46	52	2
Upper Miocene	39	61	0
Pleistocene	0	29	71

Table 5 shows that the decrease in the number of extinct genera appears to be a simple function of time. While 50 million years ago 8 per cent of the genera were identical with Recent ones, at the beginning of the Pleistocene, 1 million years ago, all genera were Recent. But the Pleistocene contains as many as 29 per cent of extinct species (and sub-species), which elucidates to some extent the rate of change in the lowest systematic categories. The East African copal contains a few species not known to be living, but this may be due to insufficient knowledge of the Recent East African fauna.

The most informative Pleistocene site is Starunia, in the Carpathians, of early Upper Pleistocene age and belonging to a cold phase. Its *Saltatoria* have been studied in detail[2].

The nine species represented can be classified as follows:

Extinct, related to, but more highly specialized than, a localized Recent species in Montenegro	1
Extinct, intermediate between a Recent species in Montenegro and one in Altai Mountains	1
Very close to Altai species, perhaps a geographical race	1
Identical with Recent Asiatic species	1
Identical with Recent holarctic mountain species	5

No progressive evolution is present, but extinction has occurred of one species and of one geographical race whereby a cline was disrupted so that two separate Recent species are recognized.

Finally, the Isle of Jersey, Channel Islands, which has been separated from the Continent for about 7,000 years, has produced, among 21 species of *Saltatoria*, 2 distinct geographical sub-species and one dwarf form which is not morphologically different from the Continental form[3]. In addition, there is a local colour form of a shrew, and the red deer evolved an insular sub-species in the Last Interglacial which is now extinct[4]. This evidence suggests that minor geographical sub-species or ecological forms have appeared within 7,000 years, but that for the evolution of full species a hundred thousand years are (normally) insufficient, while half to one million years is a reasonable estimate for the time required. No insect species are known with certainty to have survived from the Miocene, but as an example of abnormal, slow evolution, the honey bee must be recalled. Generic differences have not appeared during the Pleistocene but Recent genera are known to have lasted for at least 50 million years, among them *Drosophila*.

In mammals, evolution appears to have been somewhat faster than in insects; in marine groups (Mollusca) it proceeds more slowly. The slow rates suggested by palæontological evidence suggest that the apparently fast rates deduced in some groups from Recent evidence apply to stabilized populations of ecotypes, to isolated communities exhibiting insignificant mutations, and, in plants and, in very rare cases, in animals, to polyploids.

Speciation studies in animals have been particularly illuminating in mammals, in birds and in butterflies, and each of these groups was dealt with by a separate speaker.

Dr. G. B. Corbet (British Museum (Natural History)), speaking of recent work on the British microtine rodents, emphasized how often breaks in the regularity of a cline are correlated with geographical discontinuities. Thus the short-tailed field mouse (*Microtus agrestis*) is represented by two parallel north–south clines in Britain and Scandinavia, affecting the pattern of the upper molar teeth. In Britain the greatest discontinuity on the mainland is across the Scottish Great Glen. Presumably the chain of lochs and rivers in the Great Glen is a barrier to gene flow. Other discontinuities separate island races, and it is

hoped to extend the investigation by applying more rigorous quantitative techniques to fossil material.

Prof. K. H. Voous (University of Amsterdam) showed that in birds the speciation process in Europe differed markedly from the adjacent palæarctic region to the east (Asia). Some indication of post-Tertiary species-formation can be detected in 43 per cent of the birds at present breeding in Europe. While Europe was the scene of active speciation, Asia provided a reservoir of 'old' species, only a few of which remained as relics in Europe. Notable among these relics is the Corsican form of the black-capped nuthatch, *Sitta canadensis whiteheadi*, which, as Löhrl[6] has shown, has developed marked differences in social and reproductive behaviour. That southern Europe formed a refuge for birds during times of glaciation, and was during these times isolated from the Asiatic reservoir, is shown by the interaction of the green woodpecker (*Picus viridis*) with the grey-headed woodpecker (*P. canus*). The former has re-colonized Europe from the south, the latter from the east. In the greater part of central and eastern Europe, and in small areas of Scandinavia, they now occur alongside each other, and behave as distinct though sibling species, with some degree of ecological differentiation[7–9]. Other cases with a similar history are listed in Table 6*a*. In yet other cases, the period of isolation has not resulted in a fully specific differentiation. In many the subsequent re-colonization from the two directions has resulted in zones of hybridization between sub-species in which the amount of individual variation is extremely wide (Table 6*c*). There are also certain cases where the degree of differentiation achieved during the period of isolation seems to be intermediate between that of the species and that of the sub-species (Table 6*b*).

Table 6

Species or race re-colonizing from the South	Species or race re-colonizing from the East
(*a*) Pairs in which specific differentiation has been achieved (ref. 9):	
Picus viridis	*P. canus*
Milvus milvus	*M. migrans*
Alectoris rufa	*A. graeca*
Porzana parva	*P. pusilla*
Strix aluco	*S. uralensis*
Galerida theklae	*G. cristata*
Regulus ignicapillus	*R. regulus* (ref. 10)
Parus caeruleus	*P. cyanus*
Certhia brachydactyla	*C. familiaris*
Emberiza cirlus	*E. citrinella*
Fringilla coelebs	*F. montifringilla*
Luscinia megarhyncha	*L. luscinia* (ref. 10)
(*b*) Pairs in which differentiation is intermediate in rank between species and sub-species	
Buteo [*buteo*] *buteo*	*B.* [*buteo*] *vulpinus* (ref. 11)
Corvus [*corone*] *corone*	*C.* [*corone*] *cornix* (ref. 12)
(*c*) Pairs in which sub-specific differentiation has been achieved	
Dendrocopos major pinetorum	*D. major major* (ref. 8)
Dendrocopos minor hortorum	*D. minor minor* (ref. 8)
Lanius excubitor galliae	*L. excubitor melanopterus* (refs. 13 and 14)
Aegithalos caudatus aremoricus	*A. caudatus caudatus* (ref. 15)
Sitta europaea caesia	*S. europaea europaea* (ref. 16)
Pyrrhula pyrrhula europoea	*P. pyrrhula pyrrhula* (refs. 15 and 17)
Garrulus glandarius glandarius	*G. glandarius brandtii* (ref. 18)

The southern refuge area was further complicated by the fact that the intricate Mediterranean coast-line, with its three isolated peninsulas, led to some differentiation into West Mediterranean and East Mediterranean forms.

Mr. L. G. Higgins (Chobham) recognized three contrasting patterns in the speciation of European butterflies. The first group (Holarctic) is an extension of the fauna of northern Asia to most of Europe, including the Arctic and the Central Alps, and often to North America. Despite the vast distribution area, there is little regional variation, and sub-species are rare. Some, like *Colias palaeno* L., show ecological modifications. The second faunal pattern is developed in Mediterranean areas, and has originated in the Near East. There are many localized indigenous species, and sub-specific differentiation is common. This fauna includes the Lycaenids; the taxonomic situation is complicated by a great diversity in chromosome numbers. Finally, there is a unique pattern of speciation in the mountain ringlets of the genus *Erebia*, which, despite a holarctic distribution, shows a concentration of well-defined species in the Central Alps. Some of these are confined to one or two alpine valleys. Others, with a wider distribution, show marked geographical variation. They appear to have originated in the Alps from pre-Quaternary stocks, and active speciation is now in progress.

Three speakers dealt with the processes of post-Tertiary speciation in flowering plants. Dr. V. H. Heywood (University of Liverpool) pointed out that speciation is essentially an experimental term and implies the origin and development of reproductively isolated gene-pools. The species utilized in most phytogeographical considerations are taxonomic species and are based on morphological discontinuities in the variation pattern. They are known to represent very different evolutionary situations. In considering post-Tertiary speciation we are bound to use data concerning the present-day distribution of species which are seldom known from an evolutionary point of view. As a consequence, whether speciation is considered to have occurred or not is a matter of taxonomic judgment. This was illustrated by reference to recent studies on the cytology and taxonomy of British and European *Leucanthemum vulgare*, where different polyploid races are only with considerable difficulty separable on morphological criteria such as pollen-size. Such cryptic 'species' may come and go in the fossil record without our even knowing it, relying as we do on exomorphic features.

In considering the post-Tertiary speciation of Angiosperms the following lines of evidence should be considered: (1) Present-day distribution and ranges of species which can at best provide indirect evidence concerning what happened in the past. This relies heavily on taxonomic judgments. (2) Palæobotanical data which will tell us what happened, when the material available is adequate and there are good sequences. This approach is well illustrated by the Quaternary studies of Godwin and the Cambridge school providing factual information concerning the successions and migrations of floras in the post-glacial periods and earlier. (3) Cytology, genetics, ecology and physiology of contemporaneous species tells us how speciation happens to-day and, by extrapolation, how it probably happened in the past. The most fruitful approach to an understanding of post-Tertiary speciation will come from a study of present-day populations by experimental cultivation to assess their ranges of variability in different parts of their area, their possible biotype de-pauperation during migrations, the influence of geographical isolation; by cytogenetic studies to tell us about their fertility relationships; and by morphogenetic and physiological studies to tell us about their tolerance and powers of extension. Such a combined approach will, as Böcher has said recently, raise many discussions of the evolution of taxa from a purely theoretical level to a factual basis.

The bearing of polyploidy on speciation was then considered in detail, since the polyploid nature of present-day taxa is one of the main lines of evidence for estimating the relative age of taxa. It is widely agreed that diploid taxa, especially in montane or arctic regions, originated in the Tertiary with subsequent formation of polyploids in the Quaternary and up to recent times. Polyploidy is almost always an irreversible phenomenon, making it all the more important that the cytological status of populations should be known. Examples are known of taxa once regarded as recent endemics which have turned out on investigation to be diploid relics while major populations from which they were thought to have been derived are found to be polyploid and themselves derived.

Conditions in the Quaternary are likely to have favoured hybridization and intraspecific crossing of differently adapted populations or ecotypes, especially in montane habitats, with the consequent formation of allopolyploids. New habitats opened up by the glaciations allowed the establishment of these new polyploids and changed selection pressures would allow the further crystallization out of the polyploid derivatives. The

sequence of hybridization and polyploidy was probably followed, however, by subsequent hybridization between polyploid components with the result that the morphological gaps between the original taxa were filled.

Polyploidy is often, therefore, a cyclical phenomenon and the evidence from many cases seems to point to the desirability of considering polyploidy as a progressive condition that can arise within species or species-groups. It is, in Huxley's terminology, not simply cladogenesis but also anagenesis, although neither term quite applies. It was pointed out that in polyploid complexes such as *Dactylis glomerata*, *Phacelia magellanica*, etc., relationships are normally shown diagrammatically as anagenesis not cladogenesis. The implications of such a view of speciation by progressive polyploidy were discussed and it was suggested that in many such cases speciation had been polytopic and not monotopic as we have long been conditioned to think. If we looked for simple origins of species in many instances of polyploidy, it was probable we were asking the wrong questions.

The structure and dynamics of a typical angiospermous eupolyploid racial complex was demonstrated by F. Ehrendorfer (Vienna) using the example of the Mediterranean–European Section Lepto-Galium (Rubiaceae). Lepto-Galium consists of 6 series, corresponding to good Linnean species, and about 40 micro-species; its history must go back a considerable way into the Tertiary. Three special cases were chosen to bring out some important general evolutionary features of Lepto-Galium: (1) The closely related alpine groups of *Galium baldense* and *G. anisophyllum* have undergone quite a different course of evolution. The *G. baldense* group is represented mainly by diploids and only one tetraploid ($2x + 4x$); it is strongly depleted in biotypes and has a very disjunct distribution in or near unglaciated areas. *G. anisophyllum*, on the other hand, is represented mainly by polyploids ($4x + 6x + 8x$) with only a few diploids ($2x$) persisting, and one advanced local decaploid ($10x$). It is very polymorphic and has a continuous distribution with the diploids around unglaciated areas and the ascending polyploid series progressively in marginal to central glaciated areas. (2) Detailed cytotaxonomical analysis of Lepto-Galium races in north-eastern Central Europe and adjacent Scandinavia has revealed that what was formerly regarded as one species consists of a number of micro-species. The diploids of *G. cracoviense* on limestone rocks of the Cracovian Jura and *G. oelandicum* in open Alvar vegetation of the Island of Oeland are closely related. Furthermore, there is the diploid *G. suecicum* in grasslands and open pine forests with a disjunction between the north-east German plains and southern Sweden. A tetraploid extension of the southern *G. valdepilosum* has reached the warm open oak woodland of the Hartz Mountains and central Jylland. Finally, there is the widespread octoploid, *G. pumilum*, which has its main habitats in mixed acidophilous oak forests of Central Europe but was introduced with grass seed farther north. These races seem to represent remnants of four waves of post-glacial invasion into formerly glaciated territory. They obviously correspond to well-known phases of post-glacial vegetation-succession and may be reconstructed with the help of ploidy sequence, ecological requirements, and by taking into consideration the post-glacial palæogeography of the Baltic Sea area:

G. cracoviense → *G. oelandicum* ($2x$): ± 7500 B.C., cold tundra and grassland.
G. suecicum ($2x$): 7000–6000 B.C., open boreal pine woodland.
G. valdepilosum ($2x$–$4x$): 4000–3000 B.C., thermophilous oak woodland.
G. pumilum ($8x$): 2000 B.C. → present, mixed oak climax forest and adventitious.

(3) The origin of the relatively recent high polyploid *G. pumilum*, the most widely distributed microspecies of the section, can be linked to diploid and tetraploid relic groups of progenitors which to-day are found partly in south-eastern European refugial areas. In addition, there is ample evidence for hybridization on the octoploid level with other parallel polyploid lines (*G. rubrum*, *G. marchandii*, *G. anisophyllum*), resulting in further increase of variability and plasticity of *G. pumilum*. While the primitive diploids and tetraploids are found mainly in permanent rock, talus and grassland associations, the derived polyploids are concentrated in open climax forest and corresponding successional associations.

One may generally conclude that cytogenetical, morphological, chorological and ecological aspects of racial structure and evolution in Lepto-Galium are closely tied together. While basic divergent evolutionary differentiation occurs predominantly on the diploid level, polyploidy linked with intra- and inter-specific hybridization seems very important for the mobilization of genotypic plasticity blocked on the diploid level by various internal isolation factors. This blockade is overcome by the origin of hybridogenous and polymorphic polyploid daughter-races. From a morphological point of view, the diploids form a discrete racial basis while the polyploids build a diffuse racial superstructure. The diploids are depleted in biotypes, show less colonizing activity, are often left behind in refugial areas and have disjunct distribution patterns. The polyploids with an increasing biotype reservoir are aggressive, have re-colonized extensively glaciated areas and show continuous distribution patterns. The diploids have been conserved mainly in stable environments and in permanent 'marginal' associations where successional changes of soil and vegetation are checked by specific edaphic conditions. The polyploids, on the other hand, mainly originate and are dispersed as members of widely expanding 'central' climax associations and corresponding labile successional stages.

Dr. G. Haskell (Genetics Department, Scottish Horticultural Research Institute) discussed the relationships between the effects of glaciation in Great Britain and North America and the present distribution of the genus *Rubus* (*R. fruticosus* agg.). Particular emphasis was paid to the prevalence of polyploidy and apomixis in this successful genus. In Europe there are now only a few diploid, sexual primary species which are considered to have survived the last glaciation, *R. tomentosus* and *R. ulmifolius* alone being widespread. Of these only *R. ulmifolius* occurs in Britain and is more frequent in the south, its distribution decreasing north of the last glaciation boundary (very roughly a line from the Wash to the Bristol Channel). When the vice-county frequencies of the 344 contemporary British species, listed in Watson's *Hand-book*, are plotted, the centre of diversity is revealed as south-east England, and the highest frequencies are generally in the non-glaciated areas. It is possible, however, that nunataks occurred north of the glaciation line.

The life-cycle of a representative sexual genus, stable at the diploid level, was illustrated by *Ribes nigrum*, and a facultative apomictic Rosaceous genus by *Malus zumi* and *M. sieboldii*. Competition between apomictic and sexual cells was demonstrated with one or other type ultimately winning. Fertilization is necessary in *Malus* to form a sexual endosperm, even that of an apomictic embryo, although such an embryo receives no contribution from the pollen. This is recognized genetically in *Rubus* as pseudo-heterosis in the young maternal-like offspring, and depends on the pollen parent. Hence all apomictic *Rubus* are pseudogamous; that is, they require pollination by fertile pollen.

Diploid primary *Rubi*, although sexual, are either partially or completely self-incompatible. Hence following the glaciation, with the consequent paucity of pollinating agents and the infrequency of individual plants, there would be a reduction in the opportunity for cross-pollination in the species, leading to poor seed setting. As polyploidy usually overcomes incompatibility, and as diploid *Rubi* are known to produce unreduced gametes, there would be encouragement of any polyploids produced by these diploids. Although they would still require pollination they could then set self-seed. This would,

however, have led to gradual inbreeding depression, known in diploid and tetraploid *Rubi* (for example, *R. idaeus* [2*x*], Merton Thornless [4*x*]), so there would be selection for those polyploids able to maintain themselves as heterozygotes with the consequent hybrid vigour. In other words, the apomictic pseudogamous types with good pollen would be at a selective advantage.

The apomictic mechanism in *Rubus* may or may not involve crossing-over and recombination, namely, apospory and diplospory respectively. Hence variation, although somewhat limited, can still occur and selection since the time of the last glaciation has been able to act on these forms. Hence evolution has given rise to the numerous apomictic 'species' which have proved so adaptable in re-colonizing land once under the ice. It is known that some British species of apomictic *Rubi* are widely spread and constant breeding: these are the predominantly aposporous types. The unstable apomicts, often represented by clusters of related minor 'species' with limited and scarcer distribution, are those segregants of a more diplosporous nature.

The whole gamut of these polyploid apomictic *Rubi* must have a fairly limited original parentage from diploids failing to survive glaciation. This is indicated by pollen of all species so far tested (providing the pollen is fertile) being able to function on the stigmas of other species, regardless of which of the 5 sub-genera they belong to. Furthermore, most of these apomicts are still facultative sexuals, so providing increased adaptability.

Finally, although polyploid apomictic *Rubi* were at an advantage compared with sexual diploids following the last ice age, some tetraploid species, for example, *R. rosa* (4*x*) from North America, are now regularly producing secondary diploids, which are as vigorous as their tetraploid sibs. Attempts are now being made to extract diploids from the highly apomictic *R. caesius* (4*x*), believed from its cytology to be allotetraploid. In this way it is hoped to expose the original diploid parental species, which must have failed to survive glaciation, but which have been maintained biologically intact through the aposporous mechanism for thousands of years.

During the discussion which ensued it was very clear that mechanisms through which speciation occurred differed markedly in animals and in plants. Polyploidy and apomixis had clearly played an extremely important part in the development of the vigorously colonizing plant species in post-glacial times, whereas neither process is suspected to have occurred to the same effect in the animal stocks concerned. Polyploidy and chromosome complications in insects, mentioned by both Prof. Zeuner and Mr. Higgins, seem to have been adapted to a different evolutionary purpose. Yet, surprisingly, the distribution patterns shown by the active post-Tertiary migrants, whether of animals or plants, had many points in common. It seemed that the environmental control called for a similar response, and this was supplied regardless of the mechanism concerned.

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NUCLEAR MAGNETIC RESONANCE IN MOLECULES OF MODERATE AND HIGH MOLECULAR WEIGHT

THE third series of talks on magnetic resonance organized by the Department of Chemical Technology, Bradford Institute of Technology, was held during April 25–26. This year the title was "Nuclear Magnetic Resonance in Molecules of Moderate and High Molecular Weight". About seventy graduates attended from universities, colleges of advanced technology, Government establishments, and industry.

Opening the meeting, Dr. E. G. Edwards, principal of Bradford Institute of Technology, pointed to the number of academic institutions and of branches of the chemical industry represented—including colloids, fibres, organic chemicals, paper, petroleum, plastics, resins, rubber and soap—as some commentary on the wide acceptance of nuclear magnetic resonance in academic and industrial research. Nuclear magnetic resonance spectrometer manufacturers also sent representatives: three from the United Kingdom and others from Japan, Switzerland and the United States.

In his introductory talk, Dr. D. W. Jones (Bradford Institute of Technology) summarized the magnetic properties of nuclei which underlie nuclear magnetic resonance spectroscopy and showed how the chemical applications arise from interactions between the resonating nucleus and its magnetic environment—whether of the order of gauss in, for example, a solid polymer, or of milligauss in a liquid sample. As molecular size increases, the high-resolution spectrum does not invariably become complicated—for example, the phosphorus-31 spectra of some organic phosphorus compounds—but proton spectra of large molecules usually are complicated and low solubility or high viscosity may provide further problems. Such spectra with overlapping fine structure can often be unravelled with the aid[1] of high laboratory magnetic fields, integration of peak areas, and the double resonance technique.

In addition to the spectrometer components which have their analogues in more familiar regions of the electromagnetic spectrum, nuclear magnetic resonance needs a magnetic field of high intensity, homogeneity and stability. Dr. R. Freeman (National Physical Laboratory) first dealt with these requirements in his talk on experimental methods, and then outlined the radio-frequency bridge and crossed-coil systems as means of achieving minimal effective transmitter noise in steady state nuclear magnetic resonance experiments. With signals several kc/s broad, genuine nuclear magnetic resonance signals could be emphasized by modulating the field over a narrow band to yield a differential curve on sweeping. With liquid lines less than 1 c/s broad, the first pair of side bands produced by putting an audio-frequency of low modulation index on the resonant frequency of, say, 60 Mc/s has been used for calibration, base-line stabilization and control of field/frequency ratio. Finally, Dr. Freeman showed that it was feasible to use modulation techniques to achieve spin–spin decoupling[2] (frequency sweeping[3] one radio-frequency) and to observe the spin coupling effects on hydrogen-1 of carbon-13 in, for example, acetic acid molecules in which one of the two carbon nuclei is a magnetic isotope.

The second session was devoted to more detailed treatments of—chiefly high-resolution—nuclear magnetic resonance in various branches of chemistry. Dr. J. A. S. Smith (University of Leeds) opened his review of inorganic applications with a reference to susceptibility measurements of liquids by nuclear magnetic resonance, as first suggested by Feher and Knight and more recently by Frei and Bernstein[4], a topic which attracted attention in the subsequent discussion with regard to the validity of the calculations to samples in close contact. A variety of problems, including general stereochemistry, detailed bonding, and exchange processes, was described, starting with hydrido-complexes in which direct hydrogen-1-metal bonds are characterized by a proton peak of very high τ-value. The metal alkyls exemplify the use of both of the commonly measured nuclear magnetic resonance high-resolution parameters: coupling of the magnetic platinum-195 nucleus in aqueous trimethylplatinum nitrate[5], for example, gives a 77·3-c/s doublet across the methyl singlet corresponding to platinum nuclei with zero spin; while chemical shifts between methylene and methyl protons in X–CH_2–CH_3 compounds have been correlated with electronegativity. Among π-complexes, such as Zeise's salt and *cyclo*pentadienyl complexes, Dr. Smith showed that a comparatively simple hydrogen-1 resonance criterion can sometimes give a useful structural indication. Most of the talk dealt with diamagnetics, but mention was made of some paramagnetic complexes in solution, for example, the nickel (II) aminotroponeiminates, in which the relaxation times permit quite well-resolved high-resolution hydrogen-1 spectra to be recorded, from which spin densities have been deduced[6].

Speaking about applications to organic structure determination, Dr. A. R. Katritzky (University of Cambridge; professor designate, University of East Anglia) first showed how phosphorus-31 resonance in monothiopyrophosphates can give a simple proof of the symmetry of a product. He then concentrated on hydrogen resonance in a limited range of nitrogen heterocyclic compounds of fairly low molecular weight, starting with the information that τ-values and multiplicities can give about disulphonylaminopyridines. Dr. Katritzky directed attention to the effects of tautomeric rates on nuclear magnetic resonance spectra and to the importance of interpreting these spectra in conjunction with those from the infra-red and ultra-violet, instancing 3,4-dimethyl-Δ^3-pyrrol-2-one and related compounds[7]. Finally, Dr. Katritzky showed how the rapid molecular rearrangement of benzofuroxan manifests itself in proton resonance as a change from an unsymmetrical $ABCD$ spectrum at low temperatures to a symmetrical A_2B_2 spectrum at 130° C (ref. 8).

Polymers have been investigated by both broad-line and high-resolution nuclear magnetic resonance techniques. Dr. I. M. Ward (Imperial Chemical Industries, Ltd., Fibres Division) first discussed the measurement over a temperature range of the width, second moment, and relaxation times of comparatively broad polymer lines, which can often lead to information about the motion of the structure and to a nuclear magnetic resonance parameter related to crystallinity. A molecular interpretation of the nuclear magnetic resonance transitions tends to be more readily obtained for the main glass transition than for processes at lower temperatures. With polypropylene as an example[9], the advantages of making measurements on a number of deuterated samples and of correlating nuclear magnetic resonance and dynamic mechanical data were outlined. The same polymer was used to illustrate the desirability of being able to interpret high-resolution spectra of polymers in solution in terms of local stereochemistry and so predict stereoregularity in the bulk of the polymer. However, undeuterated vinyl polymers are liable to give overlapping spectra complicated by coupling from protons on the α-carbon[10]; most published nuclear magnetic resonance investigations of stereoregularity have been on more amenable polymers, such as polymethyl methacrylate or deuterated polypropylene.

In the concluding session, several manufacturers described the potentialities of commercial nuclear magnetic resonance spectrometers. Mr. J. M. Babb (Varian AG) outlined equipments extending from a 2–16-Mc/s variable frequency broad-line instrument through the 60-Mc/s analytical spectrometer to the 100-Mc/s system; Dr. I. Stelzer (Delviljem, Ltd.) presented the less-familiar but almost equally extensive Japan Electron Optics range; and Mr. H. J. M. Fitches (Associated Electrical Industries, Ltd.) discussed a British spectrometer based on a 14,000 gauss electromagnet and capable of resonating at various frequencies. Among permanent magnet high-resolution spectrometers, Mr. L. Minikin (Mullard Equipment, Ltd.) described a relatively cheap 9,000 gauss instrument intended for proton resonance, and Mr. L. K. Parker (Perkin-Elmer, Ltd.) introduced a 14,000 gauss instrument designed to operate at the frequencies of several nuclei either separately or for spin decoupling.

D. W. JONES

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SHRINKPROOFING WOOL

A COLLOQUIUM on shrinkproofing sponsored by the Commonwealth Scientific and Industrial Research Organization and the University of New South Wales was held on March 13 at the Organization's Division of Textile Physics, Sydney. This was the third in the series of wool research colloquia which the C.S.I.R.O. initiated in 1961; the first two dealt with the structure of wool and the setting of wool respectively. Prof. J. B. Speakman, of the University of Leeds, gave the opening address. Only three formal papers were presented and, in addition to discussion periods associated with the papers, an entire session was reserved for informal contributions from the sixty invited delegates.

Prof. Speakman dealt with the three main methods of obtaining shrink-resistance in wool fabrics—cross-linking, resin deposition and chemical degradation. He pointed out that the last method, the most commonly used, was least satisfactory in its effect on other important fabric properties such as resistance to wear. No major advance had been made in the theoretical understanding of this approach in the twenty-five years since the suggestion was put forward that the only necessary reaction was rupture of disulphide bonds. The optimum result was obtained when this reaction was confined as much as possible to the surface layers of the fibres, to produce modification of the scales. Recently, processes of this type had been adapted for continuous treatment combined with continuous dyeing.

The other approach to surface modification was to mask the scales by depositing resin. Such a treatment could be used to improve resistance to wear and resistance to

creasing of wool fabrics at the same time as it conferred shrink-resistance. Combined in the one process, these effects would enable development of easy-care tropical worsted fabrics. For a given weight of polymer, the optimum benefits in both shrink-resistance and abrasion-resistance would be obtained only if the resin were polymerized by a method which at the same time anchored it chemically to the fibres.

Prof. Speakman suggested that cross-linking had the greatest potential as a shrinkproofing technique. No industrial process of this type had been developed, and this was due to lack of knowledge of the distribution of the reactive side-chains in keratin. Moreover, in past investigations insufficient attention had been given to the ratio of the amount of cross-linking reagent to the amount of wool. Unless this was strictly controlled, optimum cross-linking might not be obtained. If these problems were overcome, a process could be developed which would be satisfactory for all end-uses except those requiring crease-resistance.

Dr. E. F. Denby and Dr. M. W. Andrews (C.S.I.R.O.) reported progress in analysing fibre movement during washing of untreated fabric and fabric shrinkproofed with the C.S.I.R.O. potassium permanganate/saturated sodium chloride process. They claimed that the direct effect of washing was fibre movement and showed that the treatment caused a reduction in the rate of fibre movement. For a given level of treatment this rate was constant up to at least 10 per cent linear shrinkage. Thus there was considerable fibre movement during the induction period (the period at the commencement of washing when no shrinkage occurs). The relation between shrinkage and the average fibre movement was independent of the level of the treatment. This indicated that the mechanism of felting was the same in the treated and untreated fabrics.

The force required to move the fibres during washing depended on their elastic properties, their frictional properties and the distribution and pressure of inter-fibre contacts. Average values of this force were obtained by pulling fibres from the fabric in the against-scale and with-scale directions. The against-scale force for the treated fabric was considerably less than the value for the untreated fabric, whereas the with-scale force was only slightly altered by the treatment. The force was also altered by washing the fabric. A significant increase in the against-scale force occurred during the induction period. It was suggested that this increase was due to changes in the surface of the fibres brought about by the mechanical effect of the washing, and that an increase in the with-scale force, which was observed at the end of the induction period, might be associated with the formation of 'entanglements' of the type suggested by Shorter.

Developments and progress in shrinkproofing were reviewed by Dr. J. R. McPhee (C.S.I.R.O.). He suggested that research into the chemical degradation methods should be directed towards making simpler and cheaper processes, keeping in mind the disadvantages that still existed in processes of this type. Although resin treatments appeared to offer genuine advantages, these advantages tended to be outweighed in practice because the cost and complexity were high. The most promising was the formation of polyamides on the fibre surface by a technique called 'interfacial polymerization'.

The informal discussion period was led by Prof. A. Datyner (University of New South Wales) and Dr. A. J. Farnworth (Australian Wool Bureau), who briefly discussed problems and anomalies encountered in shrinkproofing research. The main impression gained from this discussion period was of wide gaps in basic knowledge. At present many phenomena have not been explained, and in some cases sound basic ideas have not been developed to the stage required for industrial use. Dr. Farnworth listed several instances; for example, the efficiency of the simple oxidation processes would be considerably increased if the reaction could be restricted entirely to those chemical sites the modification of which is essential for changes in the frictional properties of the fibres; however, little is known about these sites. Delegates reported that the effect of surface contaminants on the efficiency of many processes is not fully understood; nor did they know how to explain the pattern in which *p*H, temperature, etc., of the felting media affect felting rates. Above all, there is very little information about what happens physically in a fabric during felting.

To conclude on this note would be to deny the sound progress made in this field. The manufacturer has now come to a realistic appreciation of the need for careful quality control. Dr. McPhee reported that there are at least six processes in common use producing good results for a wide variety of applications; several of these processes do not impair the 'handle' of the wool. Thus the textile chemist has disdained the luxury of inactively waiting for basic research to point the way to the process *ne plus ultra*.

E. F. Denby
M. W. Andrews

ENERGY AND THE EUROPEAN ECONOMIC COMMUNITY

A BROADSHEET written by J. Hartshorn, entitled *An Energy Policy for E.E.C.*, and issued by Political and Economic Planning*, reviews briefly the national energy policies of Belgium, France, Western Germany, Italy, Luxembourg, the Netherlands and the United Kingdom, the search of the European Economic Community for a co-ordinated energy policy up to the 1962 proposals for a common market in energy, and finally considers the prospects for a policy.

While during the past three years the concepts on which a logical and coherent policy might be based have been steadily refined, there is no certainty that the member nations can be brought to accept one. Nobody, even coal interests, expects to be able to expand domestic fuel-producing capacity and productivity enough to keep pace with the growth of energy requirements to the extent that, for example, European farm interests can confidently be hoped to expand in domestic farm products. Energy demand per head shows less sign of levelling off within rising standards of living than food demand does.

The French have some objections of principle to the concept of an open common market in energy, and are not convinced that this is the surest way of arriving at the lowest energy costs if that means giving the non-European oil companies free play at a time when the Community's energy resources are on the point of being used up. Reliance on the diversification of sources of oil supply is a real safeguard, but its importance can be exaggerated. Diversification may raise the question of possible preference to particular oil-producing areas. In the context of scarcity, the 4–6 month level of supplies suggested for stocks by the European Economic Community has much logic, but may not be cheap even if it supplies sufficient insurance against the interruption of Soviet supplies as well.

Apart from the vested interests of the European nations concerned, Mr. Hartshorn sees cogent arguments for delay, and points out that it is difficult not to share some

* *Planning*, Vol. 29. No. 469 (18th March, 1963). *An Energy Policy for E.E.C.* By Jack Hartshorn. Pp. 55–98. (London: Political and Economic Planning, 1963.)

of the reservations of the Governments asked to commit themselves to these oil policies. An oil policy for Europe fitting the provisions of the Treaty of Rome and a coal policy fitting a possibly revised Treaty of Paris could exist, but he hesitates to suggest that this will be the outcome. What is remarkable, moreover, is the almost complete absence in the Broadsheet of any reference to nuclear energy.

PETROLEUM IN 1975

ON April 27, 1963, Esso Petroleum Co., Ltd., celebrated its seventy-fifth anniversary and a special issue of the *Esso Magazine* was published to mark this milestone in the Company's history.

The leading article, entitled "A Look Ahead to 1975", makes some interesting forecasts for the petroleum industry in Britain. If world economic progress during the next twelve years continues at the same rate as in the past twelve years, it is estimated that world oil demand will rise from the 1962 figure of 1,200 million tons to 2,200 million tons in 1975; in Britain the demand will approximately double the 1962 figure of 52 million tons to 102 million tons by 1975. New sources of oil may stem from the discovery of large natural gas deposits in Holland in 1961 and this has inspired an under-water search for oil off the British coast in the North Sea, a joint venture by Esso, Shell and British Petroleum. Esso is at present exploring possibilities in Kent, Sussex and Hampshire.

In the matter of transporting crude oil from overseas to refineries in Britain, tankers of 26,000 tons d.w. were among the largest afloat in the 1950's; now 80,000-ton tankers are already a feature of the Esso fleet and an 86,000-ton ship is scheduled for service early next year. The economic advantages of tankers up to 150,000 tons d.w. are at present under consideration, and the possibility of nuclear-powered tankers being used by 1975 is by no means remote.

If the forecast of demand for petroleum products doubling itself in the next twelve years is correct, then Britain's refining capacity will have to be increased to take care of at least another 50 million tons of oil by 1975, involving a capital outlay in the region of £200 million. In the distribution of refined products, pipe-lines are destined to play an increasingly important part in the years ahead; it is estimated that by 1975 about two-thirds of the 'white' products marketed by Esso alone in south-east England and the Midlands will travel by pipe-lines.

Fuels for engines of all kinds, ships' bunkers, cars, lorries, locomotives, aviation fuels, etc., are expected to increase the demand by 75 per cent, oils for heating about 70 per cent greater in 1975 than in 1962. Regarding heavy oils for industry, apart from traditional fuels, new methods of generating electricity are anticipated by 1975, such as magnetohydrodynamics, which converts the energy into hot gases, produced by burning fuel, directly into electricity in a strong magnetic field; the fuel cell would convert petroleum products into energy at a much higher efficiency than 45 per cent, the maximum efficiency that at present is achieved in the modern power station. Finally, the capital expenditure in Britain to cover all these developments in the oil industry is anticipated to be more than £1,000 million during the next twelve years.

H. B. MILNER

PRE-FLUVIAL, AUTOGEOSYNCLINAL SEDIMENTATION IN THE NAMURIAN OF THE SOUTHERN CENTRAL PROVINCE

By B. K. HOLDSWORTH

Department of Geology, University of Keele

SINCE the publication of Gilligan's[1] now classic study, British stratigraphers have stressed the deltaic and terrestrial aspect of Namurian rocks developed in the Central Province of England—'The Millstone Grit Series'.

While coarse, cross-bedded arkosic sandstones—the 'fluvial grit facies' of Trotter[2]—such as those investigated by Gilligan undoubtedly record terrestrial, fluvial activity (cf. ref. 3) and represent an important stage in the evolution of the Central Province, it has long been realized that in the south of the Province a complex history of Upper Carboniferous sedimentation preceded the first appearance of fluvial conditions[4–6]. An important newly published account of Carboniferous rocks in the Ashover district[7] together with earlier work in the Staffordshire–Derbyshire region and recent investigations in north Staffordshire and south-west Derbyshire allows the period between the close of the Dinantian and the first establishment of fluvial environments in the region to be seen in clearer perspective.

Hudson and Cotton[8] stressed that Dinantian successions in the area are of two contrasting aspects—'basin' and 'massif'. The sedimentation contrast was apparently related to a northerly projection of the Carboniferous Central Land Barrier (St. George's Land). On the submerged surface of this projection from the ancient block developed the limestones of 'massif facies'. To the north and west lay the basin area.

During *D*1(*B*2) time a part of the western and northern margin of the block projection in Derbyshire became marked by a line of limestone-reef building activity. To-day the belt of 'reef-knolls' is almost completely exposed from the upper Dove Valley to the Castleton district. East and south of the belt in Derbyshire, back-reef, massif limestones are exposed (cf. refs. 9 and 10). West and north of the belt, in north Staffordshire and north Derbyshire respectively, borings show contemporaneous limestones and shales of basin aspect[8].

A slight increase in differential movement between the geotropically negative basin- and positive massif-areas probably explains the *D*1(*B*2) reef-building episode and it appears that this movement continued into *D*2 time[8]. By late *P* time, however, the positive tendency of the massif projection appears to have been lost and rather similar, relatively argillaceous limestones accumulated over earlier basin- and earlier massif-areas alike[8].

The framework which was to govern the distribution of sediment accumulation during some two-thirds of Namurian time now developed, probably in late *P* time, through recrudescence of differential movement between basin- and massif-regions. Uplift along the earlier reef-building line resulted in an essentially monoclinal fold. Erosion exposed the line of *D*1(*B*2) knolls and gave rise to the local *D*1(*B*2)–*E*2 (Namurian) unconformity at the massif margin in north Derbyshire[11] and the upper Dove Valley[8].

The re-establishment of a positive tendency in the massif exerted a profound control on Namurian sediment accumulation. Contrast between thick earlier Namurian

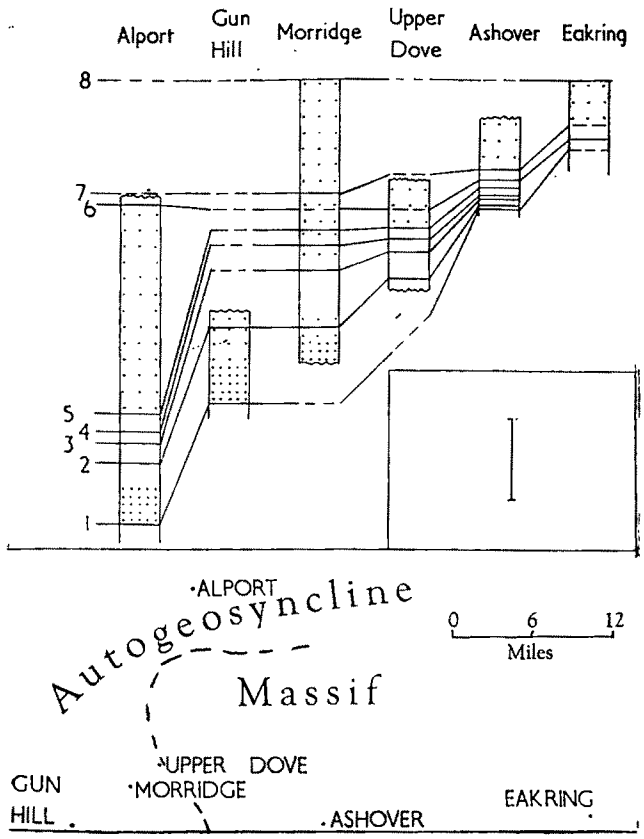


Fig. 1. Comparison between massif and autogeosynclinal successions of Namurian rocks
Faunal horizons: 1, *C. leion*; 2, *E. bisulcatum* s.s.; 3, *N. nuculum*; 4, *Hd. proteum*; 5, *R. reticulatum* s.s.; 6, *R. reticulatum* mut. α; 7, *R. reticulatum* mut. β; 8, *G. subcrenatum*
Small stipple, siltstones and fine sandstones; large stipple, sandstones. Scale (inset) represents approximately 800 ft.

successions of the basin area and the diminished accumulations of the massif is marked. Differential subsidence was now apparently greater than at any point in Dinantian time and the area of Staffordshire–Derbyshire west and north of the massif margin possessed the essential properties of an 'autogeosyncline' in the sense of Kay[12]. Only when the period of autogeosynclinal subsidence ended in approximately late Kinderscoutian (*R*1) time did fluvial grit environments become established in the Staffordshire–Derbyshire region.

Six established successions of Namurian rocks (Fig. 1) serve to illustrate thickness differences between: (*a*) massif —Eakring[13] and Ashover[7]; (*b*) autogeosyncline margin— upper Dove Valley; (*c*) points within the area of major subsidence—Morridge-Goyt area (in part from Cope[14]), Gun Hill[8] and Alport region[15].

Three phases may usefully be distinguished in the *E*1–*R*2 history of the autogeosynclinal basin.

The first phase is roughly co-extensive with the Pendleian Stage (*E*1). 600 ft. of beds were deposited at Alport, 800 ft. at Gun Hill and at least 400 ft. in the Morridge region, where the base is as yet unknown. Some 40 ft. only comprise the Stage at Ashover.

The phase appears to have been one of marine, restricted circulation conditions over the entire Staffordshire–Derbyshire region. The characteristic coarser sediments (that is, of silt grade and greater) of the basin are thinly bedded, fine calcareous sandstones and silt-stones. The petrography of these rocks at Alport and Gun Hill—known only from bore-hole specimens—was described by Hudson and Cotton[8,15] and the rock type termed 'crowstone'. Contemporaneous beds deposited closer to the basin margin and exposed in the Morridge area prove to be of uniformly finer grain, better laminated but essentially similar in detrital and secondary mineralogy.

Material preserved from the Alport and Gun Hill borings, re-examined with the permission of the Geological Survey of Great Britain, provides little evidence regarding depositional processes. The strongly laminated contemporaneous beds of the Morridge area, however, appear to be late-stage sediment differentiates of turbidity currents and it is highly probable that the majority of coarser Pendleian beds are of turbidity current origin. Siliceous spicules, endothyrid foraminifera and radiolarians contained in the beds suggest local, intrabasinal derivation of the sediment.

Scarcity of autochthonous faunas in the inter-turbidite shales and mudstones and absence of sole-trails suggest that layers of bottom water were probably toxic, highly charged with hydrogen sulphide, and acidic. Carbonates crystallized in abundance only, apparently, in response to slight rises in *p*H as bottom water was 'freshened' by turbidity current inflow. Conditions appear to have been equally inimical to life over the massif—a submarine 'high' unaffected by the turbidity flows of the basin[7].

Deposits of the second phase in the history of the autogeosyncline—Arnsbergian (*E*2) to early Kinderscoutian (*R*1)—are more variable both vertically and laterally. In the north Derbyshire region no coarser sediments accumulated, but sedimentation was still distinctly heavier than that over the massif area (Fig. 1). Subsidence may have been slightly less than during the first phase, and fossiliferous shales with goniatite–lamellibranch faunas and horizons of early diagenetic limestone point to at least periodic improvement in water circulation and aeration within the basin. A rather similar succession has recently been established at the basin margin in the Upper Dove Valley. Here the south-westerly directed marginal slope prevented the accumulation of the types of coarser sediments which were being deposited only some one and a half miles to the south-west in the north-east Morridge region and more heavily farther to the south and west in North Staffordshire (see, for example, ref. 16).

The fine and medium grained, relatively thin-bedded orthoquartzitic and protoquartzitic sandstones which characterize the second phase in north Staffordshire are essentially of Trotter's[2] 'grit-shale facies'. Traditionally (for example, refs. 4 and 17) the rock type has been known as 'crowstone' and contrasts strongly with the type characteristic of phase one and termed 'crowstone' by Hudson and Cotton[8,15].

Both thinness of bedding and absence of current bedding appear to reflect deposition of the sands in a still relatively rapidly subsiding area. Farther from the source and close to the margin of the basin in the north-east Morridge region units of coarser sediments are still more thinnly bedded and show many typical turbidite features. Sole structures clearly indicate transport from generally southerly points.

Episodes of grit-shale ('crowstone') and turbidite deposition probably occurred during periods of reduced salinity but good bottom aeration—as evidenced by abundant sole-trails. Much plant material in the marginal turbidite lithofacies and thin coals in the 'crowstones' (cf. ref. 17) point to a heavily vegetated southern land surface of low relief. Mineralogically the sands and silts are highly mature. Plagioclase feldspars occur in trace quantities only and potassium feldspar is absent. Garnet is very rare in heavy mineral fractions. The scarcity of unstable detrital minerals is believed to reflect extensive acidic alteration of material under the vegetation cover of the source area. The acme of proto-quartzite deposition in the Morridge area occurred during Chokierian (*H*1) time, corresponding with a brief appearance of swampy terrestrial conditions and shelly marine faunas (cf. ref. 18) at Congleton Edge, lying at the south-western margin of the autogeosynclinal area.

While the rapidly subsiding north Staffordshire region was receiving the highly modified coarser detritus of the

southern land surface and a relatively thick succession of marine shales and limestones was accumulating in north Derbyshire beyond the influence of the land area the massif-region persisted as a submarine high.

Sands of phase three—upper Kinderscoutian (*R*1) to lower Marsdenian (*R*2)—contrast markedly with the southerly derived phase two material. Potassium feldspar and biotite appear for the first time: plagioclase becomes more abundant and garnet very much more so. Sedimentary structures and thickness distribution suggest supply from northerly points and phase three sands appear to mark the first appearance in the Staffordshire–Derbyshire region of coarse, relatively unmodified detritus transported across the Central Province from the northern continent of crystalline rocks[1,3].

Early in the phase, subsidence was still sufficiently rapid to produce a typical turbidite lithofacies in north Derbyshire[19]. Slightly later the massif margin in south-west Derbyshire underwent burial by sub-arkosic sands of 'grit shale facies' but farther east the massif-area probably preserved a positive tendency and no coarser sediments accumulated[7,13]. Late in the Kinderscoutian, fluvial conditions were established in north Derbyshire. The separate histories of autogeosyncline and massif were now virtually at an end. The foundations had been laid for the Province-wide fluvial episodes of the upper Marsdenian and Yeadonian (*G*), and for the widespread paralic environments of the Westphalian.

I thank Prof. F. W. Cope, Dr. F. M. Broadhurst, Mr. T. G. Miller, Dr. W. H. C. Ramsbottom and the Leeds office of the Geological Survey of Great Britain for advice and assistance.

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ACCURATE TIME FOR FIELD ASTRONOMY

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METHODS for the precise timing of optical observations have been investigated, and used for the determination of astronomical latitude, longitude and azimuth at any point in the field.

The most important aspect of the information gained is that the method developed is very accurate, can be used at any terrestrial station, requires little additional equipment, and is equally suited in all other cases where precise timing of optical observations is required, as, for example, in the optical tracking of satellites.

Observation signals and time pulses from a light-weight portable crystal chronometer built to specification by Messrs. Communication Systems, Ltd., together with all relevant field data, are recorded on magnetic tape, using a standard tape recorder, which has been found to be more accurate than a chronograph, and is also cheaper and more convenient.

Whereas conventional time signals and the seconds pulses on standard frequency transmissions are used for comparison of the time indicated by the crystal chronometer and for its calibration, their direct application as time supply in the field is dispensed with. This is because of the unpredictable reception conditions and periodical interruptions of signals and their transmissions at selected times only.

The working frequency of 16 657·006 c/s of the crystal chronometer is converted to give 61 pulses/min, of which 60 are audible as tone output of about 1,040 i.p.s., and one of 260 i.p.s., which is used to operate the minute clock and minute tone. A further output of 250 i.p.s. is provided as a marker signal which is operated by a key via a relay. The audible pulses are arranged at intervals of octaves from the standard pitch of $2^4 \times 16$ Hz. Recorded on tape at a speed of $7\frac{1}{2}$ in./s and played back at half or quarter of this speed all output tones are within the audible range of the human ear.

Three methods of timing were investigated, all of which could be used to compare the crystal chronometer pulses with time signals received by radio. Two of these methods could also be used to measure the time of an observation as indicated by the pulse produced by depression of the marker key.

In the first method pulses recorded at $7\frac{1}{2}$ in./s wore played back at quarter speed, and timed with a stop watch. The accuracy of time extraction achieved was approximately 0·03 sec.

In the second method the time vernier, arising from 61 sub-divisions of one minute as obtained from the crystal chronometer, and seconds pulses transmitted by radio, is used as a coincidence meter. The aural determination of the approximate coincidence of both audio signals, crystal chronometer and time reception, at slow re-play, achieved an average accuracy in calibration of the chronometer of about 0·02 sec.

Increased accuracy of time measurement was achieved using the third method. Magnetized impulses on the coated side of the magnetic tape are made temporarily or permanently visible with metallic powder suspended in volatile fluid (hydrocarbons) (Fig. 1). Thus the recorded time differences obtained from synchronization of the chronometer or from field observations are converted to measures of length. The sharply defined cycles of the visible pulses permit a scaling accuracy of 0·1 mm and better, even in the presence of radio noise. The mean square error of time-intervals thus extracted was of the order of 2×10^{-4} sec. The time vernier, which was originally introduced for the reason given here, is still essential when using the third method since the permanent overlapping of signals is avoided, the chance of error when scaling is reduced, and the use of a single track on the tape recorder is permitted. The duration and pitch of output tones of the crystal chronometer were specified to facilitate the identification of the visible signals. The characteristics of the pattern of pulses visible on the tape eliminate ambiguities that arise from transmissions that are almost synchronized.

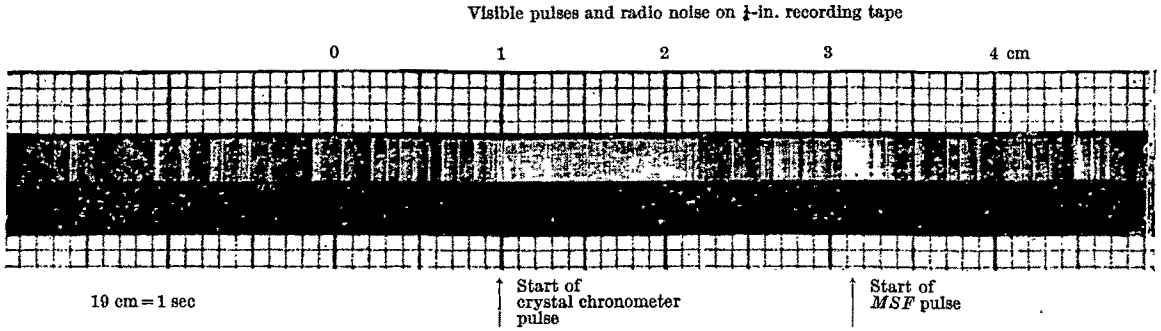
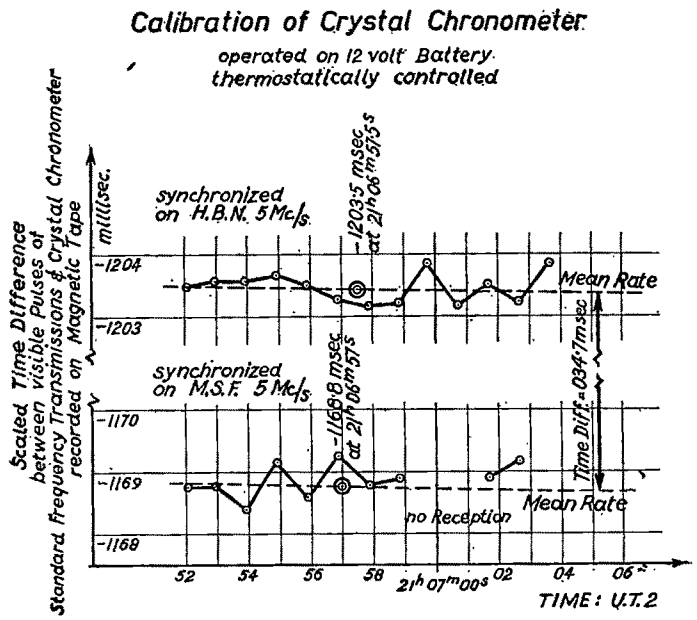


Fig. 1



MAY 5, 1961 AT: FIELD STN. TYWARNHALE MINE CORNWALL.

Bulletin No 56 & 32 R.G.O.:		
Time of Emission on U.T.2 of M.S.F. 5 Mc/s . . .	·0519 sec.	
Pulse Travel Time to Field Stn.	·0012	
Total Delay Time	·0531 sec.	
Time of Reception on U.T.2 of H.B.N. 5 Mc/s . . .	·0880	
Pulse Travel Time to R.G.O.	−·0023	
Receiver Delay at R.G.O.	−·0006	
Pulse Travel Time to Field Stn.	·0034	
Total Delay Time	·0885	
Difference of Reception Times at Field Stn.		·0354 sec.
Bulletin Horaire May 5, 1961:		
Time of Emission on U.T.2 of M.S.F. 5 Mc/s . .	·0473	
Pulse Travel Time to Field Stn.	·0012	
Total Delay Time	·0485	
Time of Emission on U.T.2 of H.B.N. 5 Mc/s	·0806	
Pulse Travel Time to Field Stn.	·0034	
Total Delay Time	·0840	
Difference of Reception Times at Field Stn.		·0355
Diff.' of Rec'. Time from visible Pulses on Tape		·0347
Discrep.'		·0007

Crystal Chronometer FAST on U.T.2 at $21^h\,07^m$, May 5, 1961: 1·115 sec. (ref. to R.G.O.)

Fig. 2

In Fig. 2 results are given of the reception of two separate sets of time signals from *MSF* (Rugby) and *HBN* (Switzerland), where the difference in the times of reception is given as 34·7 msec as measured in the field. The corresponding differences as calculated from published figures in *Bulletins* 56 and 32 of the Royal Greenwich Observatory, and the *Bulletin Horaire*, May 5, 1961, are 35·4 msec and 35·5 msec respectively, which agree with the field measurement within 0·7 msec. This and other measurements from the tape can be taken to this order of accuracy. The graphs in Fig. 2 show the results of time checks taken between radio time signals from *MSF* and *HBN* and crystal chronometer pulses. The mean difference was used to determine the discrepancy noted here. It will be seen that fluctuations, all less than 1 msec, are due mainly to tape distortion, scaling errors and reception conditions. The long-term performance of the crystal chronometer is much better, and if the chronometer is run in for 1 h before use then its rate is linear for a period of 6 h to an accuracy of ± 1 msec. Without voltage control, time comparisons before and after observations are required.

The theodolite used was a Watts 'Microptic No. 2', reading directly to 1 sec. The observation pulses were released manually at the instant a star or any heavenly body was crossing successively five calibrated grid lines engraved on the telescope's diaphragm, each subtending 30 sec. According to the star's parallactic angle these were either azimuth or altitude signals. The use of a five-line grid reduced the uncertainty of the mean square error of a single observation to 35 per cent. If the number of crossings is doubled, this would give only an additional reduction of 10 per cent and introduce optical errors. The grid diaphragm did not eliminate the personal equation, but enabled simultaneous measurements of vertical and horizontal angles to be taken. What is normally a complicated procedure was greatly simplified. Slow-moving stars were observed in the centre of the grid squares, eliminating the disturbing phenomenon of stars 'jumping the wire'. Telescope sights of transparent

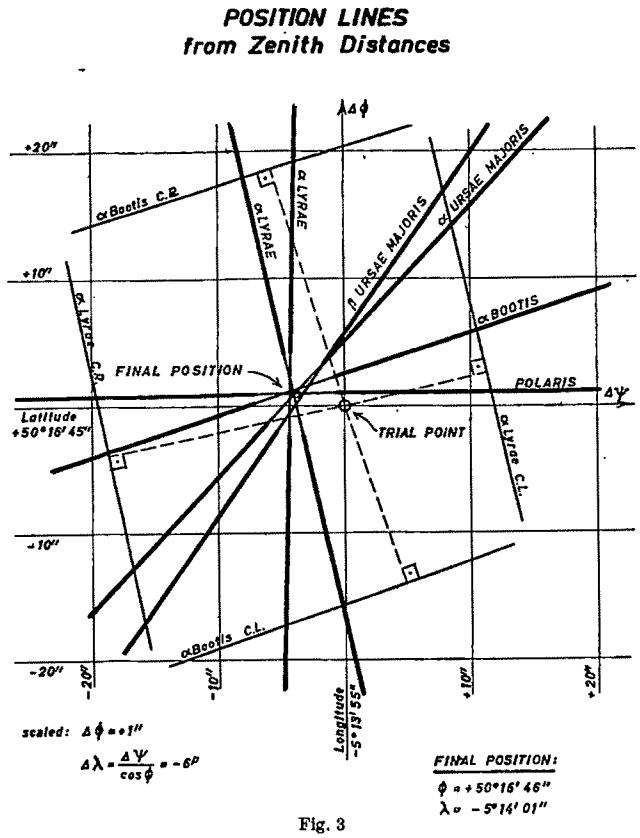


Fig. 3

synthetic resin, illuminated by the telescope lamp, were used for lining in the stars.

The personal equation and its error were determined by scaling on the tape the time intervals resulting from developed recorded mechanical chronometer clicks and marker pulses released at definite time readings indicated by the seconds hand of the mechanical chronometer.

Field results evaluated using the position line method based on zenith distances indicated that the mean of two C.L. and C.R. pointings to a single star with a single second theodolite is in error from the final adopted position by less than 1 sec (Fig. 3). The Laplace condition equation was satisfied where applicable.

The advantages of this method are as follows: Observational speed is raised so that two compensated measures of simultaneous altitude and azimuth can be taken to any visible star without the help of an assistant within 3–4 min, each observation consisting of five timed transits. The same precision can be obtained from fast-moving as from slow-moving stars (that is ignoring the personal error). The preparation of a star programme is unnecessary; star identification is easy. The chronometer error is reduced to such an extent that the only timing error of importance is the personal error. The cost of the crystal chronometer and recording equipment is moderate, being in the neighbourhood of £200.

Improvement in timing accuracy, to an extent worthwhile considering, cannot be expected with the use of an impersonal micrometer but may be possible by using some method of automatic recording of the star's transit, such as photoelectric registration.

The work was carried out at the Imperial College Field Station, Tywarnhale Mine, Cornwall. Initial trials were started in April 1959.

TESSERAL HARMONICS IN THE GEOPOTENTIAL

By IMRE G. IZSAK

Smithsonian Astrophysical Observatory and Harvard College Observatory, Cambridge, Mass.

A DETAILED investigation of the gravitational field of the Earth by analysing the motion of satellites is one of the major projects at the Smithsonian Astrophysical Observatory. The problem consists in the determination of the small numerical coefficients C_{nm} and S_{nm} in the development:

$$U=\frac{\mu}{r}\left\{1+\sum_{n=2}^{\infty}\left(\frac{a_E}{r}\right)^n\sum_{m=0}^{n}[C_{nm}\cos m\lambda+S_{nm}\sin m\lambda]\,P_{nm}(\sin\varphi)\right\}$$

of the force function of the Earth into a series of spherical harmonics; the adopted definition of the Legendre associated functions is:

$$P_{nm}(x)=(1-x^2)^{m/2}d^mP_n(x)/dx^m$$

The aim is to solve an inverse problem in celestial mechanics: Given the motion of objects in a gravitational field, derive the latter. Of course one has first to solve the direct problem—to construct an adequate orbital theory. Let us assume here that this has been done. Then we have analytical expressions for the perturbations caused by the several terms in the foregoing expansion which are functions of the geophysical constants C_{nm} and S_{nm}. Let us further assume that by some means or other we have succeeded in accounting for the most significant part of the dynamical effect of atmospheric drag, solar radiation pressure and for the perturbations by the Moon and the Sun. Then precise observations of satellites permit us to derive the coefficients of the spherical harmonics.

Many authors[1,2] have in this way computed the coefficients $C_{n0}=-J_n$, $S_{n0}=0$ of quite a few zonal harmonics. Such terms are of no concern to us here, because their primary effect is to cause secular and long-periodic perturbations in the orbital elements, easily separable from the perturbations caused by tesseral ($m\neq 0$) and sectorial ($m=n$) harmonics, the periods of which are sub-multiples of a day.

The satellite results[3–7] on tesseral harmonics published so far are contradictory and unreliable, mainly for two reasons. First of all, the observational material at the investigators' disposal was insufficient. Secondly, the adopted computational schemes were too simple. It might be some time before one can arrive at definite conclusions regarding the longitudinal dependence of the Earth's external gravity field. But there should not be any doubt about artificial satellites with a variety of orbital elements being the best means to achieve this goal.

Since last October the Smithsonian Observatory has been using a new IBM-7090 computer programme to analyse precisely reduced Baker–Nunn observations of several satellites. The input consists of the along-track and across-track residuals[8] of observations which we were unable to account for by known perturbative forces except that of tesseral harmonics. They also contain the effect of small errors in the station co-ordinates, and are about 15″ on the average. The accuracy of the observations is 2″ in position and 0·001^s in time. Because the residuals are linear in the unknown coefficients C_{nm} and S_{nm}, it is in principle a straightforward matter to compute the first few of them by the method of least squares. In practice,

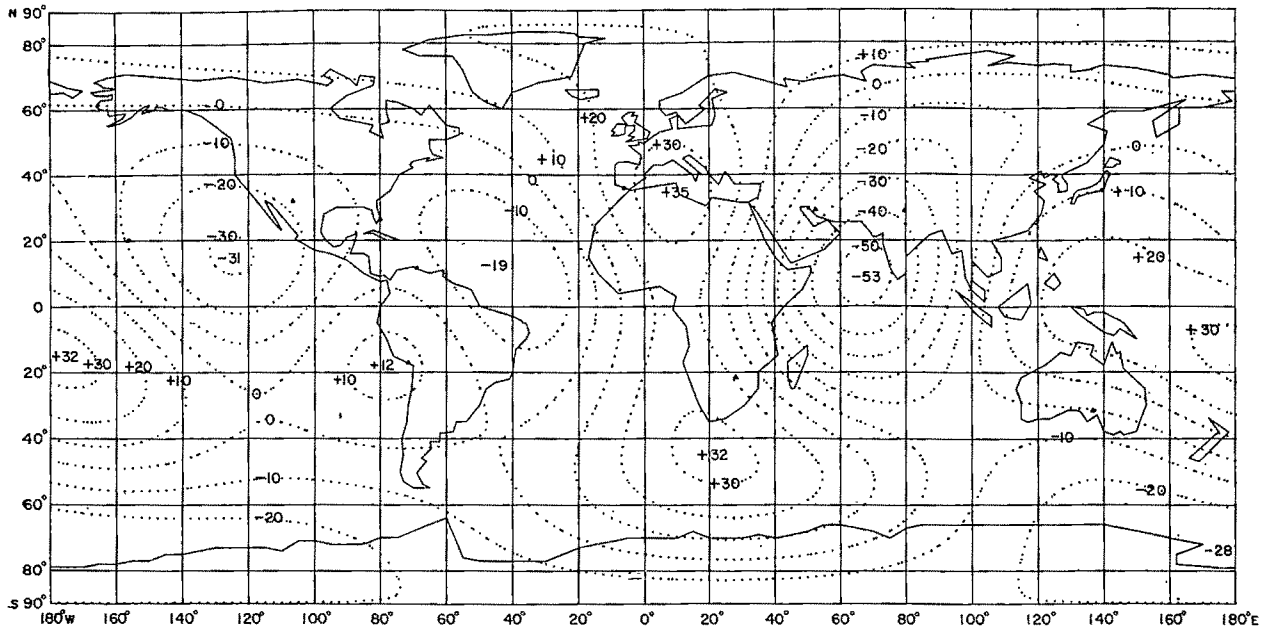


Fig. 1. Level curves of satellite–geoid heights at 10-m intervals. The small triangles indicate the positions of the 12 Baker–Nunn stations

however, one faces a rather difficult problem of data analysis. The residuals, especially those in the along-track direction, are by no means uniquely determined, because of the very nature of the differential orbit improvement which produced them. One usually gets different residuals for the same observation if two overlapping arcs of the orbit are fitted to it. For this and some other reasons, special care is necessary in this very tedious phase of the work. Then the distribution of a relatively small number of observations with respect to the arguments of the trigonometric functions that describe the perturbations is far from being uniform. Worst of all, the effect of the different tesseral harmonics is not very much different in size, and for some terms not even in shape. Therefore the perturbations caused by the various tesseral harmonics are hard to separate, a circumstance borne out by eventually considerable correlations among the unknowns. The only, but apparently rather efficacious, remedy for these difficulties seems to be the use of a large number of observations of satellites with a variety of orbital elements, with different inclinations in particular.

In selecting the observational material to be analysed one is limited not only by availability but also by what one considers an excessive machine-time needed for the execution of the computations.

Table 1 gives a short list of the observations we have been recently using.

Table 1. Observational Material

Satellite	i	e	a	Observations
1959 α1—*Vanguard* 2	32·9°	0·165	8·301 *Mm*	2,066
1959 η—*Vanguard* 3	33·4°	0·189	8·496 *Mm*	2,223
1961 δ1—*Explorer* 9	38·9°	0·115	7·985 *Mm*	2,629
1960 ι2—*Echo* 1, rocket	47·2°	0·012	7·972 *Mm*	1,465
1961 $\alpha\delta$1—*Midas* 4	95·9°	0·011	10·005 *Mm*	879

This material has been subjected to a variety of numerical experiments in order to obtain a feeling for the behaviour of the intricate least-squares process under discussion. Thus tesseral harmonics coefficients have been computed in different combinations; for example, C_{22} and S_{22} only; or C_{22}, S_{22} and C_{31}, S_{31}; or all sixteen of them, from the 2nd to the 4th degree. Furthermore, the observational material was divided into parts according, for example, to the individual satellites, to see how well the unknowns are determined by a smaller number of observations. We have been experimenting with weighting the observations in ways that seemed reasonable to us. In addition, the same programme can also compute corrections to the station co-ordinates. We have investigated their influence on the tesseral harmonics coefficients. Details concerning the outcome of such experiments would scarcely be of general interest. Suffice it to say that they made us more confident of the results than one could be without having carried them out. One of the latest runs of the programme gave the results shown in Table 2.

Table 2

$C_{22}=$	$9{\cdot}68\times10^{-7}$	$C_{41}=$	$-2{\cdot}88\times10^{-7}$
$S_{22}=$	$-4{\cdot}00\times10^{-7}$	$S_{41}=$	$-3{\cdot}21\times10^{-7}$
$C_{31}=$	$1{\cdot}12\times10^{-6}$	$C_{42}=$	$3{\cdot}51\times10^{-8}$
$S_{31}=$	$6{\cdot}16\times10^{-8}$	$S_{42}=$	$1{\cdot}23\times10^{-7}$
$C_{32}=$	$9{\cdot}12\times10^{-8}$	$C_{43}=$	$2{\cdot}15\times10^{-8}$
$S_{32}=$	$-1{\cdot}83\times10^{-7}$	$S_{43}=$	$1{\cdot}48\times10^{-8}$
$C_{33}=$	$7{\cdot}17\times10^{-8}$	$C_{44}=$	$9{\cdot}72\times10^{-9}$
$S_{33}=$	$1{\cdot}24\times10^{-7}$	$S_{44}=$	$1{\cdot}63\times10^{-8}$

The computed standard errors are unrealistically small, ranging from 3 to 15 per cent. The correlation coefficients of the 22- and 42-terms are extremely high, about 0·85; otherwise, one finds a few correlation coefficients to be about 0·20, the rest being less than 0·10. Including more objects with high inclinations in the analysis would undoubtedly make these coefficients smaller.

I believe that in consequence of the least-squares approach to the representation of the Earth's external gravity field, the individual coefficients C_{nm} and S_{nm} are not very meaningful in themselves; they should rather be considered a set. In other words, in comparing different solutions one should consider the combined contribution of the spherical harmonics to the force function U. A convenient way of doing this is to compute satellite–geoid heights. By satellite–geoid I mean an equipotential surface of the geopotential:

$$\Psi = U + \tfrac{1}{2}\omega^2(x^2+y^2)$$

as revealed by the motion of artificial satellites. Consider an approximate geopotential, $\tilde{\Psi}$, the equipotential surface $\tilde{\Psi}=C$ of which is a spheroidal Earth[9]. A suitable choice of the latter's flattening makes the finite and first-order part of $\tilde{\Psi}$ to coincide with that of Ψ. Then satellite–geoid height is the second-order positive or negative elevation of the equipotential surface $\Psi=C$ relative to the chosen spheroid.

A programme has been developed to compute, from a set of spherical harmonics coefficients, the level curves of geoid heights and gravity anomalies. Fig. 1, obtained on an EAI data-plotter, shows the level curves of satellite–geoid heights at 10-m intervals for the foregoing set of tesseral harmonics coefficients. The coefficients of the zonal harmonics are those given by Kozai[1]. Despite considerable differences in the individual coefficients these level curves compare favourably with results obtained by Jeffreys[10] and Uotila[11], based on surface-gravity data. The agreement of the new coefficients is best with the results of Kaula[12].

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OPTICAL ROTATORY POWER OF THE POLYPEPTIDE α-HELIX: A RECONSIDERATION OF THE MOFFITT THEORY

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IN 1956, Moffitt[1] analysed the optical rotatory power of macromolecules composed of helically arranged chromophores by means of coupled oscillator theory, in connexion with the alteration in optical rotation and rotatory dispersion observed[2,3] when polypeptides change from the random-coil to the α-helix conformation. The theory, predicting an abnormal rotatory dispersion for the α-helix conformation, was found[4] to be in quantitative agreement with the data then available, but shortly afterwards Moffitt, Fitts and Kirkwood[5] discovered that, owing to the use of cyclic boundary conditions, important terms contributing to the rotatory power of the α-helix had been neglected in the previous treatment. The additional and the original terms appeared to be of comparable magnitudes, and these authors suggested[5] that the agreement between Moffitt's theory[1] and experiment[4] was illusory. However, subsequent experimental work[6–9] supports further predictions of Moffitt's original theory[1], and it is now shown that the later treatment[5] does not alter significantly the conclusions of that theory.

Moffitt considered[1] a helix composed of N amide residues, and he showed that of the N possible electronic transitions corresponding to a particular amide chromophore excitation only three were allowed. The quasi-symmetric coupling of the individual amide chromophore excitations gives a transition with moments directed along the axis of the helix (Fig. 1*a*), and two quasi-antisymmetric coupling modes give a degenerate transition with moments directed perpendicular to the helix axis (Fig. 1*b*). Each of the allowed transitions involves a helical displacement of charge, giving an electric and a magnetic dipole transition moment orientated along the particular polarization direction.

The optical rotatory power of an electronic transition in a dissymmetric molecule is governed by the rotational strength, R, which represents[10] the scalar product of the electric, ρ, and the magnetic, μ, transition moment, namely:

$$R = \rho\mu \cos\theta \qquad (1)$$

where θ is the angle between the directions of the two moments. The rotational strength of an electronic transition is given experimentally by the band area of the corresponding circular dichroism absorption, measured either as the differential extinction coefficient for right and left circularly polarized light ($\varepsilon_l - \varepsilon_r$), or as the molecular ellipticity [ψ].

For a right-handed helical polypeptide, and for a particular amide chromophore excitation with an electric moment, ρ, and a frequency, ν, the resultant rotational strengths, per residue, of the transitions polarized parallel and perpendicular to the helix axis, $R_{\parallel}$ and $R_{\perp}$, respectively, are given by[1]:

$$R_{\parallel} = -R_{\perp} = \pi\nu d\rho^2 \cos t \cos v \qquad (2)$$

where cos t and cos v are, respectively, the tangential and the vertical cosines of the transition moment vector in each individual amide chromophore, which lies at a radial distance, d, from the axis of the helix. The terms, tangential and vertical, refer to a cylindrical co-ordinate system, in which the outward radial vector, the tangential vector, and the vertical direction of the advancing screw, form a right-handed set of Cartesian axes (Fig. 2).

Peptides and other amides give broad $\pi \rightarrow \pi$ absorption bands[11,13] near 1900 and 1600 Å, with oscillator strengths, in the case of dimethylformamide[13], of 0·237 and 0·209, respectively. Studies of the crystal spectrum of myristamide, measured with plane-polarized light[13], indicate that the most probable orientation of the 1900 Å transition moment is at an angle of 9·1° to the nitrogen–oxygen direction, in the sense that a parallel line through the nitrogen atom cuts the carbonyl bond (Fig. 3). The α-helix[14] contains some 3·6 amide residues per unit turn, during which the helix, with a radius of 1·6 Å, rises through 5·4 Å. The disposition of a given amide group in the cylindrical co-ordinate system of a right-handed α-helix (Fig. 2) gives the direction of the transition moment responsible for the 1900 Å amide absorption radial,

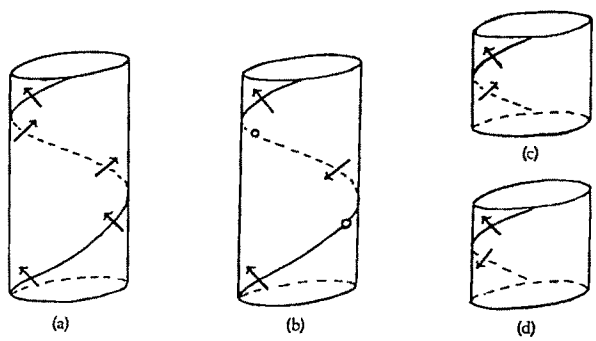


Fig. 1. The transition moments of the polypeptide α-helix giving the 1900 Å amide absorption due to: (*a*) the parallel, quasi-symmetric, coupling mode of the residue excitations; (*b*) one component of the doubly-degenerate, perpendicular, quasi-antisymmetric, coupling mode; (*c*) the quasi-symmetric coupling mode in a helical segment containing two amide residues; (*d*) the quasi-antisymmetric coupling mode in a two-residue segment

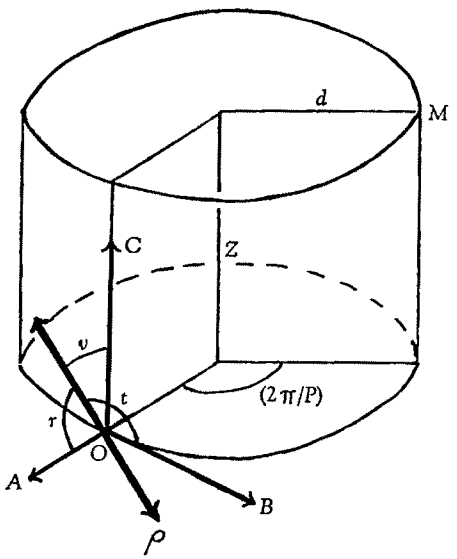


Fig. 2. The orientation of the moment vector, ρ, of the transition giving the 1900 Å absorption of an amide residue at O in the cylindrical co-ordinate system of the α-helix, defined by the radial, tangential, and vertical vectors, OA, OB and OC, respectively. The residue at M is related to that at O by the rise per residue along the helix axis, Z, the radius of the helix, d, and the angle about the helix axis $(2\pi/P)$, where P is the number of amide residues per unit turn of the helix

tangential, and vertical angles of some 90°, 140° and 50°, respectively.

For the corresponding absorption of the polypeptide α-helix, these values give the parallel-polarized transition the smaller oscillator strength and the lower frequency, Moffitt estimating[1] the energy separation, $\Delta\nu = \nu_{\parallel} - \nu_{\perp}$, to be —2,800 cm^{-1}. For the right-handed α-helix the degenerate perpendicular transition (Fig. 1*b*) should have a positive rotational strength with the value of $\sim 120 \times 10^{-40}$ c.g.s., while the parallel transition (Fig. 1*a*) should have a negative rotational strength of the same absolute magnitude. Since the parallel and the perpendicular transitions of the α-helix have a small energy separation and rotational strengths of equal magnitude and opposite sign, the two terms which they contribute to the expression for the molecular rotation give, when combined, an equation denoting an abnormal dependence of the rotation on the fourth power of the wave-length[1], namely:

$$[M']_\lambda = b_0\lambda_0^4/(\lambda^2 - \lambda_0^2)^2 \qquad (3)$$

where $[M']_\lambda$ is the contribution of the allowed transitions due to excitation-coupling in the α-helix to the molecular rotation per amide residue at the wave-length λ, and λ_0 is the average of the wave-length maxima of the absorption given by the transitions, b_0 being a constant.

The abnormal rotatory dispersion observed[4] for poly-L-glutamic acid and other polypeptides derived from L-amino-acids in solvents favouring the α-helix conformation give experimental values of $b_0 \simeq -640°$ and $\lambda_0 \simeq 2100$ Å for the constants of equation (3), compared with Moffitt's theoretical values[1], $b_0 = -580°$ and $\lambda_0 = 2000$ Å. The polarized absorption spectra[6] of orientated films of poly-L-alanine and poly-γ-methyl-L-glutamate place the absorptions due to the parallel and the perpendicular transitions of the α-helix at 2060 and 1910 Å, respectively, corresponding to an energy separation, $\Delta\nu = -2{,}700$ cm^{-1}, in agreement with Moffitt's theoretical value[1]. (A later, corrected, value is $\Delta\nu = -3{,}700$ cm^{-1}; Doty, P., and Holzwarth, G. M., private communication.) The spectrum of the α-helix form of poly-L-glutamic acid in aqueous solution[7] shows that the absorption due to the parallel transition appears as a shoulder at 2050 Å on the more intense band at 1900 Å due to the perpendicular transition (Fig. 4), supporting the theoretical oscillator-strength ratio for the two transitions.

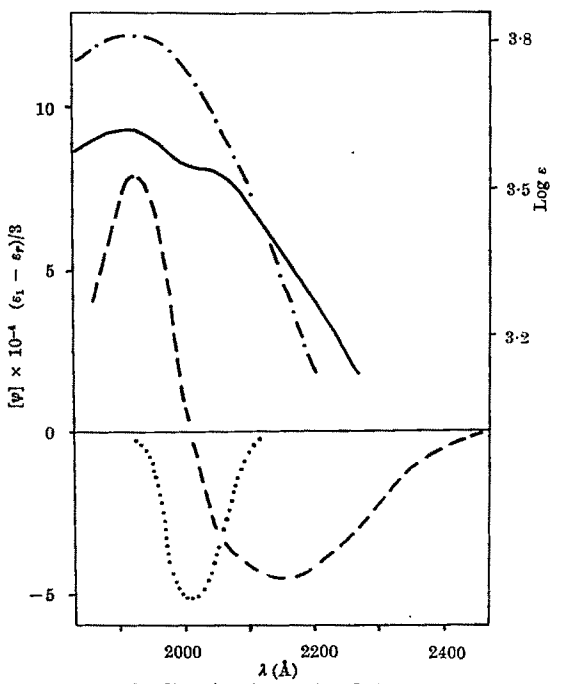


Fig. 4. The circular dichroism (– – – –) and the absorption spectrum (———) of the α-helix form of poly-L-glutamic acid, and the circular dichroism (· · · ·) and the absorption spectrum (—·—·—) of the random coil form (based on data recorded in refs. 7 and 8)

The corresponding spectrum measured with circularly polarized light[8] gives two circular dichroism absorptions with maxima at 2160 and 1920 Å (Fig. 4), and from the areas of the circular dichroism bands the rotational strengths of the parallel and the perpendicular transitions of poly-L-glutamic acid in the α-helix conformation are estimated[8] to be −41 and +36, respectively, in units of 10^{-40} c.g.s., while the rotational strength of the amide absorption due to the random-coil conformation[8] is −14 in the same units. Since the energy interval (2,700 cm^{-1}) between the parallel and the perpendicular transitions is less than one-half of the band width of the resultant absorption, the respective circular dichroism bands overlap, and, having opposed signs, they mutually cancel to some degree, as is shown by the relatively large separation (5,800 cm^{-1}) between the two circular dichroism maxima observed. Owing to the cancellation in the wave-length region of overlap, the absolute magnitudes of the two rotational strengths due to excitation-coupling in the α-helix are larger than the values obtained experimentally from the solution circular dichroism measurements, which thus afford only a semi-quantitative support for the Moffitt theory.

From the agreement between his theory and the evidence available, Moffitt[1,4] suggested that polypeptides derived from the L-amino-acids should form a right-handed α-helix, and the prediction was afterwards verified by the X-ray diffraction study[9] of myoglobin.

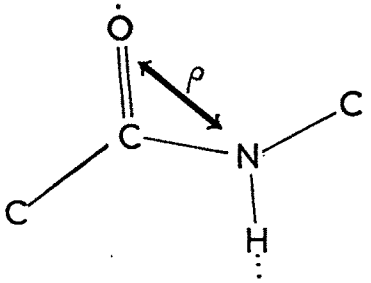


Fig. 3. The orientation in the amide group of the transition moment, ρ, giving the 1900 Å peptide absorption

Much of the evidence supporting Moffitt's theory was not available at the time of the amended treatment of Moffitt, Fitts and Kirkwood[5], and hitherto the subsequent experimental studies have given only a phenomenological status to the earlier theory. However, the physical model of the theory appears to be valid, for the additional terms contributing to the rotational strength of the polypeptide α-helix in the Moffitt, Fitts and Kirkwood treatment are important only in a helical segment consisting of a few amide groups.

The quasi-symmetric coupling of the $\pi \rightarrow \pi$ excitations of two amide chromophores composing a segment of the α-helix (Fig. 1*c*) makes two contributions to the rotational strength of the segment. Both contributions are negative in sign, and one is directed parallel, $R_{\parallel}^{s}$, and the other perpendicular, $R_{\perp}$, to the axis of the helix, namely:

$$R_{\parallel}^{s} = 2\pi\nu d\rho^2 \cos t \cos v \sin^2(\pi/P) \quad (4)$$

and:

$$R_{\perp}^{s} = -\pi\nu Z\rho^2 \cos^2 t \sin(2\pi/P)/2 \quad (5)$$

where Z is the rise per residue along the helix axis, and $2\pi/P$ is the angle about the helix axis between two adjacent amide residues (Fig. 2).

Similarly, the quasi-antisymmetric coupling of the two amide-group excitations makes two contributions to the rotational strength of the segment (Fig. 1*d*). Both the contributions have a positive sign, and both are directed perpendicular to the helix axis:

$$R_{\perp}^{a} = -2\pi\nu d\rho^2 \cos t \cos v \sin^2(\pi/P) \quad (6)$$

and:

$$R_{\perp}^{a'} = \pi\nu Z\rho^2 \cos^2 t \sin(2\pi/P)/2 \quad (7)$$

The original terms of Moffitt's theory[1] are those of equations (4) and (6), and the additional terms of the Moffitt, Fitts and Kirkwood treatment[5] are those of equations (5) and (7).

For an extended array of amide residues in the polypeptide α-helix there are, in general, two residues adjacent to any given amide group, disposed at angles to it of $\pm (2\pi/P)$ about the helix axis. The interaction of the excitation moment of the amide group with those of the adjacent residues contributes terms to the rotational strength of the α-helix according to equations (4) to (7). The contributions determined by equation (5) cancel one another, and so do those governed by equation (7), since the contributions depend in each case on $\sin(2\pi/P)$, which is negative for one of the adjacent residues and positive for the other. On the other hand, the contributions governed by equation (4) or (6) are additive, as they depend in each case on $\sin^2(\pi/P)$. Similar considerations apply to the coupling of the excitation moment of a given amide group with those of its two *n*th neighbours, and to interactions involving the terminal residues. The coupling of the excitation moment of the first residue in the α-helix with that of a given neighbour makes a contribution, governed by equation (5) or (7), which cancels the corresponding term derived from the analogous interaction involving the *N*th residue.

Physically the sum of the contributions to the rotational strength determined by equation (5) or (7) vanishes for n turns of the helix, such that nP is integral, or, more generally, when N becomes large, because the terms of either equation depend only on the tangential components of the amide excitation moments. The tangential components, $\rho \cos t$ (Fig. 2), interact in the quasi-symmetric coupling mode of the α-helix (Fig. 1*a*) to give only a magnetic moment, and in the quasi-antisymmetric coupling mode (Fig. 1*b*) to give only an electric moment, so that in neither case are the conditions (equation 1) for a non-zero rotational strength fulfilled.

It is concluded that the only significant contributions to the rotational strength of the α-helix are the terms of equations (4) and (6), which form the basis of Moffitt's original theory[1]. Equations (5) and (7), from the amended treatment[5] of Moffitt, Fitts and Kirkwood, contribute terms which sum to zero for a polypeptide α-helix with N large or with nP integral, and for a helix with nP non-integral they contribute only a small end-correction term with a value of the order of $1/N$ of the main terms governed by equations (4) and (6). Thus the 'Moffitt plots' of $[M']_\lambda(\lambda^2 - \lambda_0^2)$ against $(\lambda^2 - \lambda_0^2)^{-1}$ (equation 3) have a physical and not merely a phenomenological basis, and they may be relied on to give[4], from the sign and the magnitude of the constant, b_0, the proportion of a given polypeptide assuming the α-helix conformation in the particular conditions studied, and the chirality of the helix formed.

I thank Prof. P. Doty for permission to reproduce Fig. 4.

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HIGH-SENSITIVITY DETECTION OF AMINO-ACIDS BY GAS CHROMATOGRAPHY AND ELECTRON AFFINITY SPECTROMETRY

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SINCE the classical work of Sanger in 1945[1] on the determination of the structure of insulin the technique of end-group analysis of proteins via formation of 2,4-dinitrophenyl amino-acid derivatives (DNP's) has become a basic procedure in this field. The method, in its present-day refinements, offers a mean of selectively 'tagging' the free amino-groups of peptide chains which on hydrolysis yield coloured amino-acid derivatives with a relatively high absorption in the ultra-violet. This phenomenon is due strictly to the very active chromophoric 2,4-dinitrophenyl group attached to the amino nitrogen. These derivatives are now easily chromatographed by either appropriate paper or column techniques. Because of their colour, their position is readily discernible and identification is facilitated by comparing retention factors with those of pure standards. Following their elution and isolation they are determined quantitatively by ultra-violet spectrophotometry.

An extension of this technique would be the complete hydrolysis of the peptide prior to reaction with 1,2,4-fluorodinitrobenzene, the DNP forming reagent, thereby yielding the DNP derivatives of all the amino-acids present in the hydrolysate[2]. This technique is similar to the at present more popular method of Moore and Stein for automatic amino-acid analysis[3,4], whereby the ninhydrin derivatives of amino-acids are simultaneously

chromatographed and quantitatively determined by virtue of their high absorption in the visible region.

Certain disadvantages of these techniques are inherent, particularly the length of time necessary for a complete analysis (18–24 h for the Moore–Stein method or 20 h for the recently automated DNP technique[5]) and the sensitivity of the method or minimum amount of each amino-acid component required for detection. For the Moore–Stein technique about 10^{-7} moles of sample are needed[3], while approximately 10^{-8} moles of DNP derivatives are required to yield a proper colorimetric analysis of these compounds[6].

In the past few years great strides have been taken in order to adapt amino-acid analysis to the rapidly developing realm of gas chromatography[7–9]. Preparation of a number of derivatives of amino-acids, usually the combination of *N*-acyl esters, has enhanced their volatility and promoted their elution from gas-liquid columns in about 60 min. The concurrent use of the highly sensitive ionization detectors has increased the level of detectability for amino-acids to about 10^{-12} mole/sec, which in practical terms signifies that approximately 10^{-10} moles of the protein or peptide would be required as starting material[8].

Because of the high molecular weight of most biologically important proteins and the difficulty of isolating them in pure form in large amounts it seemed desirable to improve further the sensitivity of detection of amino-acids to lessen the burden of procuring workable quantities for analysis. From past experience[11,12] it was reasoned that the use of the electron capture detector[10] in conjunction with gas chromatography could readily satisfy the need for higher sensitivity. The only major problem that remained was to find a volatile amino-acid derivative that possessed an electrophoric group with a high affinity for free electrons. Although most amino-acids do not contain such groupings, certain derivatives can be prepared to satisfy this requirement. This concept is not new to protein chemists who have used and continue to use chromophoric derivatives for colorimetric or ultraviolet analysis as cited here. However, it is relatively unique to gas chromatographic analysis, having only recently been applied to the analysis of sterols as their chloroacetates[11]. Indeed, from a theoretical point of view *N*-chloroacetyl amino-acids also could be used for the same purpose. This will be the subject of another communication.

From earlier work with this detection system, although not unanticipated, it was noted that many well-known chromophoric groups are also highly electrophoric when measured by this device. An excellent example of this phenomenon proved to be the 2,4-dinitrophenyl group, which was originally measured as 2,4-dinitrophenol[10]. Since a great deal is known about the formation of DNP amino-acids and their behaviour under various conditions of hydrolysis a logical extension and improvement of the present methods of amino-acid analysis using these compounds was the application of gas chromatography in conjunction with electron affinity spectrometry.

The compounds tested were obtained commercially as the 'chromatographically pure' free DNP acids (Mann Research Laboratories, New York) and converted to the methyl esters by reaction with ethereal diazomethane. Where poor solubility was encountered, as noted by slow evolution of nitrogen, methanol was added to speed the reaction. The compounds were diluted in methanol to a known concentration after being taken to dryness under nitrogen to remove excess diazomethane and ether. They were then applied to the gas chromatographic column by injection with a 1-μl. syringe.

The DNP amino-acid methyl esters were chromatographed at a temperature of 220° C. Injection temperature was 275°–285° C. The most useful stationary phases for this preliminary study were the neopentyl adipate or neopentyl sebacate polyesters[9,13] or the polar nitrile containing silicone, '*XE*60'. 3 per cent of the phase was coated on 90–100 mesh 'Anakron *ABS*' (Analabs, Hamden, Conn.). Once it was demonstrated that the DNP tagged amino-acid esters could be eluted under reasonable conditions their response toward detection by the electron capture device was determined. The standard 1-cm parallel plate detector previously described and studied was used[12]. Sensitivities were determined at an optimum potential of 14 V applied to the detector, which was maintained at 200° C. The rate of flow was 200 ml. argon/min. Sensitivity here is defined as the minimum detectable amount based on a peak height of twice the overall noise-level, which essentially is a composite of stationary phase emerging from the column and electronic noise. The latter was substantially reduced by placing a 0·001-μF capacitor across the input of the electrometer–amplifier commonly used with this equipment. This reduced the noise-level to 6×10^{-13} amp.

When the response exceeded 30 per cent of the saturation current, the sample was further diluted to reduce the signal and to place it within the linear range of the detector. This permitted valid calculation of the sensitivites which are reported in Table 1. The linear range for this system is about 2,000–4,000 times these minimum detectable amounts.

Table 1. Sensitivity of DNP Amino-acid Methyl Esters

Amino-acid	Minimum detectable amount ($\times 10^{-16}$ mole/sec)
Alanine	3·60
β-Alanine	2·65
α-Amino butyric acid	3·24
γ-Amino butyric acid	2·92
Aspartic acid	3·22
Cystine (di-DNP)	3·30
Glutamic acid	2·78
Glycine	3·07
Isoleucine	3·36
Isovaline	3·12
Leucine	2·94
Lysine (di-DNP)	2·45
Methionine	3·41
Norvaline	3·96
Ornithine (di-DNP)	3·60
Phenylalanine	2·13
Proline	3·27
Valine	3·21
Average (± *S.D.*)	3·12 ± 0·41 (13%)

Significantly, the minimum detectable quantities of the various DNP amino-acids were of the same order of magnitude, namely, 3×10^{-16} moles/sec, with a relative standard deviation of ± 13 per cent. This is well within the experimental error expected in measurements of this type, and clearly indicates that the sensitivity is strictly governed by the affinity of the 2,4-dinitrophenyl group for free electrons. The remainder of the molecule apparently does not exert any influence in this regard. This is a very valuable asset when using a specific type of sensitive ionization detector, since previous experience with both the conventional 'flame' and 'argon' ionization devices has shown that responses vary significantly with changes in molecular structure. However, in this instance, the electron capture device responds only to the highly electrophoric dinitrophenyl group, thus providing a predictable quantitative result. This precludes any need for calibration once reproducible optimum operating parameters are ascertained for the chromatographic column-detection system[11].

It was noted that for the lysine, ornithine and cystine di-DNP methyl esters the sensitivities were still the same as that observed for the mono-DNP derivatives. At first it was thought that these substances would evoke a two-fold increase in response since two DNP groups were present in the molecule. This was not the case, however, since structurally the two groups are far apart and incapable of any interaction that would promote electron attachment over and above that expected for either group alone. It is apparent that as soon as one of the groups has captured an electron a negative molecule-ion is formed and the possibility of such a species capturing a second electron is almost nil in the prevailing circumstances. Thus the fact that those amino-acids containing

more than one reactive amino-group respond similarly to the mono amino-acids is a further advantage of this technique.

Finally, a problem often encountered in microanalyses is the appearance and interference of impurities. Many unknown substances of an organic nature often arise from many sources and are difficult to eliminate. Often, these are impurities that are carried along in the initial extract and cannot be removed by even the best purification techniques. In addition, after certain isolation procedures there are those that appear from either the solvents or the column materials used. Fortunately the unique sensitivity of the electron capture detector to the DNP derivatives makes ultimate purification unnecessary. This is possible because when operated at optimal conditions the detector will selectively respond only to those compounds capable of forming a DNP derivative or to those rare compounds with comparable electron affinities. Thus a multitude of other substances need not be eliminated by oft-times tedious preliminary purification procedures, since the unusual properties of the electron capture detector will accomplish this end by itself. Such an approach has already been used most effectively in other areas of gas chromatographic analysis using this type of sensing system, for example, insecticides, lead compounds.

In conclusion, it is evident that the advantages of electron capture spectrometry in conjunction with the gas chromatography of DNP amino-acids are three-fold. These are: (*a*) the extreme sensitivity not heretofore reported for any other biologically significant group of compounds; (*b*) the uniformity of response toward all the DNP amino-acids; (*c*) the inherent selectivity of the detector that eliminates interference from impurities that would otherwise be troublesome at the exceedingly low levels of detection that can now be used.

Although the dinitrophenyl group is quite polar and adds significantly to the molecular weight of many amino-acids which already are difficult to volatize it was nevertheless found quite feasible to analyse them by gas chromatography at a reasonable temperature. The use of the butyl or amyl esters[8] of the DNP amino-acids would further increase their volatility compared with their methyl esters, thus enhancing the rapid elution of some of the higher molecular weight compounds. Before this can be done, however, it is necessary to determine the ease of esterification and its quantitative aspects. At present, the use of diazomethane, with its known rapid, quantitative formation of methyl esters seems quite sufficient for the amino-acids already tested. With the extremely small samples that can now be applied to the gas chromatographic column, the possibility of adsorption of the sample on the column can become a serious problem. It is therefore essential that adsorption effects are minimized in order to obtain the highest sensitivity. This can be achieved for the most part by the use of glass columns, pre-treated solid supports (acid and base washed and siliconized), the proper liquid phase (polar) and amount thereof, and the careful elimination of dead volume from the entire system. It is conceivable that under ideal operating conditions the limits of detection and linear range may be extended by one or more orders of magnitude than that reported here at this time.

Work is now proceeding to determine the most convenient conditions under which all the important DNP amino-acid esters can be resolved by gas chromatography. If this is readily accomplished the method will undoubtedly become the one of choice for the ultramicroanalysis of amino-acids.

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PLUTONIUM-239 AND OTHER NUCLIDES IN GROUND-LEVEL AIR AND HUMAN LUNGS DURING SPRING 1962

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IN nuclear weapon explosions, some of the fissile nuclides may not undergo fission, so that these nuclides, together with neutron capture products, may be dispersed from explosions in the atmosphere. Many of these nuclides are α-emitters[1], but the comparatively high levels of natural α-activity complicate their identification and measurement. In particular, the α-energies of plutonium-239 (5·15 MeV) and plutonium-240 (5·16 MeV) are very close to that of polonium-210 (5·30 MeV), the naturally occurring α-emitter which follows the β-emitters lead-210 (radium D; half-life, 20 years) and bismuth-210 (radium E; half-life, 5 days) in the decay of the radon-222 series. The residence time in the atmosphere of the Rn-222 series is sufficiently long for an appreciable activity of polonium-210 (half-life, 138 days) to be formed in the atmosphere[2]. A technique, described in this article, has been developed so that plutonium-239 and/or plutonium-240 may be measured directly in ground-level air at concentrations as low as 5×10^{-17} c./kg despite the presence of polonium-210. Since, in these measurements, an attempt has been made to distinguish plutonium-239 and plutonium-240 they are referred to, for convenience, so plutonium-239. The method uses an α-spectrometer with an energy resolution and sensitivity capable of distinguishing plutonium-239 and polonium-210 at the low activities associated with such samples. The results of preliminary measurements with this technique, together with other measurements of zirconium-95 + niobium-95 and plutonium-239 in human lungs, are reported and discussed here.

Alpha-particle spectrometry. A parallel plate, Frisch-grid, ionization chamber, accommodating four interchangeable sources each of up to 200 cm², was used in the measurements described in this article. Less than 0·005 pc. of plutonium-239 or polonium-210 doubled the effective background counting rate for these nuclides and α-spectra with 40–50 keV half-widths (full width at half the height of a spectral peak) could be obtained from a thin source at 2π geometry. The head amplifier used was based on the design published by Cottini *et al.*[3].

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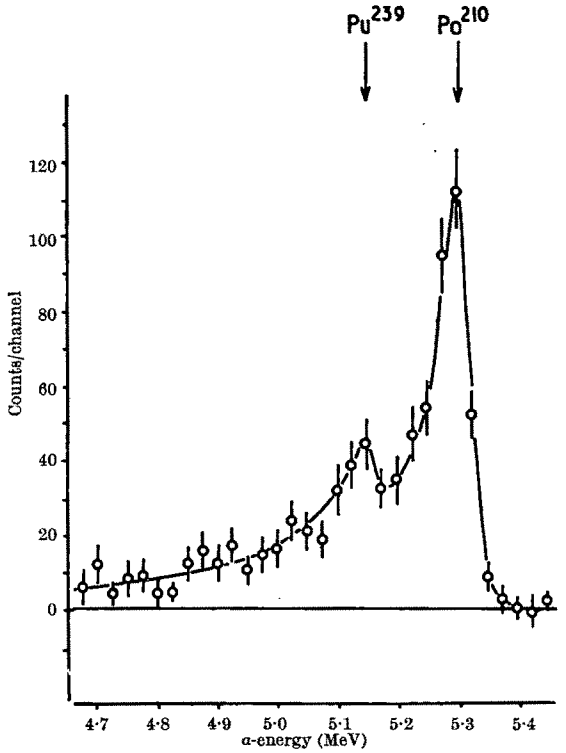


Fig. 1. α-spectrum from atmospheric dust collected during January 30–February 1 and counted 55 h

Subsequent measurements are being made with a spectrometer which has recently been designed and constructed in the form of a gridded cylindrical ionization chamber. This instrument, the details of which will be given elsewhere, has a source area of 1,800 cm² and half-widths of the order of 30 keV can be obtained.

Collection and analysis of atmospheric dust samples. As part of the programme of air monitoring by this Department[4], samples of dust from ground-level air at Sutton, Surrey, are collected on the plates of an electrostatic precipitator (efficiency 98 per cent as measured for methylene blue particles). For β- and γ-counting, the dust is removed from the plates by washing[4]; but sources in a form suitable for α-particle spectroscopy can be prepared by collecting the dust directly on aluminized melinex ('Mylar') sheets stretched over one of the precipitator plates. In this manner, 200 cm² sources having 20–30 μg dust per cm² are obtained in 40–50 h from 300–500 kg of air.

The sampled air mass was estimated for each sample by comparison of the γ-activity of the fission products zirconium-95 + niobium-95 collected on the melinex sheet with that collected by the total precipitator, for which the rate of flow was known. These γ-activities were measured with a 3-in. sodium iodide crystal in a low-background counting facility described previously[4].

Another method of estimating the sampled air mass was to measure the total α-activity of an aliquot from the total dust sample using a zinc sulphide scintillation technique[5] and compare this with the total α-activity on the spectrometer source as calculated from the α-spectrum. The overall agreement between the two methods was within 10 per cent.

The collection of the short-lived daughters of the naturally occurring radon-222 and radon-220 necessitated a delay of several days after the sample was collected, before commencement of the α-analysis in the spectrometer described above. By following the long-term build-up of total α-activity in the dust samples collected by the precipitator, the concentration of lead-210 in air and the ratio of polonium-210 activity to lead-210 activity at time of sampling were also estimated.

Analysis of lung samples. Complete pairs of lungs, together with the associated lymph nodes, were obtained from human adults from southern England who had died suddenly and had not had a long period in hospital immediately prior to death. The samples were ashed at 450° C in a muffle furnace and the zirconium-95 + niobium-95 γ-activity of the ash was measured using the 3-in. sodium iodide crystal in the low-background counting

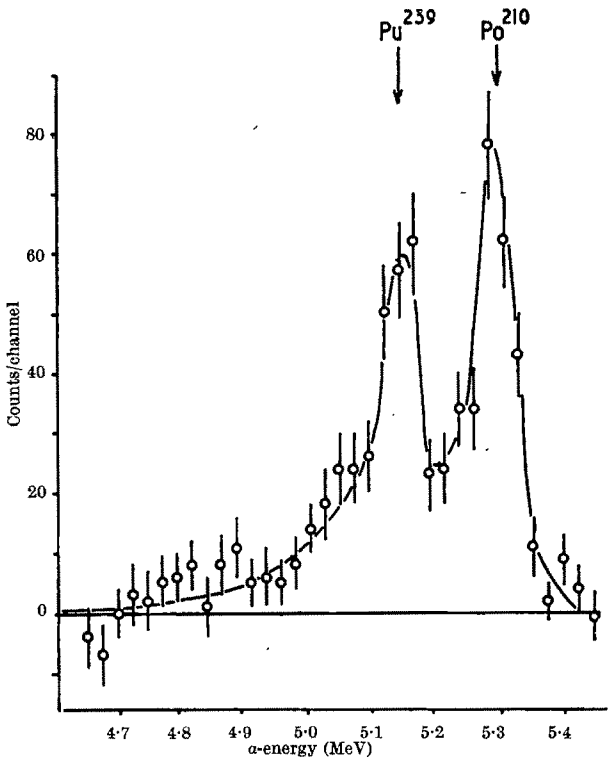


Fig. 2. α-spectrum from atmospheric dust collected during May 7–9 and counted 55 h

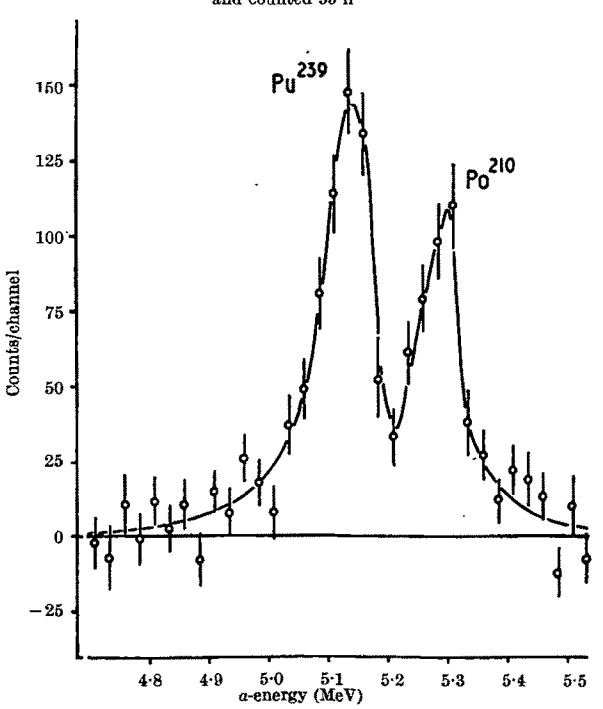


Fig. 3. α-spectrum from atmospheric dust collected during June 18–20 and counted 116 h

facility mentioned here. Because of the very low specific activity of plutonium-239 in lungs it was necessary to separate the plutonium chemically prior to assay. The separation, which will be described in detail elsewhere, was based on the selective absorption of plutonium (IV) from 7·5 N nitric acid by a column of strong base anion exchange resin. The plutonium was then eluted and further purified by solvent extraction with 2-thenoyltrifluoroacetone (T.T.A.) in benzene. Finally, the plutonium was electroplated on a stainless steel disk and the activity measured in the α-spectrometer. This allowed assay of the plutonium-239 without interference from any polonium-210, which is known to be present in soft tissues in appreciable concentrations[7] and which may have come through the chemical separation. Assay by α-spectroscopy also enabled accurate determination of the overall recovery of plutonium to be made: the sample was spiked with plutonium-238 (α-energy, 5·50 MeV) prior to the separation and the activity of plutonium-238 in the α-spectrum was measured. (The results of the air measurements reported here and also those by other workers[6] indicate that the activity of any plutonium-238 present in the atmosphere is much less than that of plutonium-239.)

Results. Spectra obtained from atmospheric dust, sampled in the manner described here, are shown in Figs. 1–3. Generally the activities analysed were less than 10^{-13} curies so that, although the counting times were 50–120 h, the standard deviations from counting statistics (shown in the spectra) were quite large. From the α-energies of the observed peaks, the long-lived activity was attributed to plutonium-239 and polonium-210. The plutonium-239 concentrations in the air, measured at approximately weekly intervals, are shown in Fig. 4, and the estimated lead-210 concentrations and polonium-210/lead-210 ratios are summarized in Table 1.

The results of the measurement of plutonium-239 and zirconium-95 + niobium-95 in lungs are shown in Table 2.

Plutonium-239 in the atmosphere. The levels show a rather drawn-out spring peak similar to that observed[9] for fission products. The average of the plutonium-239 concentrations measured during the period February to July 1962 was 20×10^{-17} c./kg air and this corresponds to approximately 0·08 per cent of the maximum permissible level of continuous exposure for large populations[8]. The mean ratio of cæsium-137 activity (estimated[9] from the measured zirconium-95 + niobium-95 concentration and the age of debris) to plutonium-239 activity for the samples measured during this period was 122 ± 8, which is not very different from previously reported ratios[10,11].

Polonium-210 and lead-210 in the atmosphere. The average lead-210 concentration in air observed during the period of these measurements was $3{\cdot}2 \times 10^{-15}$ c./kg, which is very close to that reported previously by Burton *et al.*[2]. However, except for the last month (June), the polonium-210/lead-210 ratio was higher than the value (0·1) reported by the same workers. The highest value observed here occurs near the commencement of the spring peak in plutonium-239 fall-out (Table 1, Fig. 4).

Table 1. Lead-210 Activity and the Ratio Polonium-210/Lead-210 in Ground-level Air, February to June 1962

Month (1962)	No. of samples	^{210}Pb 10^{-15}c./kg air	^{210}Po/^{210}Pb
February	2	3·3	0·24
March	3	2·0	0·48
April	5	3·3	0·21
May	7	3·2	0·15
June	5	3·9	0·09

Table 2. Plutonium-239 and Zirconium-95 + Niobium-95 in Human Lungs

Mass wet (g)	Death 1962	^{95}Zr + ^{95}Nb per person (pc.)	^{239}Pu (10^{-15}c./g)	^{239}Pu average per person (pc.)
1,500	March	—	0·12	0·15
860	April	—		
890	June	234	0·21	0·17
685	June	194		
1,100	June	302	0·13	0·15
1,300	July	246		
920	July	241		
1,080	July	251	—	—

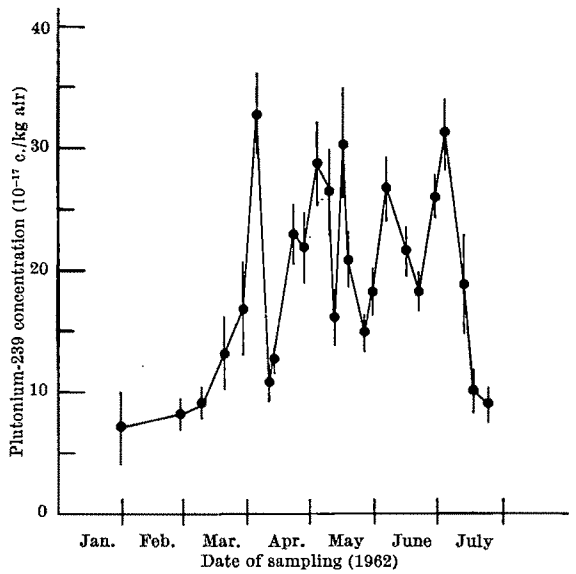


Fig. 4. Concentration of plutonium-239 in ground-level air, measured at weekly intervals during 1962. Vertical lines are standard deviations from counting statistics only

Burton *et al.* (*loc. cit.*) have suggested that some of the lead-210 measured at ground-level may have circulated through the stratosphere with a mean residence time of several months in a manner similar to debris from nuclear weapon tests in the atmosphere. The other component of the lead-210 at ground-level has been formed and has remained in the troposphere with a mean residence time of only a few weeks. The high polonium-210/lead-210 ratios observed here may, therefore, be a result of a higher contribution from the older stratospheric component relative to the tropospheric lead-210. The possibility exists, however, that some of the polonium-210 observed early in the spring may have been artificially produced from bismuth (bismuth-209) in weapon detonations.

Plutonium-239 and zirconium-95 + niobium-95 in human lungs. The burdens of zirconium-95 + niobium-95 at the time of death were comparable with those reported by Rundo *et al.*[12]; but the plutonium-239 levels were, in general, lower than those reported for specimens taken during 1954–59 [7,13]. The average lung burden of plutonium-239 found here (0·16 pc.) was approximately 6 per cent of the average polonium-210 activity measured in other individuals[14]. The average total α-activity of adult lungs in Great Britain has been given[15] as 8 pc.

The radiation doses to the lungs from the measured activities of zirconium-95 + niobium-95 and plutonium-239 are 2·4 mrem/y and 0·17 mrem/y respectively. For comparison, the lung dose from polonium-210 is estimated[14] to be 3 mrem/y. However, the actual distribution of the various nuclides, particularly the α-emitters, will have considerable effect on the irradiation pattern and any assessment of the significance of these activities from a biological point of view must take this into consideration.

Relation of lung burden and atmospheric concentration. The measurement of both the atmospheric concentration and the lung burden of a given nuclide provides opportunity to evaluate the validity of some of the 'standard man' parameters associated with lung retention. Here, although only a few measurements have been made, some estimations may be carried out regarding the lung retention of zirconium-95 + niobium-95 and plutonium-239.

Assuming that the International Commission on Radiological Protection (I.C.R.P.) single exponential model[8], characterized by a half-life T days, is valid for lung retention and excretion, the lung burden N pc. after inhalation of I pc./day for t days is given by:

$$N = \frac{fIT}{0{\cdot}693}\left[1 - \exp\left(\frac{-0{\cdot}693t}{T}\right)\right]$$

where f is the fraction of inhaled material which is not exhaled or quickly swept out of the respiratory tract and swallowed. Both f and T may vary considerably with the character of the aerosol.

Considering the zirconium-95 + niobium-95 results and taking an average burden per person as 245 pc., the fraction f may be estimated using the foregoing expression as follows. The average zirconium-95 + niobium-95 activity in the air at Sutton, Surrey, during the period January–May 1962 was approximately 1 pc./kg air[9]. If the biological half-life (T_b) is much greater than the physical half-life (T_r) of a nuclide in the lungs, the effective half-life (T) is only slightly less than T_r. In the case of zirconium-95 + niobium-95 (T_r = 65 days), if T_b is not less than the 120 days suggested by the I.C.R.P.[8] for insoluble particles, the effective half-life will be between 40 and 65 days. Supposing that the daily inhalation is the standard 26 kg air, the value of f, calculated with these parameters in the above equation, lies in the range 14–19 per cent. The I.C.R.P. suggests 12·5 per cent for f.

Assuming that the particle size distribution of plutonium-239 was similar to that of the zirconium-95 + niobium-95, so that the same value of f was effective, the lung burden of plutonium-239, at the end of June 1962, may then be predicted from the above equation. With f = 14–19 per cent, T = 120 days and taking the average activity of plutonium-239 in the air as 20×10^{-17} c./kg air for the three months prior to this time and 7×10^{-17} c./kg for the period 1955 until March 1962 from the foregoing results and others[16], the predicted burden is 0·10–0·13 pc., which is of the same order as that measured. Thus, the foregoing results and the 'standard man' parameters appear to be reasonably consistent.

Possible levels of plutonium-239 in other tissues. From ref. 16 and the results given here, the total plutonium-239 inhaled up to June 1962 by a 'standard man' in England can be calculated to have been of the order of 5 pc. Of the 14–19 per cent suggested here as being retained in the lung with an effective half-life of 120 days or longer, approximately 0·16 pc. was still there in June 1962. Absorption of plutonium via the gastrointestinal tract is extremely small[8], so, assuming only 19 per cent of the inhaled material was available for intake to the body fluids, not more than 0·8 pc. would have been absorbed and transferred to other tissues from the lungs. If it is assumed that this activity of plutonium-239 is redistributed in the proportions suggested by the I.C.R.P., then the activities in bone, liver and kidney would be less than 0·6 pc., 0·12 pc. and 0·016 pc. respectively. (The natural α-activity of these tissues has been given[7] as 400 pc., 9 pc. and 1 pc. respectively.) The only reported value which can be compared with the foregoing is a measurement of plutonium-239 in human kidney[13]. This gave ten times the activity suggested here as an upper limit.

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RETINAL PHOTOCOAGULATION BY LASERS

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WHEREAS coagulation of detached retinæ with light has been performed for many years, the present-day techniques suffer from long exposure time, large size of the lesions, and awkward and expensive apparatus. With the advent of lasers the use of a high-energy light source which can deliver the required amount of energy over a short time and cause lesions of small size has become possible. Furthermore, the overall instrumentation can be compacted to the size of conventional ophthalmoscopes.

Photocoagulation is used largely for the treatment of retinal detachment. This common eye disorder occurs in thousands of people each year and, if allowed to develop fully, ultimately results in blindness. The technique offers advantages over previous methods of retinal coagulation. For example, retinal coagulation by diathermy needle takes several hours and general anæsthesia is required. Furthermore, this is often a 'blind' procedure in the sense that the portion of the retina which is to be coagulated is not viewed directly during the use of the needle. Also, the recuperation period is prolonged. In contrast to this method, photocoagulation is a very simple procedure which can be accomplished in a few minutes. Moreover, a general anæsthetic is never required, the spot which is to be coagulated is always directly in view, the affected area is relatively small and well defined, and the recuperation period is not painful and rarely exceeds a few days.

Investigations of damage by light to the retina[1] were carried out as early as 1867 when an attempt was made to burn the retina of rabbits with sunlight. However, it was not until 1945, when intense artificial light sources

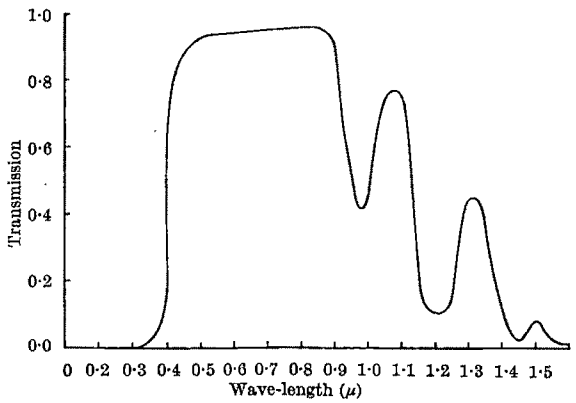


Fig. 1. Spectral transmission curve of the refractive media of the human eye

were available, that therapeutic light coagulation appeared promising. By 1949 Meyer-Schwickerath[2] and Littman had developed a technique and an instrument for practical therapeutic light coagulation. In this instrument, light from a continuously operated high-pressure xenon lamp is transmitted along a train of optical components located in a long, movable arm which extends from the instrument. At the end of the drum is a mirror with a small hole through which viewing and aiming are done. The lens of the patient's eye causes the light to be focused on the retina where a lesion is produced. The light intensity can be adjusted to obtain the desired effect in an exposure of about 0·25–1·5 sec.

Coagulation Using Lasers

A new class of unique light source, namely, optical masers and lasers[3–5], is now available for photocoagulation work. These sources are monochromatic and emit light at various wave-lengths both in the visible and infra-red. A common solid-state laser configuration consists of an active material (polished flat and parallel at the ends) which is placed on the axis of a helical xenon flash lamp. For a period of time (typically $\simeq$ 300 μsec) an intense flash from the xenon lamp causes more active material ions to exist in an upper quantum state than are in the ground state. During this period, light which travels parallel to the cylinder axis is amplified and an intense, highly collimated, monochromatic, coherent light beam is emitted.

(*a*) *Selection of Wave-length.* In selecting a wave-length for retinal photocoagulation one must consider the optical properties of the eye as well as the difficulties of obtaining required amounts of light at a given wave-length. Fig. 1 shows the transmission of the refractive medium of the human eye[2]. It appears that for photocoagulation work the wave-length of light should be confined to the portion of the spectrum between 0·4 and 0·9μ or possibly to the infra-red region around 1·08μ. At these wave-lengths the light will pass through the vitreous and aqueous humours and the retina, and will be absorbed in the pigment epithelium and the choroid. At the present time there are only two laser materials which conveniently give practical amounts of energy in these regions. These materials are chromium in alumina (ruby), which emits at 6943 Å, and the neodymium in glass, which emits at 1·06μ.

The fraction of the incident light which is absorbed in the fundus varies several hundred per cent[6] from one eye to another and also varies at different regions in the retina. However, the absorption does not vary rapidly with wave-length in the visible and slowly falls towards the infra-red. Because of this and because the transmission of the refractive media of the eye is higher in the visible than in the infra-red, ruby appears to be favoured over neodymium. However, there is a possibility that instrumental considerations associated with neodymium may make its selection advantageous.

(*b*) *Photocoagulation Process.* All the biophysical details of the absorption-coagulation process are not fully understood. Certainly the effect is dependent on power although the extent is not known. Soesterberg[7] has advanced a theory which appears to be applicable to light sources less powerful than lasers. A mathematical description of the process is reported by Rose *et al.*[6] Qualitatively, the process is quite simple. During the time ($\simeq$ 300 μsec) when light energy is delivered to the fundus, very little energy can escape to the surrounding tissue either by convection within the capillary system or by conduction to surrounding tissue. Because of this, the temperature of the small amount of tissue which absorbs the energy becomes highly elevated. If a large amount of energy is delivered rapidly, tissue fluid is vaporized and the vapour erupts through the retina into the vitreous humour and a bubble is formed. A hæmorrhage or a retinal hole can also be caused under these conditions. If more moderate amounts of energy are delivered, the absorbing tissue is not vapourized and some of the stored energy is passed on to the retina causing it to be coagulated and 'welded' to the choroid.

Experimental Laser Photocoagulator

An experimental laser photocoagulator has been built to determine laser parameters pertinent to photocoagulation and also to provide an instrument for clinical testing on rabbits, cats and human beings. Fig. 2 illustrates, diagrammatically, the optical features of this instrument. Here a collimated beam of light is emitted from the active laser material and is focused by the lens of the eye on to the retina where a lesion occurs. Provision is made so that the active medium can either be a cylindrical ruby crystal or a cylinder of neodymium-doped glass. The instrument is about the size and weight of a normal ophthalmoscope so that it can be controlled easily with one hand. Direct ophthalmoscopic viewing is provided with an auxiliary light source and several examining lenses, both negative and positive. The laser configuration consists of a 0·25 in. × 3 in. rod placed between two linear xenon flash lamps surrounded by a specular reflector. For safety and reliability, the lamps are connected to independent storage capacitors although they are triggered from the same source. The capacitors are capable of delivering up to 800 joules of energy to the pumping source, that is, xenon lamps.

The energy output of the laser can be altered by varying the voltage to which the capacitor banks are charged. The result is that energy output and average power output are not independent variables, but rather they are related in some complex manner. Moreover, the instantaneous power output is a rapidly varying function of time and no attempt is made to control it[8]. In this instrument the total energy output of the laser was varied continuously from zero to 0·25 joule.

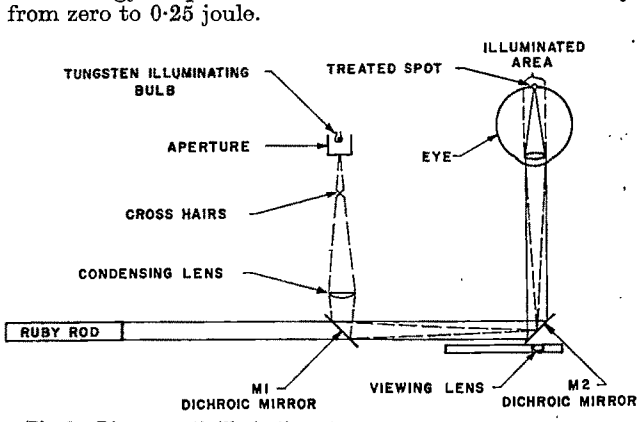


Fig. 2. Diagrammatic illustration of the optical features of the experimental laser coagulator

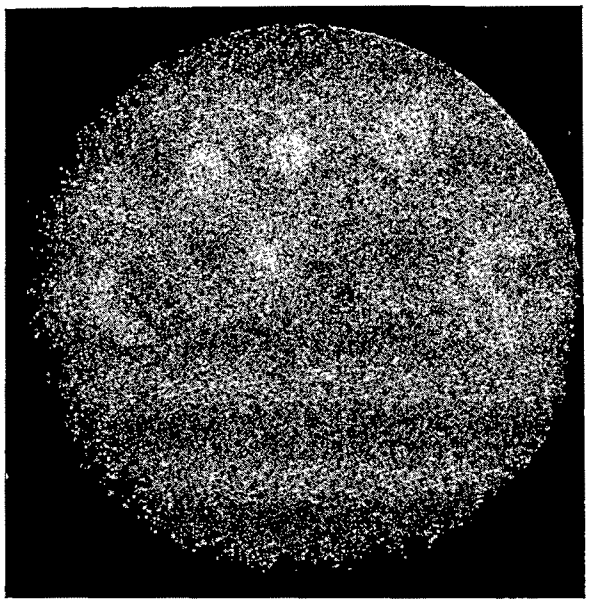


Fig. 3. Two rows of lesions showing effects of increasing laser energy on the size of lesions

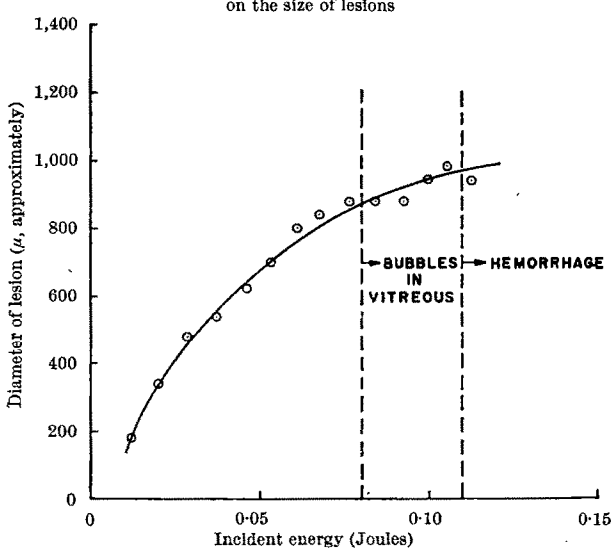


Fig. 4. Plot of the diameter of lesions versus incident laser energy. The bubbling and hæmorrhage thresholds are indicated

Experimental Results

So far all experimental work has been performed on rabbits or cats. In the experiments the animal is anæsthetized with intravenous pentobarbital and the pupils dilated with 10 per cent phenylephrine. After treatment with a laser photocoagulator and/or a Zeiss photocoagulator, photographs of the lesions are taken. Histological sections of the lesions are also made.

Immediately after laser coagulation the structure of the lesions is more apparent than in those caused by the Zeiss photocoagulator and are smaller in size. In the centre of the lesion is a dark crater, indicating a high local density, and concentric with this is a dark ring with smaller and larger white rings on either side. During the next few days the lesion changes in appearance and begins to look more like a uniform disk of affected tissue. This disk of affected tissue apparently is firmly attached to the choroid.

It was found that the coagulation threshold (minimum energy required for coagulation) varied grossly with colour of the fundus. This was expected and is related to the reflectivity and absorptivity of the fundus at 6943 Å, the wave-length of the radiation from the laser. In general, the threshold energy for a lightly pigmented fundus is higher than a darkly pigmented fundus. Variations of more than a factor of five were observed. The amount of damage caused by a given incident energy is also a function of the colour of the fundus. It is possible for a given incident energy to cause a hæmorrhage in a darkly pigmented fundus and yet not visibly damage an albino fundus. The energy-range over which good lesions can be caused without excessive damage varies markedly with the colour of the fundus. For example, for a particular Siamese cat with a pale fundus the ratio of energy required for a hæmorrhage to coagulation threshold energy was about eleven to one. This same ratio for a cat with a darker fundus was three to one. Similar variations were found with rabbits.

Investigations were carried out to determine the area of affected tissue as a function of incident energy. In general, the diameter of the lesion increases with increasing incident energy but not indefinitely. Fig. 3 is a photomicrograph showing this effect on the retina of a rabbit, on which two rows of lesions were caused. It will be noted that the energy associated with the lesions increases from left to right in both rows, with the lesion in the upper left being caused by the least amount of energy. In experiments on pigmented rabbit's retina, as the energy is increased bubbles begin to form between 0·08 and 0·11 joule, and finally hæmorrhaging occurs above 0·11 joule. Fig. 4 gives a plot of the diameter of lesions on a rabbit retina caused by varying the laser power output. The size of the lesions varies from 200μ to 1 mm when the energy is varied from 0·01 to 0·15 joule.

Histological sections of many of the lesions were prepared to determine the general character of the lesions, as well as the depth of penetration. In the process it was found that the retina usually separates from the choroid except at the sites where lesions were caused. This is evidence that the retina is indeed 'welded down' in these regions. Fig. 5*a* is a section of a typical lesion caused

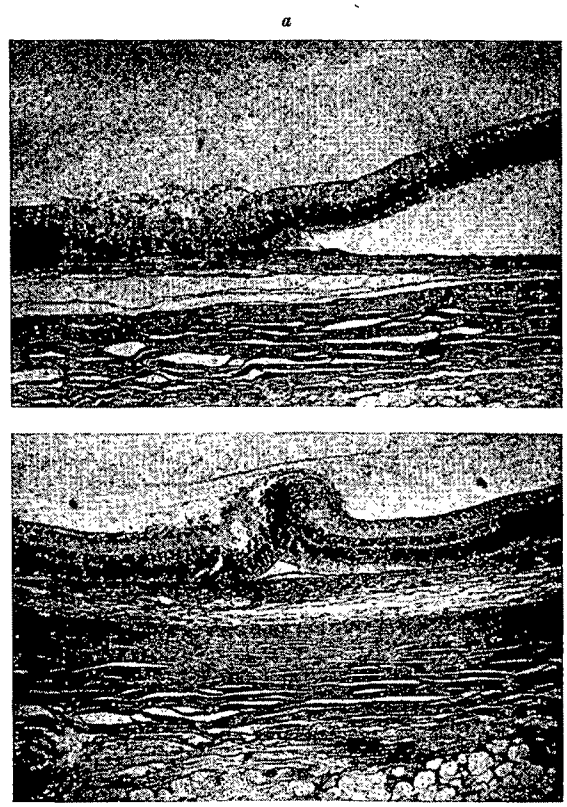


Fig. 5. *a*, Histological section of a normal lesion caused by laser photocoagulation; *b*, histological section of a lesion showing eruption through the retina due to high laser energy

in the retina of a rabbit. Scar tissue has formed above the retina and a general disruption of cells is apparent below this tissue. The retina has separated from the choroid except at the lesion. Fig. 5*b* is a section of a lesion through which a bubble erupted. It is obvious from the photomicrograph that the tissue which was vapourized was in the vicinity of the pigment epithelium and in fact probably was a portion of the pigment epithelium. The photomicrograph also shows that the retina has separated from the choroid except at the lesion. Other sections indicate that the pigment epithelium is affected at very low incident energies. Even at very high energies (0·25 joule) the damage does not appear to penetrate very deeply into the choroid.

Conclusions

Because of the large variations in colour of the fundus it will be necessary to begin any treatment at low energies and gradually increase the energy until the proper effect is caused. This change in energy-level can be caused both by varying the condenser voltage and by using calibrated filters in the beam. This procedure, however, is not objectionable and is generally followed today with the existing coagulator. There are a number of advantages offered by laser photocoagulation. The fact that the lesion which is caused is small is important when the macula of the eye is involved. The size of the lesion can be varied by varying the laser energy or by using an appropriate optical system. Also, since the lesion is caused in such a short time, the normal reaction of the eye to a bright light does not occur until after the process is over and thus the burn is not smeared across the retina. The ease of manœuvering and aiming a hand-held instrument with an ophthalmic viewing head, the fact that the patient does not need to be anæsthetized, and the potential low cost of the instrument are all important practical considerations which make retinal photocoagulation by lasers appear quite promising.

We thank N. Silbertrust for his assistance.

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PARTITION OF DIVALENT CATIONS BETWEEN BACTERIAL WALL AND CELL CONTENTS

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A DETAILED examination of the requirements of *Rhizobium trifolii* for magnesium and calcium[1] correlates well with the results we have since obtained on the disposition of these elements in the cell wall and within the cell itself, under contrasting conditions of calcium deprivation and sufficiency[2]. The information so derived supports the indications we already have that calcium has a structural role in the wall of this organism. This role can be distinguished from the less specific part it shares with magnesium in meeting a much larger requirement by the cell for divalent cations.

Portion of the analytical data[2] can be shown graphically (Fig. 1) in such a way as to indicate the concentration of both elements in walls and cells (height of histograms) and their total distribution between the two (area). For this purpose and subsequent calculations we have taken 20 per cent as a fair estimate of the proportion of total cell mass likely to be represented by wall[3].

Three things call for comment: (*a*) Calcium is more concentrated in the wall than in the rest of the cell. (*b*) When calcium is severely limiting (histogram *B*) the wall retains a reduced but appreciable amount of this element, sufficient to account for all that is found in the whole cell analysis. (*c*) Magnesium only partly replaces calcium in the wall but fully compensates for deficiency of calcium within the cell (histograms *A*, *B*). In fact, the total divalent cation thus represented by magnesium on a molar basis is significantly greater than when calcium is present as well. On an actual weight basis, Fig. 1 is about the same whether calcium is present or absent. (Total divalent cation in cells receiving calcium, 4·11 mg/g dry cell substance; without supplied calcium, 4·38.)

The apparently more particular relationship between calcium and the cell wall is, we think, significant in relation to the spherical condition of calcium-deprived cells that is quite different from the appearance of cells limited by a shortage of magnesium[4]. It is apparent that each element has a distinct role. Ultra-thin sections of the calcium-deprived cells viewed under the electron microscope show a well-defined double-layered cell wall[4] which is quite different from what we have seen in penicillin-induced spheroplasts of the same organism. Moreover, although the proportion of viable cells grown under con-

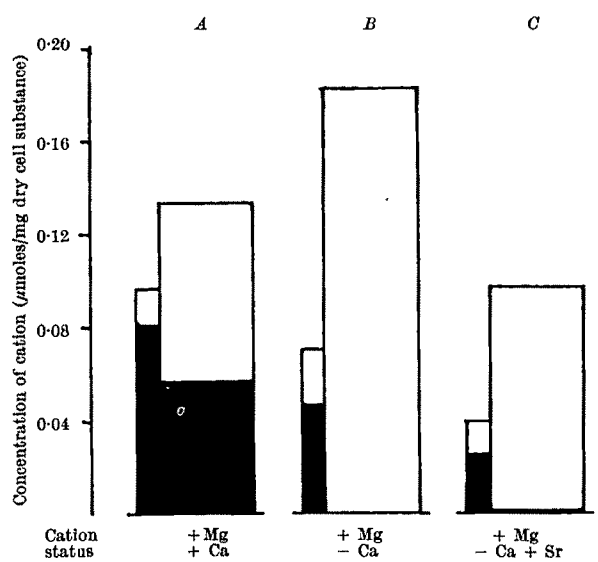


Fig. 1. Distribution of calcium and magnesium between walls and cell interior
In each histogram (*A*, *B* and *C*) the wall, narrow column at the left, is taken to be one-fifth the total cell mass. The height of each column represents concentration; the area, total amount contained in wall or cell
White, magnesium; black, calcium

ditions of calcium deprivation is significantly less than that of calcium-adequate cells[1], we have not been able to demonstrate any marked susceptibility to swelling or osmotic disruption when the deprived cells are suspended in distilled water. Therefore, although their shape suggests some loss of rigidity in the walls, they seem to retain appreciable strength. Any loss of rigidity is not due to absence of the usual '*R*'-layer components[2], and, as has since been found, the cell wall protein of calcium-deprived cells is indistinguishable from the calcium-adequate, either by qualitative analysis of amino-acids or the amount of total protein.

Cell wall calcium was stable to ordinary washing procedures, but 90 per cent of it could be removed readily enough with ethylenediamine tetraacetic acid (EDTA). Whole, non-growing calcium-deprived cells readily took up calcium when exposed to this element for 72 h and attained an internal calcium concentration of the same order as was found in cells grown with a sufficiency of this element ($0{\cdot}06 \times 10^{-3}$ μmoles/μg). Walls of deprived cells made up only half of their deficiency when given the same treatment. These facts, together with our failure to restore normal morphology by subsequent exposure to calcium, seem to indicate that its incorporation into the wall requires calcium to be present when the organism is growing[2].

There is, in fact, other evidence that calcium may be involved structurally in bacterial walls. Keerer and Gray[5] showed that labelled calcium was incorporated into the wall fraction of *Listeria*. Calcium could also be the metal the removal of which by EDTA (and not by several other chelating agents) made a number of Gram-negative species sensitive to lysozyme[6], although the inhibition of lysozyme action by restoring cation seemed to involve a wide range of metals. The EDTA-potentiation of lysozyme in the preparation of spheroplasts[7] could be due to greater exposure of the polysaccharide backbone of the '*R*' layer consequent on the withdrawal of calcium.

So far we have tended to look to the '*R*' layer as a likely site where calcium might exercise a stabilizing role, for example, by neutralizing excessive charges due to carboxyl groups, and thus permit the formation of a tightly packed structure (compare Primosigh *et al.*[8]). In fact, the found calcium seems to be of the order required by the free carboxyl groups in the model for the mucopeptide given by Salton (ref. 3, Fig. 15), taking a value of 10–20 per cent for mucopeptide content of the wall (compare Salton (ref. 3), p. 50). According to these calculations 2 carboxyl groups per mucopeptide unit would require $0{\cdot}1$–$0{\cdot}05 \times 10^{-3}$ μmoles calcium per μg wall, and one carboxyl per unit, $0{\cdot}05$–$0{\cdot}025 \times 10^{-3}$ (found content = $0{\cdot}081 \times 10^{-3}$ μmoles).

It is, however, possible that calcium effects a degree of bonding between '*R*'-layer and the outer lipoprotein, so that it may be a disruption of this, rather than rendering the '*R*'-layer labile, which clears the way for lysozyme action. Similarly deficiency of wall calcium could lead to abnormal morphology. This proposal has something in common with the mechanism of EDTA-potentiation of lysozyme suggested by Noller and Hartsell[9,10] but retains a chelating rather than a detergent role for EDTA. There is no reason for supposing, of course, that other agents could not disrupt the lipoprotein layer for quite different reasons.

Results we have recently obtained with *Rhizobium trifolii* point to calcium as an element that specifically exercises a protective action against the lytic effect of lysozyme (Table 1). Whereas calcium-adequate cells required potentiation by EDTA (columns 1 and 2) those that were deficient in calcium, though having sufficient magnesium (columns 5 and 6), were considerably lysed without the addition of this chelating agent.

Wherever the site of calcium's specific action, the failure of magnesium to replace calcium in the maintenance of normal cell morphology and in the composition of the wall

Table 1. Lysis of *Rhizobium trifolii* by Lysozyme*

Calcium status†	Plus calcium		Without calcium plus strontium		Without calcium	
Age of culture (h)	72	96	72	96	72	96
Treatments						
Lysozyme + *tris* buffer + EDTA	61%	64%	72%	66%	83%	61%
Lysozyme + *tris* buffer	17%	18%	24%	11%	69%	48%
tris buffer + EDTA	13%	17%	24%	11%	22%	12%
tris buffer	2%	10%	18%	6%	11%	7%

* Using the method and criteria of Noller and Hartsell (ref. 9). Per cent lysis = 100 (initial optical density − final optical density) ÷ (initial optical density). Conditions: *p*H 6, 45° C, 60 min. 20 μg/ml. lysozyme, 133 μg/ml. EDTA, 0·03 M *tris* buffer.
† Growth media all with 1 mM total divalent cation, either as 0·5 mM Mg + 0·5 mM Ca or Sr, or 1·0 mM Mg.

suggests a structural requirement that magnesium cannot meet. Our further experiments have shown that of the alkaline Earth elements heavier than calcium, strontium, but not barium, can take its place in permitting the development of normal cells, though less efficiently on a molar basis. Growth data (Table 2) showed a similar substitution by strontium (with about one-fourth the efficiency), and columns 3 and 4 of Table 1 again reveal strontium as a substitute for calcium, this time in its protective role against lysozyme.

Table 2. Growth Response of *Rhizobium trifolii* to Variable Amounts of Calcium, Strontium and Barium

Concentration of varied cation (mM)†	Growth* in the presence of: Exp. 1 Ca	Exp. 1 Sr	Exp. 2 Ca	Exp. 2 Ba
0	29	29	23	23
0·0025	43	34	35	25
0·01	74	41	64	28
0·04	90	74	74	37
0·16				28

* Turbidity at 100 h. 1 unit = approx 50×10^6 cells/ml.
† In all cases the medium contained 0·5 mM Mg. The second divalent cation was supplied as Ca or Sr (Exp. 1); Ca or Ba (Exp. 2).

Cells deprived of calcium, but normal in morphology as a result of being supplied with strontium, were analysed for calcium and magnesium, with the result shown in histogram *C* of Fig. 1. Strontium not only permitted normal growth and morphology but also evidently replaced some of the calcium that otherwise accumulated in the wall from traces of this element in the medium (histogram *B*). It has also reduced the amount of magnesium substituting for calcium within the calcium-deprived cell.

(We have not so far been in a position to undertake strontium analyses directly, but the foregoing evidence appears to be satisfactorily circumstantial.)

We can use growth and analytical data to test the approximate quantitative relationships between cells and media (Table 3). The trace of calcium known to be in the basal medium (0·003–0·006 μmoles/ml.) is accounted for by that found in the cells, practically all of which is in the walls (column 1 in Table 3). As would be expected, calcium contained in the cells accounts for only a fraction of the calcium supplied by the medium when the latter is in excess (column 2). In this case, however, the amount represented in the cell wall (0·032 μmoles in cells obtained

Table 3. Calcium represented in Cells and Cell Walls in Relation to Calcium in the Growth Medium

	Nutrient status calcium in medium (μmoles/ml.) None added (0·003–0·006*)	Excess (0·5)
Turbidity†	32	100
(1) Whole cells		
Yield (μg/ml. medium)‡	640	2,000
Ca (μmoles/ml. medium)	0·0045§	0·12§
(2) Walls		
Yield (μg/ml. medium) ‖	128	400
Ca (μmoles/ml. medium)	0·006 ¶	0·032 ¶

* Calculated as impurity: 0·006 by analysis; 0·003 by extrapolation of dose/response curve, Fig. 3 (ref. 1).
† From ref. 4, Table 1).
‡ Applying found value of 20 μg/turbidity unit.
§ Applying values $0{\cdot}007 \times 10^{-3}$ and $0{\cdot}062 \times 10^{-3}$ μmoles/μg dry cells/ml. medium, respectively (ref. 2).
‖ Taking walls as one-fifth whole cells.
¶ Applying values of $0{\cdot}047 \times 10^{-3}$ and $0{\cdot}081 \times 10^{-3}$ μmoles/μg dry cells/ml. medium, respectively (ref. 2).

from 1 ml. of medium) is particularly interesting because it agrees very well with the level on the dose/response curve (ref. 1, Fig. 3) below which the yield becomes dependent on calcium concentration (0·025–0·031 μmoles/ml.). This result again points to the likely importance of calcium in the normal cell wall and the influence it can thereby exert on the growth of the organism.

Beyond the growth-limiting concentration calcium appears in the cell interior, sharing with magnesium a non-specific part that both can play in maintaining sufficient divalent cation. By a similar calculation to those given in Table 3, the cells grown with both calcium and magnesium in excess can be estimated to contain total divalent cation equivalent to 0·25 μmoles/ml. medium. This is within the range of supplied divalent cation in which the total growth responds sharply to concentration of divalent cations (ref. 1, Table 3) and represents considerable concentration within the cells (100–200-fold). Magnesium has, of course, its own specific and quantitatively important role[1].

Our experience with calcium and *Rhizobium* contrasts with that of Wyatt *et al.*[11] with *Staphylococcus*. These authors were unable to show any significant uptake of labelled calcium by a method that would detect removal of $0{\cdot}004 \times 10^{-3}$ μmoles calcium/μg dry cells, an amount very much less than the calcium values we were concerned with. This difference might reflect an association between calcium and lipoprotein in the cell wall of our Gram-negative organism, lipoprotein being absent from the Gram-positive *Staphylococcus*.

The rhizobial cell has specific requirements for calcium and magnesium, each satisfied by a relatively low concentration in the medium. Calcium deficiency is expressed in abnormal morphology (but not a true spheroplast condition), withdrawal of calcium from the cell interior, lowered concentration in the wall, and susceptibility to the action of lysozyme without potentiation by EDTA. A role for calcium in the structure of these walls is thereby suggested. Possibilities are that calcium is involved in stabilizing the '*R*'-layer by bonding with excess carboxyl groups or linking lipoprotein and '*R*'-layer components of the wall. Strontium, but neither magnesium nor barium, can substitute for calcium in its cell-wall function, though less efficiently.

Quantitative relationships between growth and analytical data show that the calcium represented in the walls of deprived cells accounts approximately for the calcium known to be present in the deficient medium. The concentration of calcium below which growth is controlled by this element is close to that concentration at which the cell's need for wall calcium is satisfied.

Calcium shares with magnesium the ability to satisfy the cell's larger non-specific requirement for divalent cations. The total of these within the cell represents considerable concentration from the medium.

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PATHWAY OF REDUCTION OF METMYOGLOBIN AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE BY ILLUMINATED CHLOROPLASTS

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In photochemical reductions by isolated chloroplasts the first naturally occurring co-factor to be described was the leaf-protein shown to be highly active in catalysing the reduction of methæmoglobin and metmyoglobin[1]. The 'methæmoglobin reducing factor' was purified[2] and obtained in crystalline form[3], and it has been shown[4] to be identical with 'photosynthetic pyridine nucleotide reductase', a leaf protein fraction found by San Pietro and Lang[5] to mediate the aerobic reduction of substrate amounts of nicotinamide adenine dinucleotide phosphate (NADP) by illuminated chloroplasts. More recently Tagawa and Arnon[6] have described a modified method for preparing the protein and have made further observations on its redox properties. They have concluded that the leaf protein is similar to, but not identical with, an iron-containing protein 'ferredoxin' isolated by Mortenson, Valentine and Carnahan[7] from *Clostridium pasteurianum*, where it was shown by them to serve as an intermediate catalyst between hydrogen, activated by hydrogenase, and a number of hydrogen acceptors.

In experiments where the hydrogenase system in darkness replaced illuminated chloroplasts as a source of reducing power Tagawa and Arnon[6] found that neither the *Clostridium* nor the leaf protein could mediate NADP reduction unless the reaction mixture was supplemented either by chloroplast fragments or a flavoprotein fraction isolated from leaves. From this they concluded that the name 'photosynthetic pyridine nucleotide reductase' is an inappropriate description of the iron-containing leaf protein and they proposed that the name 'ferredoxin' should be extended to this material. This terminology will be adopted in what follows.

It has now been possible to extend the conclusions of Tagawa and Arnon[6] to a reconstructed photochemical system in which illuminated pea chloroplast fragments provide the reducing power and to show that the reduction of metmyoglobin in this system is independent of the presence of protein factors other than ferredoxin. The relative activity of intact pea chloroplasts towards NADP and metmyoglobin has already been described[4]. When such chloroplasts, prepared in isotonic sucrose medium[2], were fragmented and exhaustively extracted by repeated sedimentation from water, they were found progressively to lose their capacity to reduce NADP when illuminated in the presence of adequate amounts of ferredoxin. By contrast, their reactivity towards metmyoglobin increased as a result of the washing (Table 1).

Table 1. The Effect of Successive Water Extractions of Pea Chloroplasts on their Capacity to Photoreduce NADP and Metmyoglobin in the Presence of a Saturating Amount of Ferredoxin

Treatment of chloroplast material	Rate of reduction (μmoles/mg chlorophyll/h)	
	Metmyoglobin	NADP
As prepared in 0·4 M sucrose 0·1 M *tris*-HCl, *p*H 7·8	188	87
Extracted with water:		
twice	220	39
three times	265	25
six times	302	11

Reaction mixtures contained (in 3 ml.) chloroplast material equivalent to 0·03 mg chlorophyll ; pea ferredoxin 0·15 mg and (in μmoles) NADP 0·5 or metmyoglobin 0·025; *tris*-HCl buffer, *p*H 8·0, 150; sodium chloride, 20. Reaction measured as increase in extinction at 340 mμ (NADP) or 582 mμ (metmyoglobin) after successive periods of illumination of 50,000 lux. Gas phase air 20°.

Attempts were made to re-activate NADP reduction by restoring to the assay system protein fractions prepared by ammonium sulphate fractionation of the pooled supernatant fluid obtained in the chloroplast extraction procedure. A fraction precipitated within the limits 50–66 per cent saturation with respect to the salt and then subjected to prolonged dialysis to remove excess ammonium sulphate was found to restore completely the original activity towards NADP, but to be without effect on metmyoglobin reduction. The activity of this fraction towards NADP was destroyed by heating to 80° for 5 min.

Once it had been established that this reactivation could be attributed to protein extracted from the chloroplasts, it was found more convenient to prepare the fraction in quantity from the material which passed through a DEAE-cellulose column when ferredoxin was absorbed in the method described for its preparation by Tagawa and Arnon[6].

Both pea and spinach leaves have been used in the preparation with similar results. The active material was shown by the method of De Luca, Weber and Kaplan[8] to contain flavin-adenine dinucleotide and will be referred to as the flavoprotein fraction.

Using chloroplast fragments depleted of protein by washing, it was then possible to examine ferredoxin and the flavoprotein fraction as two variable quantities required for the reactivation of NADP reduction. In the experiment represented in Fig. 1 all the reaction mixtures contained a sufficient quantity of ferredoxin to saturate the particulate photochemical system. Increasing amounts of added flavoprotein then produced a linear increase in the reduction rate until the system became saturated with respect to this second factor. At the saturation point the rate of reduction, in terms of chlorophyll concentration, is of similar magnitude to those obtained with freshly prepared intact pea chloroplasts where ferredoxin is the only catalytic factor required. With high concentrations of the flavoprotein in the reconstituted system, dark reoxidation of reduced NADP became significant. The extent of the reactivation of the photochemical reaction can be better appreciated in the curve in Fig. 1 to which corrections have been applied to take account of this dark reaction.

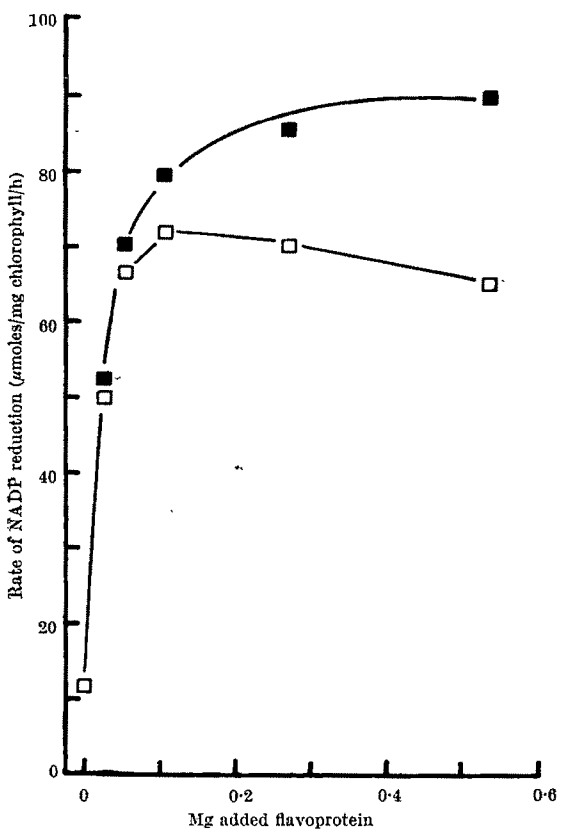


Fig. 1. Activity of pea-leaf flavoprotein fraction in restoring the capacity of protein-depleted pea chloroplast fragments to photoreduce NADP in the presence of saturating concentration of ferredoxin. Experimental conditions as for NADP reduction in Table 1 except that each cell contained pea chloroplast fragments extracted six times with water and equivalent to 0·025 mg chlorophyll. □, Experimental; ■, corrected for dark oxidation

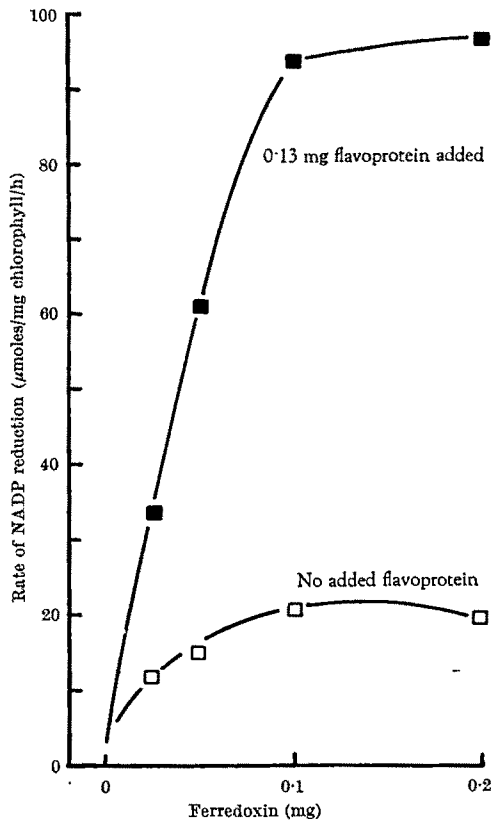


Fig. 2. Activity of pea ferredoxin in mediating NADP photoreduction by protein-depleted pea chloroplast fragments. □, With no further additions; ■, in the presence of a saturating amount (0·13 mg) of a pea-leaf flavoprotein fraction. Experimental conditions as Fig. 1

In a second series of experiments the level of ferredoxin was varied in the presence and absence of a saturating amount of the flavoprotein fraction. The result, shown in Fig. 2, confirms that both leaf proteins must be restored in saturating amounts to the washed chloroplast system for maximum reactivation of NADP reduction to be attained.

A further line of evidence that a factor additional to ferredoxin is involved in the photoreduction of NADP by intact chloroplasts was obtained by the use of organic mercurial compounds as selective inhibitors of this additional factor *in situ* within the chloroplast. It has already been shown that mercury compounds inactivate ferredoxin[2]; their inclusion in the reaction mixture would therefore not be expected to produce differential inhibition of metmyoglobin and NADP reduction. In order to localize the action of the heavy metal, pea chloroplasts

Table 2. The Effect of Treating Intact Pea Chloroplasts with Phenyl Mercury Acetate on their Capacity to Photoreduce NADP and Metmyoglobin

Chloroplast treatment	Rate of reduction (μmoles/mg chlorophyll/h) Metmyoglobin	NADP
Untreated	168	60
Treated with PMA	170	20
Inhibition (%)	0	66

Reaction mixtures as Table 1 except that chloroplasts contained 0·045 mg chlorophyll.

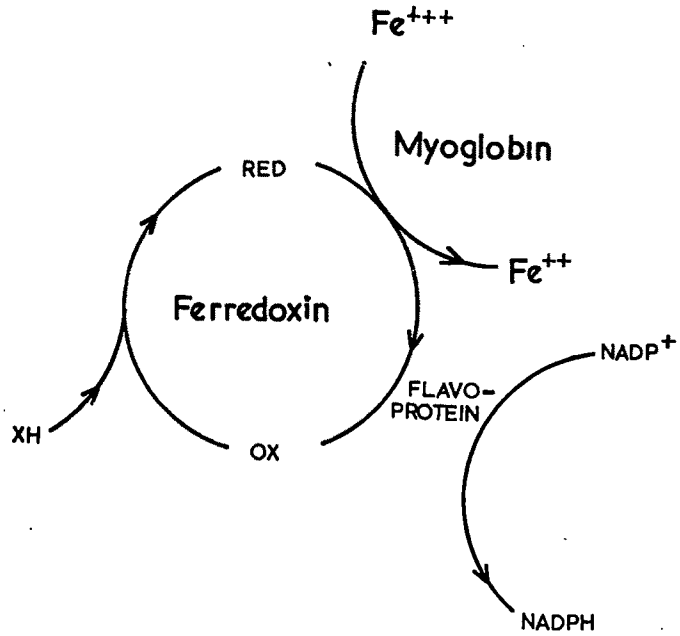


Fig. 3. Diagrammatic representation of the reduction of NADP and metmyoglobin by illuminated chloroplasts

which had been prepared and washed once in isotonic sucrose, and which were therefore substantially free from ferredoxin, were incubated for 5 min in the sucrose medium containing 5×10^{-4} M phenyl mercury acetate, (PMA). Excess PMA was removed from the chloroplasts by two further washes with the sucrose medium and their photochemical activities were compared with those of pea chloroplasts which had undergone a similar sequence of washes in sucrose alone. Table 2 shows that the ferredoxin-catalysed reduction of metmyoglobin was unaffected by the treatment with PMA whereas reduction of NADP underwent a 66 per cent inhibition, suggesting that the mercury was reacting at a site in the chloroplast essential for NADP but not for metmyoglobin reduction.

Although results similar to those in Table 2 have been obtained with chloroplasts prepared from spinach (*Spinacea oleracea*) these proved to be less satisfactory than pea chloroplasts in the type of experiment summarized in Table 1. It appeared that, whereas ferredoxin is readily released into the suspending medium from the chloroplasts of both genera, the flavoprotein is held more tenaciously by the spinach chloroplasts.

The extracted flavoprotein has the properties of a partially purified preparation of the $NADPH_2$-specific diaphorase extracted by Avron and Jagendorf[9] from spinach chloroplasts. In addition to the range of electron acceptors listed by these authors, it was found to mediate the reduction of pyocyanine and, in a nitrogen atmosphere, 1,1′-ethylene-2,2′-dipyridylium dibromide($E'_0 = -349$ mV). It has not yet been possible to demonstrate a net reduction of ferredoxin but, in the presence of this protein, or of pyocyanine, the flavoprotein actively mediates the reduction of metmyoglobin when $NADPH_2$ serves as hydrogen donor. Lazzarini and San Pietro[10] have reported a similar activity where a preparation of spinach leaf transhydrogenase, possessing diaphorase activity, was found to mediate the reduction of cytochrome *c* by $NADPH_2$ only in the presence of ferredoxin. Attempts to demonstrate transhydrogenase activity in the pea flavoprotein fraction, using the assay method of Keister and San Pietro[11], have not been successful.

The foregoing observations are consistent with the proposals of Tagawa and Arnon[6] for the electron transport pathway leading to the photochemical reduction of NADP. The position of metmyoglobin in the sequence of reductions is expressed diagrammatically in Fig. 3.

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CYTOCHEMICAL MEASUREMENT OF PROTEIN SULPHYDRYLS IN CELL LINES OF DIFFERENT RADIOSENSITIVITY

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THE method for histochemical detection of protein-bound sulphydryl groups by 2,2′-dihydroxy-6,6′-dinaphthyl disulphide (DDD) was introduced by Barrnett and Seligman in 1952 [1,2]. They showed that the disulphide of this compound reacts with protein sulphydryls at an alkaline *p*H. The chromogenic naphthol moiety, transferred to the protein, becomes part of the reaction product, and is readily converted to a coloured azo product by either tetrazotized diorthoanisidine[1,2] or diazotized 4-amino-3,6-dimethoxy-4′-nitroazobenzene (*Echtschwarzsalz*)[1]. The reactions, accomplished according to the procedure developed by Barrnett and Seligman, were shown[2,3] to be specific for protein-bound sulphydryls. Based on the results of a series of methodological experiments using *Echtschwarzsalz*, Bahr[4] concluded the method to be suitable for quantitative histochemical work at a cellular level.

Microspectrographic measurements of the amount of protein sulphydryls in individual cells, as revealed by Bahr's procedure, using DDD in combination with *Echtschwarzsalz*, were carried out in closely related tumour cell lines of different radiosensitivity. A hyperdiploid Ehrlich ascites tumour, denoted *ELD*, with a modal chromosome number 46 and an average DNA content of $11{\cdot}2 \times 10^{-9}$ mg/cell, and its three sub-lines were used. The sub-line *ELT* represents the hypertetraploid clonal population of an endoreduplicated cell, isolated from *ELD*, and has a modal chromosome number 92 and an average DNA content of $20{\cdot}5 \times 10^{-9}$ mg/cell. *SELD* clone *g* and clone *j* originated from single cells, which were isolated from *SELD*. *SELD* was derived from *ELD* by serial irradiation of the cells with sub-lethal X-ray doses during the course of 23 intraperitoneal passages. Both *SELD* clones had a modal chromosome number 45. The average DNA content was 11·32 (clone *g*) and 10·99 (clone *j*) $\times 10^{-9}$ mg/cell. The details of the isolation of the clones and several characteristics of the cell lines, including the growth characteristic, chromosomal variation, radio-

sensitivity, DNA and non-protein sulphydryl content, have been examined, and are described in previous reports[6–9].

Smears were prepared from freshly withdrawn ascites 7–12 days after the intraperitoneal inoculation of the respective tumour into hybrid mice, and fixed and stained according to the method already referred to[4].

The total extinction of individual cells was measured at 5800 Å in a rapid-scanning universal ultramicrospectrograph[5]. The instrument was fitted with Zeiss achromatic ultra-violet optics.

Fig. 1 shows the frequency distribution of the total extinction of individual cells at 5800 Å of four separate *ELD* populations. The term 'total extinction' refers to the total amount of absorbing material within the cell at the wave-length used. One hundred cells were measured in each of two populations (populations I and II), and 50 in each of the two other populations (III and IV). It can be noted that the values of all four populations are distributed within a closely similar range between 10 and 70, with means varying between 36·9 and 44·6. The distributions seem to approach the shape of normal distribution curves with the exception of population III, the curve of which shows an extra peak near the doubled mean value.

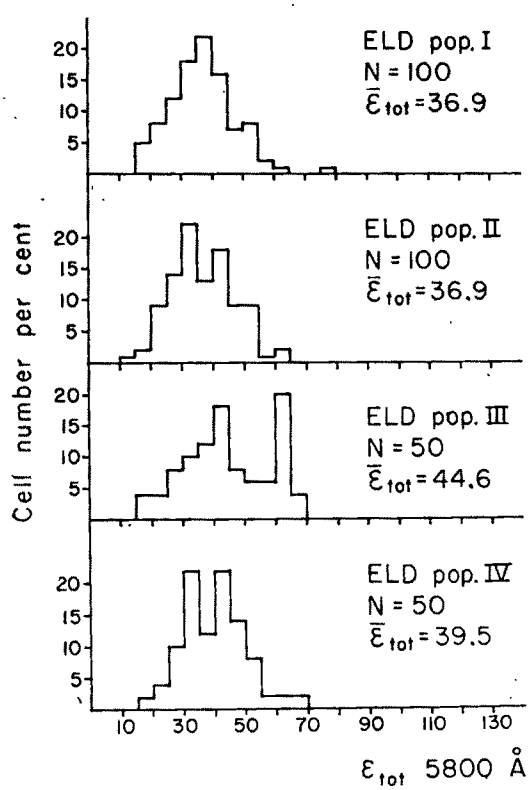


Fig. 1. Frequency distribution of the total extinction of individual cells at 5800 Å in DDD-*Echtschwarzsalz* stained specimens of four separate *ELD* populations obtained, fixed and stained at different times

Fig. 2 shows the frequency distribution of the total extinction of individual cells at 5800 Å of the different cell lines examined. The upper curve is the total of *ELD* populations I–IV already mentioned. The two middle curves show the distribution of single populations of the two *SELD* clones. The lower curve was obtained by measuring two separate *ELT* populations of 100 cells each. A comparison of the frequency diagrams indicates that the distribution pattern and the means of the *SELD* clones are closely similar to that of *ELD*. The *ELT* values, however, are distributed over a range which is roughly twice that of the other populations (25–150) and the mean value of 64·6 is 1·6 times larger than the other mean values.

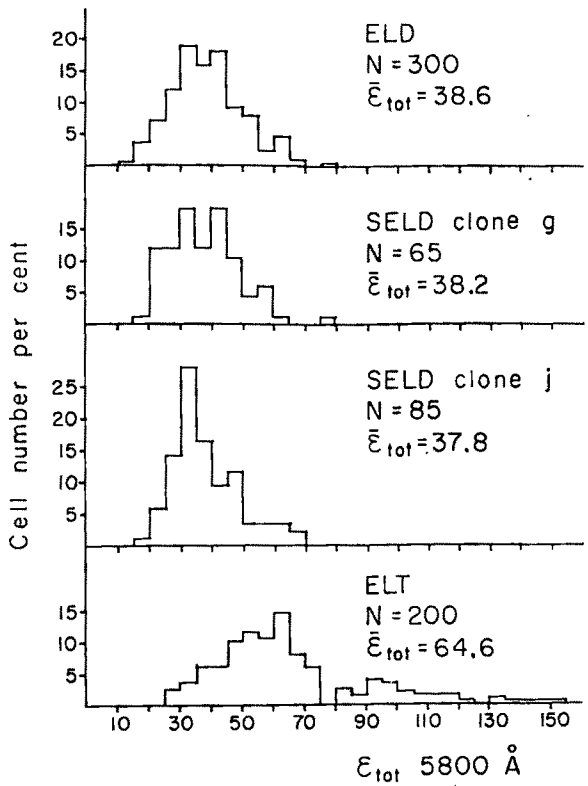


Fig. 2. Frequency distribution of the total extinction per cell in DDD-*Echtschwarzsalz* stained specimens of the pooled values of four separate *ELD* populations shown in Fig. 1, and of three *ELD* sub-lines: *SELD* clone *g*, *SELD* clone *j* and *ELT*. Each of the two *SELD* frequency distribution curves represents a single population, whereas the *ELT* curve represents a combination of two separate populations

The good reproducibility of the data of repeat experiments shown in Fig. 1 can be considered as indicating the suitability of the method for quantitative cytochemical investigations. The total extinction values indicate, furthermore, that the protein sulphydryl level is of a closely similar magnitude in the two *SELD* clones and the parental *ELD* line, but is nearly duplicated in the *ELT* sub-line with a doubled genome. There appears, thus, to exist a direct relationship between the protein sulphydryl level and the modal chromosome number or the average amount of DNA of the cell lines. The elevated protein sulphydryl level of *ELT* is in conformity with previous observations that the doubled genetic material in these cells is associated with a 1·6–2-fold increase of the amount of several enzymes, total lipid fatty acids, cholesterol and non-protein sulphydryls[6,7,9,10].

Since the average non-protein sulphydryl content per cell in *SELD* clones *g* and *j* is, as previously reported[9], 24 and 34 per cent larger, respectively, than in *ELD*, the results imply an increased non-protein sulphydryl/protein sulphydryl ratio in the *SELD* clones as compared with *ELD* or *ELT* (Table 1). Considering the radioprotective effect of low molecular thiol compounds[11], it is conceivable that the increased non-protein sulphydryl concentration per unit protein sulphydryls, as well as per

Table 1. Relationship between the Average Cellular Content of Non-protein-bound and Protein-bound Sulphydryl Groups; and the Average Cellular Content of Non-protein-bound Sulphydryl Groups and Deoxyribonucleic Acid (DNA) in Three Radio-resistant Sub-lines (*ELT*, *SELD* Clones *g* and *j*) of the *ELD* Ascites Tumour

ELD sub-lines	*ELT*	*SELD* clone *g*	*SELD* clone *j*
Non-protein sulphydryls / Protein sulphydryls	1·08	1·25	1·37
Non-protein sulphydryls / Deoxyribonucleic acid	0·99	1·22	1·37

(The figures indicate the values relative to *ELD*, and have been calculated from the data presented in Fig. 2 and in a previous publication (ref. 9).

unit DNA, may result in an increased protection of the function and structure of proteins and genetic material against radiation. This would explain the greater resistance of the serially irradiated *SELD* sub-lines relative to the original *ELD* line, to the growth inhibitory effect of anoxic X-irradiation[8]. Since, in contrast, the non-protein sulphydryl concentration per unit protein sulphydryls or DNA is of a closely similar magnitude in *ELD* and *ELT*, another explanation may be relevant for the relative radio-resistance of the latter line[7]. Considering the dominant role of damage to the genetic material in determining the cytocidal effect of radiation[10,12], the possible requirement to inactivate a larger number of homologous, sensitive sites in the exactly duplicated genome of *ELT* may account for radio-resistance in this case, as suggested previously[8].

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QUANTITATIVE RELATIONSHIPS IN TUMOURS INDUCED BY A VIRUS, AND BY OTHER AGENTS

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BRYAN'S investigations[1,2] on the Rous sarcoma (chicken tumour 1) provide quantitative data which permit the formulation of a model that may shed light on the mechanism of action of carcinogenic viruses. Bryan finds the relationship:

$$\frac{1}{t_d} = b \log M \qquad (1)$$

where M is the dose of virus, t_d the time from dosage to appearance of tumour, and b a constant. The time to appearance of tumour represents growth from an unmeasured initial volume, V_0, to an observed volume, V_d (approx. 1 mm^3); hence the reciprocal of t_d is a measure of the growth rate of the tumour. The production of virus follows a similar dose-response curve[3].

Growth of tumours depends basically on cell proliferation by duplication of cells. In normal tissues the increase in number of cells is nicely balanced by destruction of cells; but if proliferation exceeds destruction, tumours must form. Although Eversen and Iversen[4] suggest that tumours could result from decrease in destruction of cells without increase in rate of proliferation, this would not account for fast-growing tumours such as are dealt with here, and for various reasons is unlikely in most cases[5]. Thus, it seems safe to assume that in the course of growth of tumour the rate of proliferation is more rapid than it is in normal tissues. The information which directs the duplication of cells and their parts must be carried in templates which are themselves replicated in cell division; and information determining the basic rate of cell division must be one of the things carried, although extraneous factors may, of course, have transient effects on rate.

Viruses depend for their replication on the cell into which they are introduced. The virus provides the template according to which it is replicated; but the work of replication is paid for in terms of the metabolism of the cell, by means which the virus itself lacks. The greater the number of virus particles replicated by the cell, the greater the load on cell metabolism, which must have definite limits. Thus, the rate of replication of virus templates, r, may be expected to increase in inverse proportion to the number present, and since the number introduced is proportional to the dose M, we may write:

$$dr = \frac{dM}{M} \qquad (2)$$

and:

$$r = \log M + \log C \qquad (3)$$

The rate of cell proliferation R is proportional to the number of cells, and hence to the volume of the tumour. Thus:

$$R = \frac{dV}{V dt} \qquad (4)$$

Those viruses which produce tumours must increase the rate of cell proliferation, so we may assume that this is proportional to the number of particles in the cell. The increase in rate of cell proliferation entails further load on the machinery of replication, pushing it toward the limit of its capacity that is implied in expression (2). Thus we may substitute, introduce appropriate constants and integrate to obtain:

$$\log \frac{V}{V_0} = gt \log qM \qquad (5)$$

where g represents the assumed proportionality between rates of virus and cell proliferation, and q represents the proportionality between dose and number of virus particles.

Experimentally, comparison at time t_d is made at the constant volume V_d, and if we may assume that V_0 is also constant, as would be the case if the tumour grew, say, from a single cell, the left-hand term in expression (5) may be replaced by a constant k. Then substituting and rearranging:

$$\frac{1}{t_d} = \frac{g}{k} \log qM \qquad (6)$$

which corresponds to expression (1). The model appears compatible with the experimental findings.

It should be noted that, since M has been held constant during the integration to obtain expression (5), it is implied in the model that the number of virus particles is the same for each tumour cell as the number originally introduced into the parent cell. In other words, the cell appears to replicate the virus particles as though they were templates of the cell itself, for example, genes. Since the carcinogenic viruses increase the rate of proliferation of tumour cells, which might be a principal characteristic that distinguishes them from other viruses, it would seem that their proportional replication with each cell division would be essential. Bryan's finding[3] that the amount of extractable virus per unit of tumour volume is greater for fast-growing tumours, and that the relationship parallels quantitatively that described in expression

(1), adds further support for the model, since faster-growing tumours should be those which received the greater initial infection.

The model presents a very different picture from the run-away replication of viruses, such as might be expected for, say, bacteriophage, which would not at all satisfy the relationship indicated in (1); the replication of prophage might, however, follow this model. On the other hand, the latter relationship (1) is paralleled in the development of mouse encephalitis[6].

When the virus particles are introduced into the tissues we may expect many cells to be infected, but with different numbers of particles, according to chance distribution. Thus, a number of clones should be formed, the cells of which proliferate more rapidly than do the uninfected cells of normal tissue; but only the more rapidly growing of these clones would contribute importantly to the growth of the tumour. At high doses the growth of these tumours is so rapid, on the other hand, that it is unlikely they arise as a single clone. It might be expected that the number of cells infected would be proportional to the dose, that is, V_0 would be proportional to M; but from inspection of expressions (5) and (6) it is seen that this should not change the relationship essentially, and that there would still be agreement with the experimental findings[1]. This would not be true if tumour cells were recruited by further infection during tumour development; but it seems unlikely that this was an important factor in these experiments, since it would be difficult in that case to account for the quantitative relationships observed for both tumour development and extractable virus. Recent experiments of Bergs and Groupe[7] also indicate that tumour growth depends on the magnitude of the initial infection.

The induction of tumours by ultra-violet light, which requires many repeated doses, presents a very different quantitative picture describable by:

$$\log \frac{V}{V_0} = \frac{sUt^2}{i} \qquad (7)$$

where s is a constant, U the individual dose of ultra-violet light, and i the interval between doses. This and other relationships found for these data indicate that the rate of cell proliferation is increased at every repetition of dosage, and that we deal, here, with a cumulative process[5].

Dose-response relationships for induction of tumours by chemical agents appear to be quite different from those for ultra-violet light or virus carcinogenesis; they are approximately described in form corresponding to expressions (5) and (7) by:

$$\log \frac{V}{V_0} = wA^xt \qquad (8)$$

w and x having different values according to the carcinogen and probably the animal. Up to now no satisfactory model agreeing with these data has been formulated[8,9].

A consideration of quantitative relationships (5), (7) and (8) indicates that the mechanisms for the corresponding three types of carcinogenesis are very different; and that the rather current idea that all neoplasms may be attributed to virus is not tenable. There would seem, in this, support for the idea that different kinds of cancer may be looked on as different diseases[10,11]. One still searches, however, for a common denominator in terms of transfer of information in the proliferation of cancer cells, whether regarded as autonomous or not.

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MUSCLE BIOPSY IN MAN: AN INDEX OF CELLULAR POTASSIUM?

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CELLULAR deficiency of potassium arises clinically. It may do so acutely or insidiously and in either event compromises the patient's condition. Diagnosis may be difficult; certainly the serum level is unreliable as an index of cellular content.

Potassium in the extracellular phase of muscle usually makes up less than 2 per cent of the total. Even in the presence of a raised serum potassium, excess extracellular fluid and gross cellular depletion of potassium it is still less than 5 per cent of the total. The potassium content of a biopsy is therefore a good index of its cellular potassium, but does it also afford useful information about the cellular content of potassium throughout the rest of the muscle? In a wide range of circumstances, analytical results for a single slice of rat muscle afford reliable information about the muscle sampled, other muscles, and also other tissues[1]. Is this also true for man? Certainly it is often presumed to be the case.

Analysis yields values for composition in terms of amounts per weight of tissue sample analysed, and biopsies may well contain differing proportions of their various components—muscle cells, nerve cells, fibroblasts, connective tissue fibres, ground substance, blood vessels and blood cells, extracellular water and fat. Fortunately in an adult, cells other than muscle fibres make so insignificant a contribution to the cellular phase that this may be regarded as consisting wholly of muscle cells and any effect on its average composition in a biopsy which might result from differences in the ratio of muscle cells to fibroblasts and other cells can safely be ignored. Nevertheless, the potassium content of a biopsy will be affected by the relative proportions of cellular, extracellular phases, and fat, as well as by variation in average composition of muscle cells between different samples. We may further consider that a biopsy sample taken from a patient's muscle is, weight for weight, a very much smaller proportion of the total muscle sampled than in the rat. One might therefore expect, in man, greater variability between samples taken from the same muscle than in the rat. Our studies confirm this expectation. Despite this, however, we have concluded that, properly interpreted, a biopsy can appraise one of cellular potassium throughout the muscle sampled, and in other muscles too, albeit the latter with somewhat less precision. Evidence for these conclusions are summarized here.

Three widely separated locations were sampled from muscles exposed at the beginning of operations in a number of subjects. In most subjects only one muscle was sampled; in some 3, 4, or 5 muscles were sampled. Three slices, of about 20 mg weight, were cut from each sample and analysed for water, fat, and electrolytes using the method previously described[2]. The anterior muscles

of the thigh were sampled severally in three rats. Slices were also cut serially from the right anterior tibial muscle of one rat, and triplicate slices were cut from anterior tibial muscles in 28 male rats from 2–15 months old. All samples were analysed for water and electrolytes using this same method ; fat content was not determined.

From these results coefficients of variation (per cent) were derived to describe the variability in human material of potassium within one location of a muscle, that within a whole muscle, the overall variability within and between several muscles in an individual, and the variability obtaining when samples from the same muscle, but different individuals, are considered. For the rat, coefficients of variation were calculated[1] which define variability of potassium within one location of a muscle, that within a whole muscle, and that between several muscles.

None of the subjects, data for whom are considered here, had overt abnormalities of water and electrolyte metabolism; none had neuro-muscular disorders.

Table 1 presents the coefficients of variation of a single estimate of muscle potassium obtaining when it is used as an index of composition in the immediate vicinity of the sample, throughout the whole of the muscle sampled, of other muscles in the same subject, or of the composition to be expected for the particular muscle sampled but in other similar subjects. For comparison similar information is presented in Table 1 for rat muscle.

Table 1. VARIABILITY IN POTASSIUM CONTENT OF SKELETAL MUSCLE IN MAN AND, FOR COMPARISON, RAT

Variability		Man	Rat
One individual	One location one muscle	5·8	2·6
	several locations one muscle	8·1	3·4*
	several locations several muscles	13·1	4·2*
Several individuals	Several locations one muscle	12·0	5·0

* Reference no. 1.

Values are coefficients of variation (per cent) for a single estimation. They were calculated when potassium content was expressed as m.equiv./kg wet wt. of muscle.

Differences in the proportion of fat in samples will promote variability in their potassium content. It has been suggested that this is the major source of differences in potassium content of muscle[3]; but differences in the fat content of samples did not contribute much to the variability of potassium occurring in man (Fig. 1). The samples of human muscles were all taken from inside muscles and did not therefore include fat on their outer surfaces; unlike the dog muscles, analysis of which underlay the emphasis on differences in fat as the major source of differences in potassium content[4]. Nevertheless, it would be a mistake to ignore differences in fat content of samples taken as ours were from within muscle, as a source of variation in their potassium content, for we gain in understanding if potassium content is related to the content of tissue solids, and, as will be apparent from Fig. 4 evaluation, when so related, is blunter if fat content is not known. If we examine the findings in individual subjects, it is of course necessary to know the fat content of samples, for, even when all fat is apparently removed before analysis, considerable amounts may remain. In our hands as much as 16 per cent by wet weight has occasionally persisted. It is therefore gratifying that defatting does not increase errors (Fig. 1).

Differences in water content of samples would promote variation in the amount of potassium associated with a given wet weight of muscle, say 1 kg, even when the extra water is unaccompanied by potassium; they would, however, leave unaffected the amount of potassium associated with 1 kg of fat-free tissue solids. Referring potassium content to 1 kg fat-free tissue solids did not appreciably lessen variability in potassium (Fig. 2), so that this source, too, does not make much contribution to differences in potassium content of samples from control subjects. This is perhaps not surprising since, in view of the osmotic

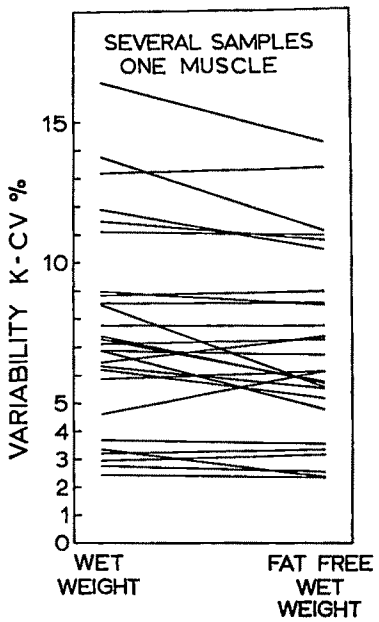


Fig. 1. Variability in the potassium content of different samples taken from the same muscle, showing the relatively small contribution made by differences in their fat content. Variability is presented as coefficients of variation when potassium content is given as m.equiv./kg of wet tissue and as m.equiv./kg of fat-free wet tissue. Each coefficient of variation is based on analyses of 6 (5 instances) or 9 (22 instances) samples from a given muscle in one subject

equilibrium across cell membranes, differences in water content of samples are unlikely, when cellular in origin, to arise in the absence of change in cell solutes—including potassium.

Neither differences in fat nor water content of samples could be responsible for the striking negative correlation encountered between the sodium and potassium contents of samples of human muscle. This is present when samples are taken from within the one muscle in the same, or different, individuals (Fig. 3); when they are from several muscles in the same individual (Fig. 3), or from different individuals (Fig. 3). The relationship is present, moreover, when potassium and sodium contents of samples are expressed as m.equiv./l. tissue solids, m.equiv./kg wet, or fat-free wet-wt., and also when expressed as m.equiv./kg fat-free dry wt. of tissue. It is absent when data are expressed as m.equiv./kg dry wt. of tissue (Fig. 4). In passing it may be mentioned that we did not find a negative correlation between the con-

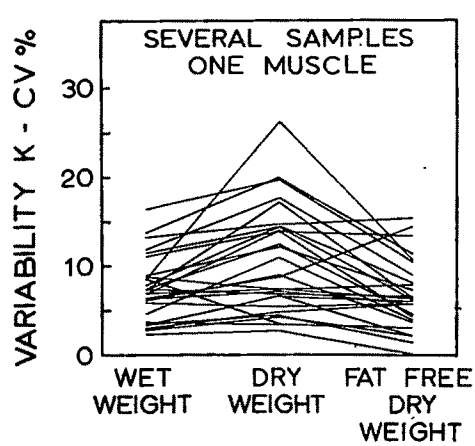


Fig. 2. Variability in the potassium content of different samples taken from the same muscle, showing the relatively small joint contribution made by differences in fat and water contents. Variability is presented as coefficients of variation when potassium content is given as m.equiv./kg of wet tissue, per kg dry wt. of tissue, and per kg fat-free dry wt. of tissue. Material presented in Fig. 1 forms the basis of this figure also. Each coefficient of variation is based on analyses of 6 (5 instances) or 9 (22 instances) samples from a given muscle in one subject

tents of potassium and sodium in samples of rat skeletal muscle (Fig. 5).

Cellular fluid is rich in potassium with but little sodium; the opposite is true for extracellular fluid. It follows that a sample with relatively more extracellular fluid will contain more sodium and less potassium than a sample with relatively less extracellular fluid, and vice versa. Differences in the amount of extracellular fluid associated with a given wet weight of tissue would arise were the average diameter of muscle fibres in samples to differ. Fourman has pointed out[5,6] that thin fibres will have a relatively greater surface film of extracellular fluid than fat ones. Variation in average diameter of muscle fibres in samples would thus occasion a negative correlation between sample contents of sodium and potassium when quoted as m.equiv./kg wet wt. of tissue, per kg fat-free wet wt., or per litre of tissue water. Unless thin fibres contain proportionately more solids than fat ones, or unless samples with a higher proportion of thin fibres also contain relatively more connective tissue, whence extracellular solids, contents of sodium and potassium will not, however, exhibit a negative correlation when 1 kg fat-free tissue solids is the reference base used. Its existence (Fig. 4) could thus be accounted for. Our findings (Figs. 3 and 4) could also be explained if the average composition of cellular fluid varied from sample to sample if it be

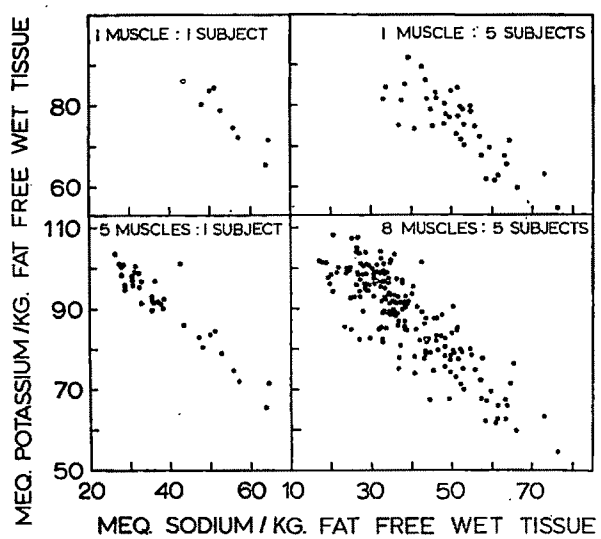


Fig. 3. The sodium and potassium contents of samples of human muscle are presented in this figure as m.equiv./kg fat-free wet wt. of tissue. A striking negative correlation exists between contents of sodium and potassium; samples with a lot of potassium have little sodium, and vice versa. This relationship obtains whether samples are all taken from the same or different muscles in an individual, whether from the same muscle but different subjects, or indeed from a number of different muscles as well as different subjects. This relationship is also present when results are expressed as m.equiv./kg wet wt. of tissue, m.equiv./l. of tissue water, or m.equiv./kg fat-free dry wt. of tissue

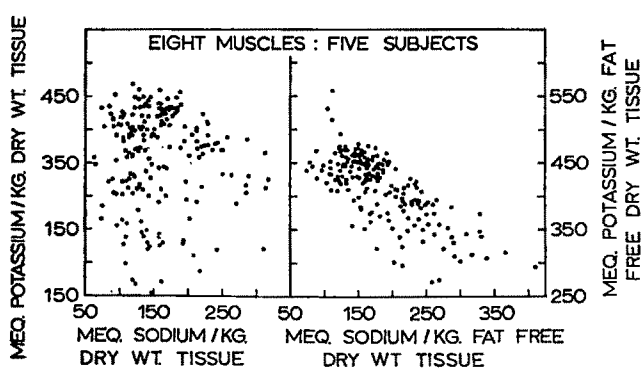


Fig. 4. Sodium and potassium contents of samples of muscle from eight different muscles, four or five of which were sampled in each of five individuals. Contents were expressed both as m.equiv./kg dry wt. of tissue and per kg fat-free dry wt. of tissue

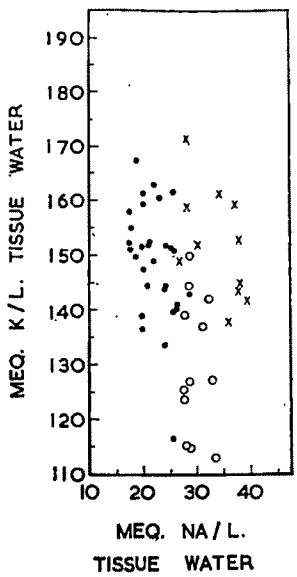


Fig. 5. The sodium and potassium contents of samples of the anterior muscles of the thigh in three healthy rats are shown in this diagram. They are presented as m.equiv./l. of tissue water as fat was not determined; essentially the same findings are noted when presented as m.equiv. kg wet wt. of tissue. The relationship seen in man between sodium and potassium contents is absent

allowed that cells containing more sodium contain less potassium and vice versa. For reasons submitted in outline elsewhere[7] we believe this to be the more important source of differences met in the potassium content of samples.

Whatever the cause of the very striking negative correlation between the potassium and sodium contents of human muscle, but for its existence one would be forced to the reluctant conclusion that biopsies afford an imprecise index of composition within muscles sampled. As it is, the coefficient of variation of a single potassium estimate, used as such an index, reduces from 8 to 5 per cent, when the sodium content is also considered. When account is also taken of the fat content it reduces still further to 4 per cent (Table 2). When used as an index of composition in other muscles of the body, the corresponding reductions in the coefficient of variation of a single potassium estimate are 13–8·5 per cent when account is taken of sodium, to 5·7 per cent when fat is also considered (Table 2). The possibility that greater variability in composition of muscle exists in patients with pronounced electrolytic or other systemic disturbances is being investigated.

Table 2. Variability in Potassium Content of Skeletal Muscle in Man; Coefficients of Variation (per cent) of an Individual Potassium Estimation, when Account is also taken of the Sodium Content of the Sample

		Reference base: 1 kg wet wt.	Reference base: 1 kg fat-free wet wt.
One individual	Several locations one muscle	5·0	3·9
	Several locations several muscles	8·5	5·7
Several individuals	Several locations one muscle	8·1	6·1

Data from which these values were calculated were expressed either as m.equiv. potassium per kg wet wt., or per kg fat-free wet wt. of tissue.

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ISOLATION AND CHARACTERIZATION OF MURAMIC ACID FROM TWO SPIROCHÆTES: *BORRELIA DUTTONI* AND *LEPTOSPIRA BIFLEXA*

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THERE is considerable confusion about the nomenclature and taxonomic position of helical, motile micro-organisms. Some are definitely classified with the bacteria (Spirillaceae)[1], others (Spirochaetales), with either the bacteria[1] or the Protozoa[2], and it has been suggested that certain members of the Spirochaetales should be classified with the Cyanophyceae[3].

One biochemical character which has proved useful in bacterial classification is the presence of cell wall mucopeptides, which on hydrolysis give a small range of sugars and amino-acids together with the amino-sugars glucosamine, galactosamine and muramic acid[4]. Apart from the true bacteria, muramic acid has been found only in the Actinomycetales[5] Cyanophyceae[6] and Rickettsiae[7]. The purpose of this article is to report that muramic acid has been isolated and characterized from two members of the Spirochaetales; *Borrelia duttoni*, the spirochæte of relapsing fever, and *Leptospira biflexa*, a free-living spirochæte found in natural waters.

B. duttoni is an old laboratory strain, maintained in young rats by syringe passage. Parasites were collected by bleeding the rats at the height of the first parasitæmia and were then separated from the blood by differential centrifugation. *L. biflexa* was cultured in Korthof's medium with added hæmoglobin, yeast extract and rabbit serum, and concentrated by centrifugation. The organisms were thoroughly washed and freeze-dried, and after extraction of lipids were treated by the method of Cummins and Harris[8] for isolation of mucopeptide from cell walls. Ribonuclease digestion was replaced by extraction of ribonucleic acid with 0·3 N potassium hydroxide for 5 h at 37° C, and deoxyribonucleic acid was extracted with 5 per cent trichloroacetic acid for 30 min at 70° C. The dried residue was hydrolysed in a sealed ampoule with 4 N hydrochloric acid for 4 h at 105° C, and dried *in vacuo* over potassium hydroxide. Muramic acid was identified in this hydrolysate by two-dimensional paper chromatography using *n*-butanol/pyridine/water (6:4:3) and *n*-butanol/acetic acid/water (63:10:27) as solvents, and spraying with ninhydrin reagent. Muramic acid was isolated from the remainder of the hydrolysate by concentration of amino-sugars on a 'Zeokarb 225 (H^+)' column, followed by further separation of muramic acid from glucosamine on a mixed 'Norit'/'Celite' column[9].

Part of the material obtained in this way was chromatographed on paper in the *n*-butanol/acetic acid/water system, together with standard preparations of glucosamine, galactosamine and synthetic muramic acid. The spirochæte material showed a spot which had the same mobility as muramic acid, and which gave positive reactions with ninhydrin and the Elson–Morgan[10] reagents. The remainder of the column eluate was finally purified by preparative paper chromatography in the same solvent system, which removed other faint ninhydrin-positive spots having greater mobility than muramic acid. The purified material eluted from the paper gave a chromogen in the Rondle and Morgan reaction[11] which had all the properties of the muramic acid chromogen[12], that is, the absorption maximum, if read immediately, was at 510 mμ and after 24 h at 505 mμ, with an approximate two-fold increase in intensity (Fig. 1). The substituted pentose obtained by treatment of the sample with ninhydrin[13] was identical in both colour and mobility with that obtained from synthetic muramic acid, when subjected to paper chromatography in *n*-butanol/pyridine/water, and sprayed with anisidine hydrochloride reagent[14].

This demonstration of muramic acid in the Spirochaetales suggests that they have a cell wall similar in structure to that of the bacteria, and provides support for their classification in this group, and not in the Protozoa. Other characters, which also point to their bacterial nature, are their general morphology, with a distinct cell wall and capsule[15], resistance to nuclear staining[16] and sensitivity to antibiotics[17] and to lysozyme[18]; these last two characters are presumed to be a consequence of the muramic acid content[4,19].

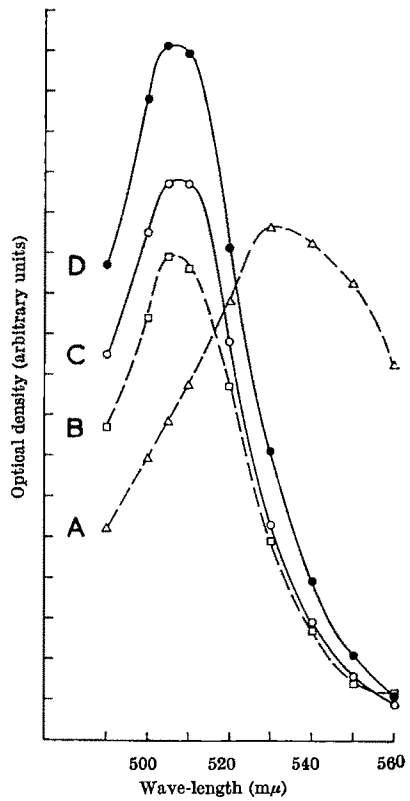


Fig. 1. Absorption spectra of the chromogens obtained in the reactions for amino-sugars described by Rondle and Morgan[11]. *A*, 20 μg of glucosamine, readings taken immediately; *B*, 20 μg of synthetic muramic acid, readings taken after 24 h; *C*, material from *B. duttoni*, readings taken after 24 h; *D*, material from *L. biflexa*, readings taken after 24 h

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APPARENT RELATIVE MOVEMENT OF 'UNSHARP' AND 'SHARP' VISUAL PATTERNS

I HAVE described[1] an after-image method demonstrating several features of the involuntary micro-ocular movements during the fixation of a test point (Fig. 1). If the eyes look steadily for some tens of seconds at the black point, and be then directed at the white point, the dark negative after-image of the white line pattern will be seen moving about irregularly.

In order to give inexperienced observers some idea of the after-image pattern to be paid attention to, a figure was constructed representing the sharp pattern together with the unsharp 'after-image' (Fig. 2). Surprisingly, the unsharp pattern appeared to move about irregularly, whereas the sharp pattern remained at rest. The sensation is evoked immediately on glancing over the pattern without previous fixation or a latency time permitting the production of clear after-images. The fact that the sharp image is white and the unsharp dark on a dark background is not essential because the illusion of relative movement is the same when, for example, both are dark on a white background. It seems clear that the apparent movements of the unsharp pattern are in some way the result of the micro-ocular movements, since these apparent movements are not unlike those of the after-image referred to here.

There are two questions to be answered. First, what information evokes the illusion of movement of the two patterns relatively to each other? Secondly, what determines which of the two patterns are seen moving, and which stationary?

It would seem that the phenomenon reported here is in some way related to that described by MacKay[2]: in a room lit stroboscopically at rates of 5–6/sec, self-luminous objects such as the glowing cathodes of thermionic valves appeared to move on pressing the eyeball with the finger, whereas the room remained relatively stable. When the retinal images of the sharp–unsharp pattern (Fig. 2) are displaced by a similar procedure the unsharp pattern seems to move with a larger amplitude. When the pattern is moved with respect to the observer—either from the left to the right, or up and down, or to and fro—the movements of the unsharp pattern are overestimated with respect to those of the sharp pattern. When the eye is moved smoothly over the pattern, for example, by following a moving fixation target (point of pencil), the sharp pattern seems to move with the pencil or the unsharp pattern seems to move in the opposite direction.

MacKay concluded from his observations that the magnitude of perceived movement is not primarily determined by the magnitude of displacement of the retinal image but by the amount of information supplied by the retinal image.

There is, indeed, some suggestive evidence from microelectrode studies of the vertebrate visual pathway—from the retina up to striate cortex—indicating that information about movement is not necessarily derived from information about locality. It seems, on the contrary, that the signals coming from the retinal receptor cells are combined and analysed by interconnexions between cells in the visual pathway in such a manner that the higher the centre the more quantitatively and qualitatively detached aspects of the original stimulus, for example, movement, becomes available[3–11].

The observations reported in this communication seem capable of interpretation along similar lines of reasoning. The different sensations of movement evoked by identical movements of sharp and unsharp retinal boundaries could arise from the larger number of movement receptor units being stimulated per unit of time and per unit of length in the stimulus gradient produced by the unsharp boundary as compared with the sharp boundary. Moreover, a given movement receptor unit might be stimulated during a longer period during the passage of the gradient produced by the unsharp boundary. The resulting differences in amount of movement information would evoke the sensation of relative movement of the sharp and unsharp boundary.

In general, the retinal pattern producing the greatest amount of movement information seems to evoke the strongest sensation of movement. In the ultimate decision about movement or rest of the objects belonging to retinal stimulus patterns two other sources of information are the motor innervation pattern of the extraocular muscles ('outflow-theory' of eye position knowledge) and the extension of the retinal stimulus patterns within the total visual field. Since there is ample evidence that proprioception in the extraocular muscles ('inflow-theory') gives no appreciable information concerning the position of the eyes[12–14], this source of information is left out of consideration.

When saccadic eye movements (visual grasp reflex) or smooth tracking movements are performed, the movements of the retina are reckoned with to some degree, with the motor innervation pattern of the extraocular muscles as information source, in deciding whether moving or stationary retinal images belong to stationary or moving objects. This results, for example, in the sensation of movement of an after-image object as soon as a voluntary eye movement is made (reafference principle[15]; cf. refs. 16 and 17). (In these considerations the sensational object is distinguished by the term 'image object' or 'after-image object' from its retinal image or after image.) Apart from, and sometimes in contradiction to, this information, there is the tendency to decide that smaller retinal patterns belong to moving objects, and that larger patterns belong to stable objects. This leads to visual illusions like the Moon 'moving' behind the 'stationary' clouds.

It is not clear to what degree the changes in the motor innervation pattern underlying the micro-ocular movements are used as a source of information in the interpretation of retinal movement information. Anyhow, an after-image, for example, of Fig. 1, does not move in a drift- and flick-like manner in complete darkness. Accordingly, the relative extension of the retinal stimulus patterns proves to have a strong effect on the sensations produced by the micro-ocular movements. A small, stable

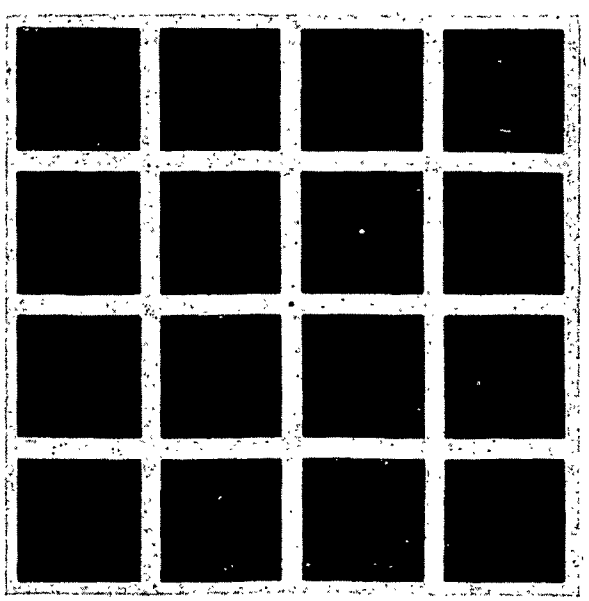


Fig. 1

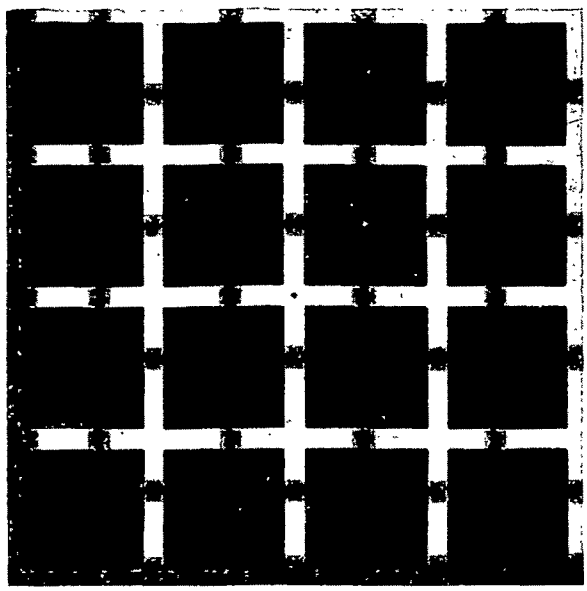


Fig. 2

light source fixed in complete darkness after the production (by electronic flash) of an after-image occupying a large part of the visual field, for example, of a pattern like Fig. 1, is generally reported to be in constant vibratory motion while the after-image object seems to be at rest. Similarly, the relative extension of the visual patterns can easily reverse the sensation of movement based on retinal information: when the unsharp part of a pattern like that in Fig. 2 is extensive and the sharp part small, for example, only one crossing-point, observers generally report that the sharp crossing-point vibrates with respect to the stationary unsharp pattern.

It seems that this illusion has something to do with Exner's *Punktschwanken* (point wobbling)[18] or that it is even identical with it. Exner distinguished between the continuous and smooth apparent movement of a spot of light in complete darkness, first described by Charpentier[19] and termed *autokinetische Empfindung* (autokinesia) by Aubert[20], and the apparent wobbling movements of a spot of light within a larger surrounding grey spot viewed in dim light. Hörwall[21] criticized Exner's idea that point wobbling would underlie the autokinetic illusion. Hörwall emphasized that point wobbling is only observed as an apparent irregular movement of small amplitude performed by the fixation point with respect to vague contours in its near environment, and that sharp contours inhibit the illusion. He ascribed the point wobbling illusion to the differences in latency time in localizing the retinal images of the vague contours and the sharp fixation point, both moving identically over the retina as a result of the involuntary movements of the eyes during fixation. It seems reasonable to suppose that differences in retinal movement information from the vague contour and the sharp fixation point, together with the different extension of both patterns, can account for Exner's *Punktschwanken*.

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The essence of Dr. Verheijen's pleasing demonstration is that a blurred and a sharp contour, moving objectively with the same velocity over the retina, do not appear to move together. This could be caused by either: (*a*) a difference in the latency, or (*b*) a difference in the magnitude of the respective responses evoked in the visual system (or, of course, by both).

As a test of the first hypothesis, we have subjected sharp and blurred contours, successively and simultaneously, to oscillatory motion by reflexion in two mechanically rocked mirrors. If a signalling time-lag alone were responsible for the illusion, the estimated amplitude of movement should be the same for either image alone. In fact, it appears greater for the blurred contour, particularly if no stationary objects are present in the field to give cues to position, as distinct from amplitude.

By adjusting the relative phase of the two mirrors, it is possible to bring the blurred and sharp images approximately into step. The amplitude of 'motion' of the blurred image can then be seen to be the greater. If a signalling time-lag were responsible for the phase-difference, the phase setting required should vary with frequency of rocking. In fact, however, the 'phase lead' of the blurred over the sharp image is found to be practically independent of frequency.

We are thus driven to hypothesis (*b*), favoured by Dr. Verheijen, that more 'velocity information' is generated by the blurred contour; but since mere blurring, as distinct from mottling, does not increase the number of contours in motion, and since it diminishes the gradient of intensity, it is not easy to see why a greater number of 'movement detector units' should be stimulated.

A simple but striking experiment suggests that the spurious information here is generated in the detectors of intensity, rather than of motion. If the image of an 'optical wedge', projected on a screen, is displaced in the darker or lighter direction, the graded region appears lighter or darker respectively, so that the apparent displacement greatly exceeds the actual. Conversely, an increase or decrease in overall illumination gives rise to an impression of displacement in the darker or lighter direction, respectively.

Both phenomena invite a simple explanation in terms of local adaptation to intensity. If the graded image is displaced in the darker or lighter direction, then each intensity-detector finds itself under- or over-adapted, respectively, and the effect of this maladaptation is to enhance the change in response (by 'successive contrast')—as if the actual image-displacement had been greater. The effect is to make the perceived image 'lead' the actual (in space). Conversely, an overall change in illumination causes changes in response and in adaptation-level locally indistinguishable from those due to displacement of the graded image.

Our suggestion would therefore be that it is not the greater number of 'movement detectors' stimulated, but the spurious enhancement of the cues supplied to the movement detecting system, that accounts for the greater mobility of a blurred image.

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LETTERS TO THE EDITOR

PHYSICS

Continued X-ray Microanalysis of Radioactive Fall-out Particles during the Year 1962

Since the beginning of atom bomb tests, the physical and chemical properties as well as the biological effects of radioactive fall-out particles have been the subject of numerous investigations[1–8]. Discussions on the mechanism of formation of these particles and on the question of their effects on the human organism after inhalation or ingestion[1,2,4], which are highly dependent on their solubility, require a knowledge of their composition[1,4,9]. Comparison of particle diameters, obtained by electron microscopy, with the content of radioactive nuclides, determined by radiochemical methods and γ-spectrometry, shows that the particles contain more inactive material than is produced by the decay of the radioactive fission products and the induced radioactive elements[2,9]. In the case of atmospheric explosions, this additional inactive component comes from unfissionable materials used in building the atomic device, and in the case of near-surface explosions also materials from the ground can be involved[1].

During an investigation into the problem of radioactive fall-out particles in the winter of 1961–62[2], we tried to obtain information on the elemental composition of a radioactive particle[9] using an X-ray microanalytical method with the aid of the electron microscope[10]. This method consists essentially of focusing the electron beam of the microscope (Elmiskop I, Siemens) on the specimen, and using the characteristic X-ray spectrum to give the elemental composition. The X-ray spectrum, which is due mainly to the $K\alpha_1$-quantum energy, is absorbed in a proportional counter and then conducted to an impulse spectroscope where it appears as a pulse-height spectrum. In the spectra taken at that time, peaks appeared at 6·4 keV and 1·5 keV. Whereas the first peak belongs to the element iron ($K\alpha_1$-quantum energy 6·4 keV), the identification of the second peak was difficult as the $K\alpha_1$ energies of the elements aluminium (1·48 keV) and silicon (1·74 keV) are only slightly different. Afterwards it was possible to attribute this peak to the element aluminium having compared them with a calibration peak obtained by focusing the electron beam on an aluminium specimen.

In view of this result, we continued to use X-ray microanalysis in our work on radioactive particles collected in 1962[11].

Ten radioactive fall-out particles were, according to a method already published[2], prepared on electron microscope grids, located by autoradiography and later analysed by electron microscopy. With a mean maximum diameter of 4·4μ, the particles had β-activities of $3 \times 10^{-10} - 1 \times 10^{-8}$ curies (Table 1). To decrease the amount of scattered radiation in the microanalysis of the particles, a beryllium window was interposed. For all nine particles analysed there were peaks at 6·4 keV and the corresponding escape peaks at 3·5 keV representing the element iron (Fig. 1). In a second series of experiments the beryllium window was replaced by a thin mylar foil in order to allow analysis of the low-energy part of the spectrum. With this arrangement, the amount of scattering is higher and resolution of the peaks is correspondingly more difficult. The analysis of two of the seven particles examined indicated the existence of the element aluminium with a high degree of probability; the identification of the peaks at 1·5 keV was made possible by comparison with an aluminium calibration peak (Fig. 2). Four particles probably also contained aluminium. This result, however, could not be verified beyond doubt because of the high

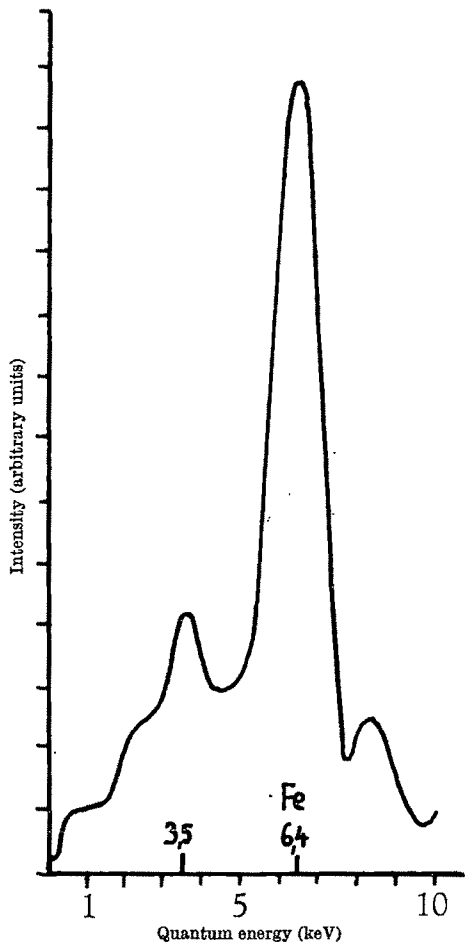


Fig. 1. X-ray microanalysis of a radioactive fall-out particle. Photometrical curve of the originally obtained pulse height spectrum. Beam voltage, 40 kV. Beryllium window. Iron ($K\alpha_1$ quantum energy, 6·4 keV.; escape peak at 3·5 keV)

Table 1. Characteristics of 11 Radioactive Fall-out Particles examined by an X-ray Microanalytical Method with the Aid of the Electron Microscope

Particle No.	1	2	3	4	5	6	7	8	9	10	11
Date of collection	31/10/61	4/8/62	4/8/62	31/8/62	17/9/62	7/10/62	8/10/62	18/10/62	18/10/62	17/11/62	19/11/62
β-activity (c.)	3×10^{-10}	$3{\cdot}4 \times 10^{-10}$	$4{\cdot}4 \times 10^{-10}$	$1{\cdot}1 \times 10^{-9}$	$1{\cdot}0 \times 10^{-8}$	$1{\cdot}3 \times 10^{-9}$	$1{\cdot}5 \times 10^{-9}$	$1{\cdot}6 \times 10^{-9}$	$1{\cdot}0 \times 10^{-9}$	$7{\cdot}8 \times 10^{-10}$	$1{\cdot}7 \times 10^{-9}$
Largest diameter (μ)	4·4	3·0	3·1	3·9	10·0	3·2	1·9	5·4	5·2	3·4	4·4
Al ($K = 1{\cdot}48$ keV)	+	not analysed	+	(+)	+	(+)	(+)	not analysed	(+)	not analysed	(−)
Fe ($K = 6{\cdot}4$ keV)	+	+	not analysed	+	+	+	+	+	+	+	+

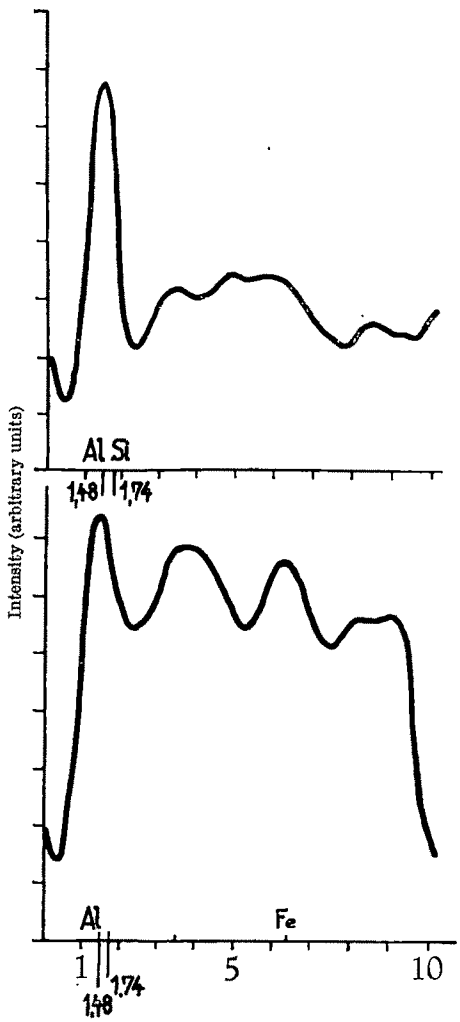


Fig. 2. X-ray microanalysis of a radioactive fall-out particle. Photometrical curves of the originally obtained pulse height spectra. Beam voltage, 40 kV. Thin mylar foil. Above, aluminium calibration peak; below, radioactive particle. Aluminium ($K\alpha_1$ quantum energy, 1·48 keV). Also discernible peaks belonging to iron at 6·4 keV and 3·5 keV

amount of scattered radiation. One particle obviously contained no aluminium. The determination of the ratio aluminium: iron in the particles contains a relatively high error; it may, however, be said that the content of both elements seemed to be of the same order of magnitude.

The results obtained permit the assumption that the elements iron and aluminium frequently appear as inactive components of radioactive fall-out particles. Both elements probably come from materials used in the construction of the atomic weapons tested[8]. Thus, for example, radioactive fall-out particles consisting mainly of Fe_3O_4 have been described, in which the characteristic fission products are homogeneously distributed[8]. The outlines of the particles which we determined by electron microscopy fit the theoretically spherical shape of the particles of Fe_3O_4 mentioned relatively well; on the other hand, we also observed crystalloid shapes in many cases[2]. From the presence of iron in the radioactive particles, and thus also in the region of the fireball, it can also be deduced that during the explosion the iron must be activated to a certain degree. In fact the presence of iron-59 has recently been demonstrated in a preparation of air filter samples, using γ-spectrometry[12]. The spectra were recorded with the permission of Dr. H. Neff and with the help of Miss Schmiedeskamp and Mr. Hellgart at the Siemens Werk für Messtechnik, Karlsruhe.

This publication is dedicated to Prof. Boris Rajewsky on the occasion of his seventieth birthday on July 19.

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Chemically Reduced Nickel Substrates for the Development of Uniaxial Anisotropy in Electro-deposited Thin Films

THE substrate surface characteristics are known to affect the hysteresis properties, the reproducibility, and the development of a uniaxial anisotropy in vacuum or electro-deposited permalloy thin (< 2000 Å) films. The primary requisites of a satisfactory substrate for development of uniaxial anisotropy in magnetic thin films are: (1) extreme smoothness; and (2) an amorphous or microcrystalline structure. Electroless nickel (chemical deposition) has been reported to be amorphous[1] as well as microcrystalline[2] in character. Consequently, chemically (electroless) deposited films of nickel were evaluated as substrates for electro-deposited permalloy films. The electroless nickel was deposited in a manner suggested by Brenner[3] on to a polyester film base.

The iron–nickel permalloy deposits were obtained from an electrolyte similar to that given by Wolf[4]. The current density was 6 m.amp/cm². The electrolyte was not agitated and was used at room temperature (27° C). All films were deposited in the presence of a magnetic field of 100 œrsteds. The composition of all deposits was approximately 81 per cent nickel–19 per cent iron. The films lacked any appreciable strain sensitivity in the easy and hard directions. The cathode diameter was 1·37 cm and the thickness of the deposits was varied from 500 to 2500 Å. This large ratio of spot diameter to thickness minimizes the magnitude of the demagnetizing fields in the plane of the film. The thickness was determined from the known geometry and the theoretical flux density.

The hysteresis parameters measured with a 60-cycle hysteresis loop tracer were: (1) the coercive force in the easy direction, H_c; (2) the anisotropy field, H_k; and (3) the maximum dispersion, αm, and quartile dispersion, αq. The dispersion was measured in the manner suggested by Crowther[5]. The hysteresis properties were examined as a function of substrate thickness and deposit thickness.

The maximum dispersion of the easy axis was found to be dependent on the thickness of the electroless nickel substrate and varied from 4° to 20°. This variation in dispersion with substrate thickness could be attributed to a substrate crystal growth irregularity or roughness. These irregularities in the substrate are replicated by the permalloy electro-deposit, resulting in a protruding grain growth and subsequent inhomogeneous composition of the permalloy deposit due to variation in the local cathode potential. The deposit non-uniformity has been characterized by determining the protruding grain-density per unit area from electron micrograph replicas of the surface at a magnification of 17,000. The correlation of maximum dispersion with deposit grain-density has been reported[6].

The results reported here show that an optimum thickness (time-temperature) of the electroless nickel substrate exists for minimum dispersion of the easy axis. Typical hysteresis loops of several permalloy deposits of various thicknesses on the optimum substrate thickness are shown in Fig. 1. The maximum dispersion was 5° and the quartile dispersion for all deposits was less than 1·5°. The hysteresis characteristics in the hard direction are shown

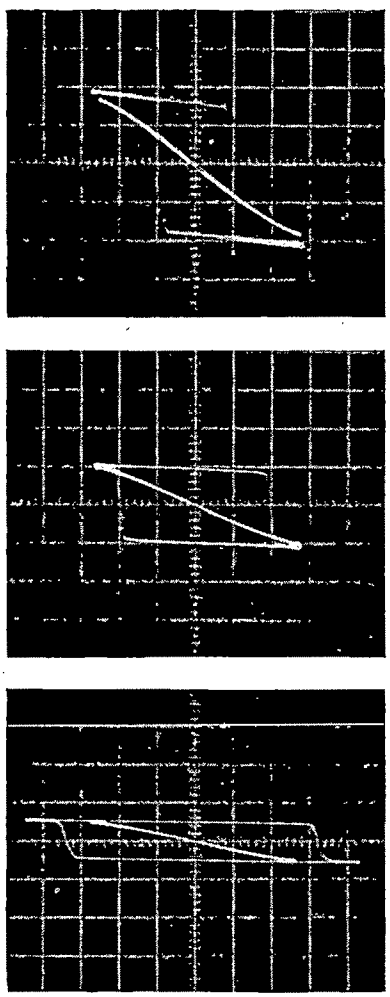


Fig. 1. Typical hysteresis characteristics of electro-deposited thin films on electroless nickel substrates *A*, 2000 Å, H_c = 0·9 œ, H_k = 2·8 œ; *B*, 1000 Å, H_c = 2·0 œ, H_k = 2·8 œ; *C* 500 Å, H_c = 3·3 œ, H_k = 3·0 œ. Maximum dispersion (α_{max}), 5°

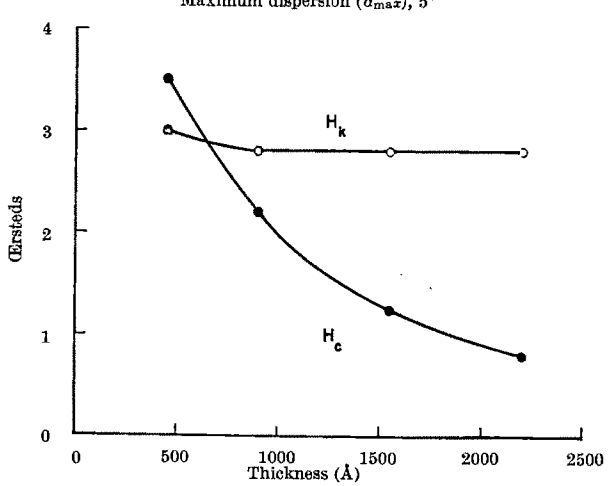


Fig. 2. Coercive force and anisotropy field of electro-deposited films as a function of thickness

to be nearly linear, indicating a high degree of coherent rotation of the magnetization or uniaxial anisotropy.

The coercive force of the deposits was inversely proportional to the thickness and varied from 0·9 œrsted at 2000 Å to 3·3 œrsteds at 500 Å (Fig. 2). The anisotropy field was nearly constant with thickness but varied slightly from 2·8 œrsteds at 2000 Å to 3·0 œrsteds at 500 Å (Fig. 2). The films were inverted, that is, the anisotropy field was less than the coercive force field, at thickness less than 700 Å. A plot of the logarithm of the coercive force versus logarithm of the thickness was linear and the slope was $-0{\cdot}45$. Thus, the coercive force may be expressed as an inverse function of the thickness to the 0·45 power. The value of 0·45 for the exponent of the thickness is considerably less than that previously reported by Lloyd and Smith for electro-deposited permalloy films[7]. The maximum dispersion is constant with decreasing thickness (500–2000 Å) in contrast to: (1) vacuum-deposited iron–nickel–cobalt films which have been reported to show an increase in dispersion with thickness below approximately 1500 Å (ref. 8); (2) vacuum-deposited iron–nickel films which show an increase in dispersion with increasing thickness (200–2000 Å) (ref. 9).

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Measurement of Thermal Accommodation of Gases on Heated Solids

The usual method of measuring the thermal accommodation coefficient (α)—defined as the ratio of the average energy acquired by a gas molecule rebounding from the solid to the maximum which it could acquire after prolonged contact—has several limitations:

(*a*) α is deduced from the measured increment in loss of energy from a heated wire *in vacuo* when a small pressure of gas is introduced. Because of the strong dependence of radiation on temperature this increment becomes an increasingly small fraction of the radiation loss *in vacuo* as the temperature is raised, for example, for tungsten at 2,700° K in helium at 10^{-3} torr the fraction is about 10^{-3}, even if α be unity.

(*b*) The hot filament is electrically heated and is used as its own resistance thermometer, so the method is not applicable to insulating solids.

(*c*) The energy is carried by the gas from the hot wire by both translational and internal degrees of freedom. So for poly-atomic molecules it is necessary to know the specific heat in order to calculate α, and the result is an average of the degree of excitation of the translational and internal modes.

Our method depends on measuring, by means of a torsion balance (sensitivity $\sim 7{\cdot}5 \times 10^{-2}$ dyne cm radian^{-1}), the mean momentum, and hence velocity, of molecules rebounding from a hot solid immersed in a rarefied gas at temperature T_0. It therefore measures directly the effective translational temperature (say T) of molecules leaving a heated solid of temperature T_1.

$$\text{Thus } \alpha = \frac{T - T_0}{T_1 - T_0}$$

The method becomes more sensitive the higher the temperature, is not confined to metals, is concerned only

with translational accommodation, and does not involve any assumptions about specific heat.

Since the deflexion of the balance depends on the impact momentum and the degree of its reversal at the balance arm, an assumption about the momentum accommodation coefficient of the latter must be made. It must lie between zero and unity, and results have been calculated on both assumptions, though the latter is much more likely, as the surface of the balance arm has not been specially cleaned.

Preliminary experiments on a tungsten wire in helium, argon and nitrogen at pressures of order 10^{-3} torr give the following values of α at 2,800° K—a temperature at which the surface of the tungsten is likely to be clean—helium, 0·07–0·12; argon, 0·23–0·50; nitrogen, 0·35–0·72, the range of values resulting from the two extreme assumptions mentioned here.

The experiments have been performed with the tungsten wire in a fixed position relative to a carefully designed system of apertures, so that only molecules coming from an isothermal and geometrically defined part of the tungsten wire can reach the balance arm directly.

Future experiments are being designed so that the heated body (for example, tungsten) can be replaced at will by the aperture of a furnace from which the issuing molecules can be assumed to be in thermal equilibrium. The degree of momentum accommodation on the balance arm can then be directly determined, made to approach unity by suitable surface treatment, and the foregoing ambiguities removed.

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Phonon Concept at High Temperatures

To deduce an expression for the lattice thermal conductivity it is usual to consider the volume of the solid as occupied by a 'phonon gas', and then to find the resistivities due to the scattering of the phonons by other phonons, electrons and structural defects[1].

Such theories assume that the phonons behave like particles, except that their number is not conserved. However, each phonon is really a group of waves. At high temperatures, defined by $\omega\tau < 1$, where ω is the angular frequency and τ the lifetime of the interacting phonon, only part of this wave train may be affected by a collision. Thus the phonon-quasi particle duality sometimes becomes invalid at high temperatures.

This is corroborated by experimental evidence[2]: a group at the Massachusetts Institute of Technology recently found that the thermal conductivity of solid solutions of $Mg_2Pb_xSn_{1-x}$ for $0 \leqslant x \leqslant 30$ per cent at room temperature did not follow the functional form suggested by Klemens's[3] equation. It becomes necessary to rely on phenomenological theories[4] to relate thermal conductivity data at high and low temperatures[5,6]. The term 'phonon' at high temperatures is frequently found in the literature when it would be better to speak of lattice waves[7,8]. Thus the limits of application of the phonon concept need further investigation.

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GEOLOGY

Velocity of Sliding of an Ice-fall

During the Nepal Himalaya *P*-29 (7,835 m) Expedition of Osaka University Mountaineering Club, 1961, we had some experience on sliding and avalanches of an active ice-fall; analysing photographs taken by a Canon 2,000 mm telephotographic lens from the base camp at 4,400 m high. Fig. 1 is a series of photographs taken at: (*a*) May 10, 1° 30′ p.m.; (*b*) May 11, 9° 30′ a.m.; (*c*) the same day 11° 30′ just after a small avalanche. By comparing the photographs (*a*) and (*b*) we can see that the glacier ice slid down as shown in Fig. 2. The distance of sliding on the original photograph was 2·0 mm. As the distance from the base camp to the ice-fall was 4 km, it corresponds to 4 m. As the estimated slope was 60°, the speed of sliding of the ice-fall became 6 m/day. Evidently this value is too large compared with the usual

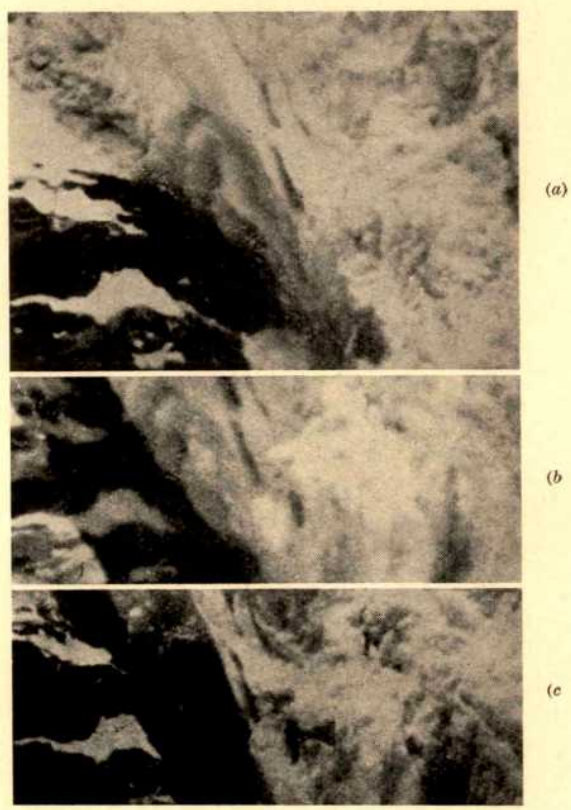


Fig. 1

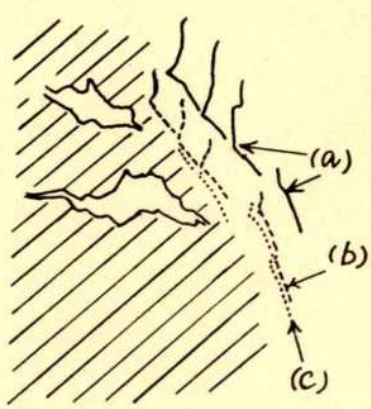
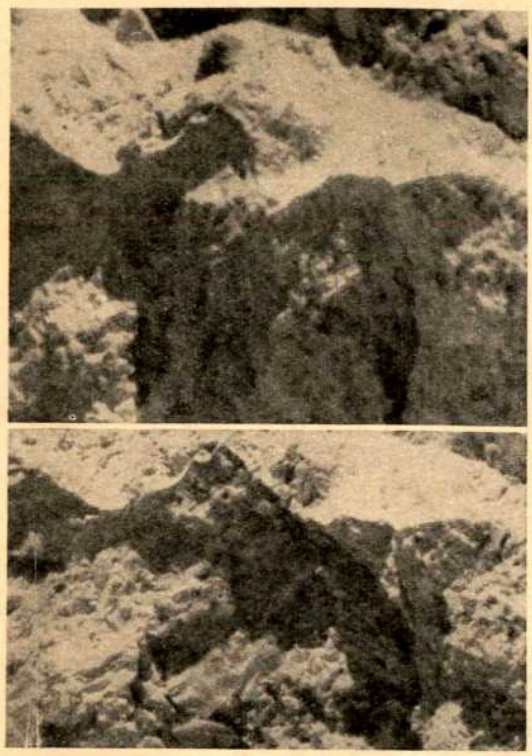


Fig. 2



(b′)

(c′)

Fig. 3

velocity of flow of a glacier. But as an avalanche occurred immediately afterwards, this value may not be unreasonable and gives some information on mobility of an ice-fall during the pre-avalanche period.

If we compare the photographs (*b′*) and (*c′*) of Fig. 3 which are on the right-hand side of (*b*) and (*c*) respectively, we can see the changes in the shape of a part of the cornice, that is, an overhanging rectangular block of ice has disappeared after the avalanche. As its volume was $8 \times 8 \times 40 \simeq 2{,}600$ m^3, if we take specific gravity of ice as 0·75, its weight was about 2,000 tons. Obviously this avalanche was quite a small one.

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Occurrence of *Kockelella variabilis* Walliser in the Aymestry Limestone of Shropshire

A collection of 1,400 conodonts collected by one of us (G. N.) in the course of a regional study of the Upper Silurian of Shropshire was submitted to the other (F. H. T. R.) for identification. Among these were found twelve fragmentary specimens not included in the fauna described by Rhodes[1] from the Aymestry Limestone. These represent *Kockelella variabilis* Walliser, originally recorded from the Silurian of Germany (from the Rheinisches Schiefergebirge, basal Orthocerenkalkes of Frankenwald and the *Cardiola* Horizon of the Karnische Alps[2]). The genus has also been reported from the Province of Malaga in Spain[3], the Lower Ludlovian of the Regganne area of southern Algeria[4] and the Silurian of the Tindorf Basin of the Spanish Sahara[5]. Ziegler[6] considered the species a good index for rocks of Middle Ludlovian age, but Walliser[7] showed it as characteristic of the Lower Ludlow. It therefore seems useful to record and illustrate the occurrence of the present specimens, which suggest an extension of the known range of the genus, even though the specimens are too fragmentary to provide any new morphological information.

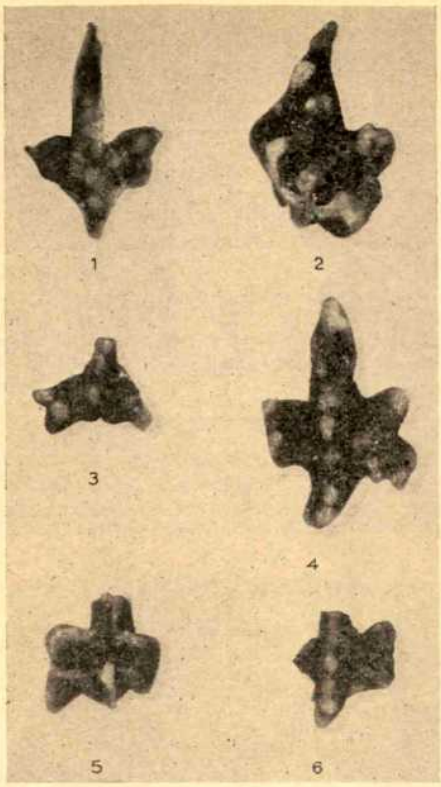
Genus *Kockelella*. Type species *Kockelella variabilis* Walliser[2]. The genus *Kockelella* has a broad resemblance to *Amorphognathus* but is distinguished by the form of the basal cavity. In *Kockelella* this consists of an anterior slit-like excavation which is expanded at about the mid-length into a wide, flaring cavity, with a subcircular outline. The lateral 'branches' which are conspicuous in the oral view are developed only on the oral surface of what is essentially a platform formed by the flaring aboral cavity, and they are quite unrepresented in aboral view. This supports Walliser's contention[2] that *Kockelella* is closely related to, and presumably derived from, *Spathognathodus*. The only substantial difference between the two genera is the development of lines of lateral denticles on what would correspond to the oral surface of the expanded 'pulp cavity' in *Spathognathodus*. Present usage of that genus includes forms with such denticulation, however. Thus Hass[8], describing *Spathognathodus*, writes "oral surface of lips or lateral expansions of pulp cavity may support denticles". The genus is, in fact, one of the most variable of all conodont genera and, in spite of Walliser's careful discussion of his reasons for distinguishing *Kockelella* from it, it is still possible that the two are synonymous. The present specimens are too few and too fragmentary, however, to provide any basis for a firm opinion.

Kockelella variabilis Walliser (Figs. 1–6); *Kockelella variabilis* Walliser[2]; *Kockelella variabilis* Ethington and Furnish[5]. The present specimens, although fragmentary, are very similar in their general form to those illustrated by Walliser.

The main denticulated blade is incomplete on all the present specimens, but on one of them (Fig. 4) the posterior blade is gently flexed in a horizontal plane, showing a close resemblance in this to some of Walliser's figured specimens (for example, ref. 2, Pl. 1, Figs. 6–10). The oral platforms of the Aymestry specimens are also poorly preserved but some specimens (for example, Figs. 2 and 4) show the presence of two well-defined rows of denticles on one side of the platform, and these appear very closely similar to those in Walliser's illustrations. Other specimens show only one row of denticles on the oral surface of the platform but in none of the Aymestry specimens are both sides of the oral surface of the platform sufficiently well preserved to determine the general character of the denticulation. One incomplete specimen suggests the presence of one row of denticles on one side and two on the other side of the platform, and another (Fig. 4) of two rows on each side. Such variation also exists within Walliser's specimens, and is regarded as infraspecific.

The form of the basal cavity (Fig. 5) in the present specimens is in all respects similar to that described by Walliser. The most striking features of this cavity are its relatively thick 'lips', its slit-like character below the median blade and its broad extension below the oral platform. This extension tends to be laterally oval and is conspicuously smaller than the oral extension, the blunted terminal teeth of which project outwards from it in basal view. It has a (very) broad resemblance to the basal view of a padded coronet, with blunted uprights projecting upwards and outwards from the circlet.

The present specimens were collected in a disused quarry at Shelderton, Shropshire (National Grid Reference: 32/418778), about 2½ miles north-north-east of Leintwardine, from a horizon some 40 ft. above the base of the quarry. The Aymestry Limestone here is equivalent to the Upper Bringewood Beds of the revised Ludlovian classification[9]. Conodonts are abundant at this horizon[1] though *Kockelella variabilis* is rare, the 12 specimens being part of a collection of more than 1,400 conodonts. The limestone is nodular and contains at this horizon a pre-



Figs. 1–6. *Kockelella variabilis* Walliser. Figs. 1–4 and 6. Oral views of specimens Nos. *C*-1–19, 16, 41, 15, 39; Fig. 5. Aboral view of specimen No. *C*-1–27. (× *c.* 20.) Catalogue Nos. refer to specimens in the Department of Geology, University of Manchester

dominantly brachiopod fauna, *Atrypa reticularis* and *Strophonella euglypha* being especially common. Phosphatic calculi, too, are very abundant[10].

At another horizon in the same quarry at Shelderton (21 ft. from the base of the quarry) *K. variabilis* occurs in the highly siliceous rock, mentioned by Rhodes[1]. *Kockelella* has not been found, however, in the lower part of the limestone, where conodonts generally are less common. *Kockelella variabilis* has also been found among conodonts extracted from the Aymestry Limestone (Upper Bringewood Beds) at View Edge (National Grid Reference: 32/425806), where it is associated with *Conchidium knighti* in the massive shell bank.

Work is at present being carried out by one of us (G. N.) on the faunal and lithological associations of *Kockelella* and other conodonts.

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CRYSTALLOGRAPHY

Doped Synthetic Diamonds—Anomalous X-ray Spikes Along <111>

Lonsdale and Smith[1] showed that type I natural diamonds give anomalous X-ray diffraction scattering along <100> directions in reciprocal space. Hoerni and Wooster[2] explained the intensities of this phenomenon in terms of impurity atoms in {100} planes, these impurity atoms having a scattering power not much different from that of carbon. Kaiser and Bond[3] published data, based on residual gas analyses, indicating that type I (infra-red opaque) diamonds (but not the transparent type II) contain up to 0·23 per cent of nitrogen-14, the amount being proportional to the intensity of the 7·8μ absorption band. They did not, however, attempt to correlate the quantity of nitrogen-14 present with the intensity (or indeed the presence) of X-ray extra spikes in the diamonds they used. Nor was this correlation made by Evans and Phaal[4], who obtained from type I (infra-red opaque) diamonds elegant transmission electron micrographs which show the presence of {100} impurity platelets, the impurity being assumed to be nitrogen.

So far we had observed no X-ray spikes in any synthetic diamond examined.

Through the kindness of Drs. R. H. Wentorf and H. M. Strong of the General Electric Co., Schenectady, we have now had the opportunity of examining very small synthetic diamonds which have been doped with boron, aluminium and nitrogen respectively, some during growth and some afterwards; and also natural diamonds doped with boron at high pressures.

All these do show X-ray spikes; but along <111> and not along <100>. Some of the diamonds are so badly distorted that the effect is a little doubtful; but it is particularly well shown by synthetic diamonds doped with aluminium during growth[5] (Fig. 1), iron being the metal 'catalyst' used to promote the diamond formation.

In Fig. 1 natural (type I, undoped) and synthetic (aluminium-doped) diamond Laue pictures are arranged in pairs. When the natural type I diamond is in such a position that the incident beam is nearly normal to (11$\bar{1}$), the 111 reflexions are accompanied by <100> spikes (cf. ref. 6). In a parallel position the doped synthetic diamonds show a configuration of spots in an isosceles triangle with a long (vertical) base. The reverse occurs for the alternative $\bar{1}\bar{1}\bar{1}$ setting, in which the natural diamond shows a triangular configuration of spots (but this time the triangle is more nearly equi-angular) and the doped synthetic diamonds show short streaks. Doped natural diamonds show the same effects as doped synthetic ones, and the geometry of these for copper K and cobalt K radiation proves that they lie along <111> directions, thus corresponding with concentrations of defects in {111} platelets.

Owing to the very small size of most synthetic diamonds and the considerable distortion found in these and in the doped natural diamonds examined which were also very small, no systematic attempt has yet been made to observe the relative [111] spike intensities for reflexions other than 111 or to correlate them with the type of impurity atom used for doping (cf. ref. 2). Attempts may be made to obtain electron micrographs for aluminium–iron synthetic diamonds, in order to find the distribution of the {111} platelets, which may well be on or near to the surface, since most diamonds have octahedral faces.

Some of the doped synthetic diamonds exhibit semiconducting properties. Among natural diamonds it is the rarer type II*b* which are semi-conducting[7] and these do not show any anomalous diffraction spikes.

Since aluminium-doped synthetic diamonds appear to give particularly good spikes it might seem curious that aluminium in natural diamonds does not seem to be concentrated in a similar way. Chesley[8] found the presence of

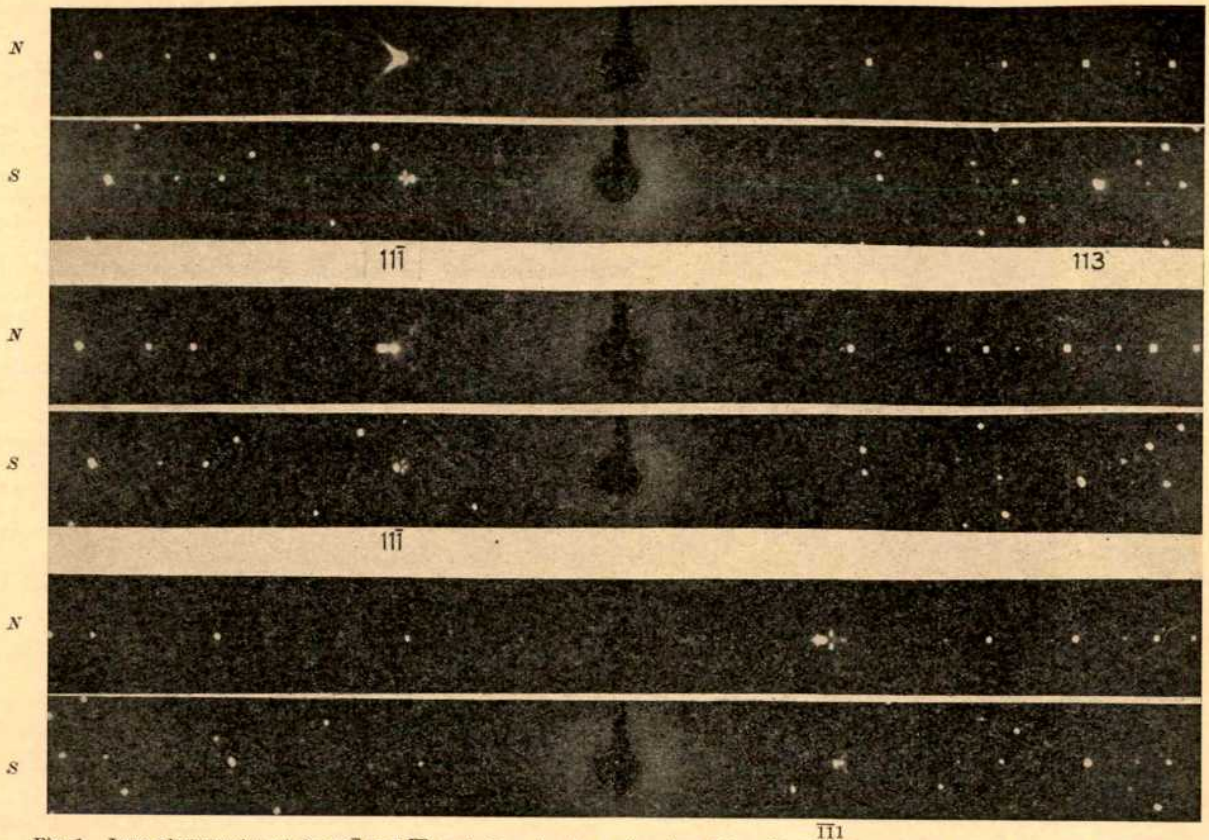


Fig. 1. Laue photographs set for 11$\bar{1}$ and $\bar{1}$11 reflexions showing spikes in <100> directions for natural type I diamond and <111> directions for aluminium-doped twinned synthetic diamond. *N*, Natural type I diamond; *S*, doped synthetic diamond

aluminium, together with silicon, calcium, magnesium, iron and other minor constituents, in many natural diamonds, but Raal[9] found no correlation between the absorption features and the concentration of these elements, which in no case exceeded 80 parts per million. Yet aluminium is abundantly present in Kimberlite minerals. This may indicate either that when natural diamond was formed most of the silicates had already crystallized, or that diamond was not formed in the silicate magma into which it afterwards migrated.

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CHEMISTRY

Structure of Silyl and Methyl Azides and Isocyanates

Evidence has been presented that the heavy atom skeletons of silyl isocyanate[1] and isothiocyanate[2] are linear while those of silyl azide[1], methyl azide[3] and isocyanate[4] are non-linear. This communication offers an explanation for these differences.

The systems are all H_3AXYZ, where A is carbon or silicon, and XYZ is either NNN or NCO (or NCS). If A is silicon the valency shell of A can be expanded beyond the octet. The linear structures possible in this case are I to VI (ref. 5). It is reasonable to suppose that a nitrogen atom can satisfactorily assume formal charges between $+1$ and $-\frac{1}{2}$, because NH_4^+ is stable in ordinary circumstances but NH_2^- only under special conditions. If this is accepted, I, II, IV and VI would be unsatisfactory structures energetically for H_3SiNNN. With III, there is a formal charge of -2 on the silicon and a triple bond between Si and N. It is therefore unlikely to be of low energy. With V there is a formal charge of $-1\frac{1}{2}$ on the silicon and a $2\frac{1}{2}$: bond between Si and N. It is also unlikely to be of low energy. Therefore there is no satisfactory linear structure for H_3SiNNN. On the other hand, I and II, and particularly IV and VI (ref. 5), are satisfactory for H_3SiNCO; with Z = oxygen, the formal charges on Z are 0, -1, $-\frac{1}{2}$ and 0 for I, II, IV and VI. The existence of H_3O^+ and OH^- shows that an oxygen atom can assume formal charges between $+1$ and -1 in stable species. In addition, the replacement of nitrogen at Y by carbon changes the formal charge at that atom from $+1$ in the azide to zero in the isocyanate, increasing further the stability.

In methyl isocyanate, the valence shell of A is restricted to an octet, and therefore, of the above six linear structures, II only is allowed. On the other hand, VII and VIII, which would be non-linear, are even more satisfactory, because the formal charges on the atoms A, X, Y, Z are respectively 0, 0, 0, 0 and 0, $-\frac{1}{2}$, 0, $+\frac{1}{2}$. In all these structures (II, VII and VIII), the number of electrons in the $AXYZ$ system is ten. Consequently, methyl isocyanate would be expected to be a hybrid of bonding VII and VIII, and bent. For methyl azide, II is impossible energetically because of a formal charge of -2 on Z; VII is most unlikely because of a formal charge of -1 on Z; on the other hand, VIII is satisfactory because the formal charges in the $AXYZ$ system are 0, $-\frac{1}{2}$, $+1$, $-\frac{1}{2}$; all these are allowable. Hence methyl azide will

be bent. In addition to VIII, because the valence-shell of silicon can be expanded beyond eight electrons, silyl azide can have the structure IX in which the formal charges on A, X, Y and Z are $-\frac{1}{2}$, 0, $+1$ and $-\frac{1}{2}$. In IX there are a greater number of electrons in bonds than in VIII so the former is probably preferred. However, both correspond to non-linear structures so silyl azide will be expected to be bent. Structures I to VI have been shown to be unsatisfactory, and VII is unsatisfactory for the same reason as for methyl azide.

$H_3A{=}X{=}Y{=}Z{=}$ I $\quad$ $H_3A{\overset{x}{=}}X{\overset{o}{-}}Y{\overset{x}{=}}Z{\overset{o}{-}}$ V

$H_3A{-}X{\equiv}Y{-}Z{\equiv}$ II $\quad$ $H_3A{\overset{x}{\underset{x}{-}}}X{\overset{o}{\underset{o}{-}}}Y{\overset{x}{\underset{x}{-}}}Z{\overset{o}{\underset{o}{-}}}$ VI

$H_3A{\equiv}X{-}Y{\equiv}Z{-}$ III $\quad$ $H_3A{-}\overset{|}{X}{=}Y{=}Z{=}$ VII

$H_3A{\overset{x}{-}}X{\overset{o}{=}}Y{\overset{x}{-}}Z{\overset{o}{=}}$ IV $\quad$ $H_3A{-}\overset{x}{\underset{|}{X}}{\overset{o}{-}}Y{\overset{x}{=}}Z{\overset{o}{-}}$ VIII

$H_3A{\overset{x}{-}}\overset{x}{\underset{|}{X}}{\overset{o}{-}}Y{\overset{x}{=}}Z{\overset{o}{-}}$ IX

The essential reason why silyl isocyanate and isothiocyanate are linear is that the multiple (or π) bonding in the SiN bond and the resulting electron distribution can be accommodated when the end atom is oxygen (or sulphur). They cannot be accommodated in silyl azide in which the end atom is nitrogen, because of its lower nuclear charge. Consequently the nature of the atom at one end of the chain affects the double (or π) bonding at the other.

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Constant Energy Differences in Atomic Structure

In a recent article Dash[1] has emphasized how three successive ionization potentials I_p, I_{p+1}, I_{p+2} in an isoelectronic series of atoms give a constant second difference which is equal to twice the Rydberg constant R divided by the square of the principal quantum number n of the electron being ionized, thus:

$$I_p - 2I_{p+1} + I_{p+2} = 2R/n^2 \qquad (1)$$

This relationship is a mathematical consequence of Glockler's[2] empirical quadratic:

$$I = \frac{R}{n^2}[\alpha Z^2 + \beta Z + \gamma] \quad (Z \text{ is the atomic number}) \qquad (2)$$

if α, β, γ are constant in an isoelectronic series, and if $\alpha = 1$ as shown empirically by Lisitzin[3] some years ago.

Dash's article provides, therefore, further empirical support for this quadratic. The evidence for it has recently been reviewed by me, and a derivation given from elementary quantum-theory using screening constants which have other applications[4]. A wave-mechanical derivation has since been given by Crossley and Coulson[5]. Both derivations explain simply why α is unity.

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Influence of Crystal Defects on the Annealing of Chemical Radiation Damage

Recent investigations have shown that crystal defects determine the fate of the fragments generated in solids by radiative thermal neutron capture as well as the kinetics of subsequent annealing reactions[1,2]. So far, there has been no experimental evidence for the influence of defects on the annealing of chemical damage produced in solids by irradiation. We have now obtained such evidence in the case of annealing in lead nitrate.

Previous workers had introduced defects by crushing, exposure to ionizing radiations, or by incorporating in the crystal small concentrations of ions bearing a different charge to the corresponding ions in the host lattice. It is known[3,4] that vacancies, and possibly interstitials, are generated in ionic solids also by compression. We compressed under 40 tons/in.^{2} crystals (30–100 mesh) of lead nitrate which had been previously dried and irradiated *in vacuo* in sealed 'Pyrex' ampoules with about 50 Mrads of cobalt-60 γ-rays. The compressed and the uncompressed samples were heated for different time intervals at 150° C. The experimental procedure for estimation of the damage was as reported earlier[5,6].

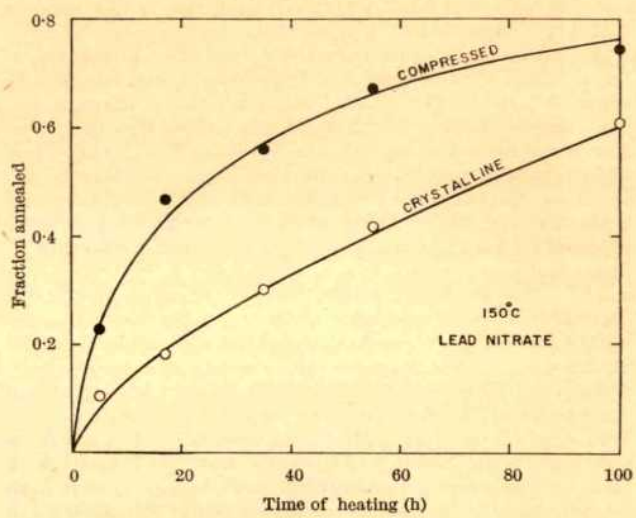


Fig. 1. Influence of compression on the annealing in lead nitrate

In Fig. 1 is shown the fraction of damage annealed against the time of heating in hours. The greater annealing of the compressed material as compared with that of the uncompressed crystals is evident. Thus, the fraction annealed over 35 h was 0·300 for the uncompressed, and 0·559 for the compressed, salt.

We thank Mr. Mata Saran for his help with the compression experiments. We also thank the Atomic Energy Establishment, Trombay, for the irradiations which were performed with the gamma-ray and sterilization unit presented by the Government of the United States. One of us (S. R. U.) gratefully acknowledges award of a scholarship by the Ministry of Scientific Research and Cultural Affairs, Government of India.

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Determination of *n*-Alkanes in Mixtures of Saturated Hydrocarbons by Means of Gas Chromatography

Methods of using molecular sieves 5*A* in gas chromatography for removing *n*-alkanes have been described previously[1,2]. According to these methods, two tests have to be performed, and the results can only be calculated after correction for differences in the size of samples has been made by means of internal markers. Such a procedure sometimes requires samples larger than those available, for example, for rock-extract analysis.

We have avoided the need for a two-fold test by placing a normal gas-chromatographic column in series with the measuring channel of a heat-conductivity cell, a 10-cm column filled with Linde 5*A* molecular sieves (50/80 mesh) and the measuring channel of a second heat-conductivity cell. This arrangement gives us two chromatograms of a single injected sample, one with and one without the *n*-alkanes. Assuming that all the compounds in the mixture of saturated hydrocarbons have roughly the same heat conductivity, we can calculate the individual *n*-alkane percentage directly from a comparison of the chromatogram areas. These areas can be determined very simply, for example, by being cut out and weighed, provided that the paper used is suitably homogeneous.

An essential condition for the successful functioning of the method is, of course, that the sieves should completely retain all the *n*-alkanes and allow all the isoalkanes and cyclanes to pass. We have found that the activated sieves do indeed retain all the *n*-alkanes, but that they unfortunately also retain a portion of the isoalkanes and cyclanes. Experiments with a number of C_{16} isomers and 2-Me-eicosane showed that when very small samples were injected (< 1 mg) as much as 50 per cent might be retained. Since the percentage always decreased as the amount of sample increased, we came to the conclusion that a certain absolute quantity of isoalkanes is always adsorbed by the sieves. This adsorption can be suppressed to some extent—but not altogether—by coating the sieves with 2 per cent silicone fluid '*GE/SF*96'. Our findings are in agreement with those of Downing *et al.*[3]. Adlard and Whitham[4] state that 2-Me-eicosane is not adsorbed from the gas phase. Their experiments were performed with samples weighing several milligrams[5], however, and with samples as large as this we have not found the adsorption of C_{16} isomers to be noticeable either.

The equipment can be assembled from various types of commercial gas liquid chromatographic apparatus. The need for two well-matched gas liquid chromatographic detection units is inherent to the method, however. Since two heat-conductivity cells rarely have exactly the same sensitivity, it will usually be necessary to shunt the more sensitive cell electrically.

The method has proved useful for the determination of *n*-alkanes in the C_{10}–C_{20} range, where preconcentration is not needed. Above *n*-C_{20} preconcentration is required, urea adduction being one method of doing this.

Full details concerning the method and the results obtained with it are shortly to be published elsewhere[6].

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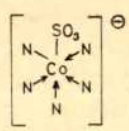
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Sulphitocobalamin

A wide range of cobalamins exist where the cyano group of the original vitamin B_{12} is replaced, for example, by water (vitamin B_{12b}), halides, nitrite, sulphur-containing groups, and alkyl groups (the coenzymes and analogues) and the nature of the ligand attached to the cobalt has a profound influence on the chemical and physical properties of the cobalamin.

Preliminary experiments directed towards the synthesis of the vitamin B_{12} coenzyme[1] consisted of a re-investigation of the action of certain sulphur-containing reducing agents on hydroxocobalamin following the report of Pawelkiewicz[2], who claimed a synthesis of the coenzyme form of Factor *B* by the sodium dithionite reduction of cobinamide, Factor *B*, in the presence of adenine. We have repeated this experiment with hydroxocobalamin and failed to obtain any product which contained adenine linked to the cobalt. However, a new light-sensitive product was obtained from the reaction, the same product also being obtained in the absence of adenine. The reaction of sodium bisulphite with hydroxocobalamin also gave the same crystalline product, the spectrum of which was somewhat similar to that of vitamin B_{12} coenzyme[3]. Electrophoresis at *p*H 8 indicated that the compound contained an additional acidic group compared with vitamin B_{12} and that it was stable both in solution and in the solid state in the absence of light. The product contained sulphur and the presence of the monoacidic sulphite ligand was suggested by the electrophoretic behaviour and by the presence of two strong bands at 983 and 1,150 cm^{-1} in the infra-red spectrum; these bands have been associated with the cobalt-sulphur linked sulphito group by Baldwin[4] in another series of cobalt-containing complexes. On this basis the sulphito compound is formulated as in the partial structure (I), the remainder of the molecule being the normal cobalamin skeleton.



(I)

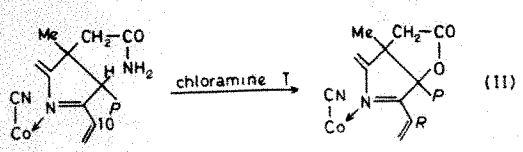
Sulphitocobalamin has been recorded in the earlier literature[5], but thought to exist only in solutions containing excess sulphite ions. More recently, Hill, Pratt and Williams[6] have described the properties of solutions of sulphitocobalamin and proposed a structure essentially similar to (I); both these workers and Bernhauer, Renz and Wagner[7] have described similar sulphito complexes from cobinamide (Factor *B*).

Like the coenzyme and its alkyl analogues[1,8], sulphitocobalamin readily reacts with acid (*p*H 2) to form a yellow protonated form (see ref. 6), but the acid solution is unstable and rapidly reverts to hydroxocobalamin. Cyanocobalamin (vitamin B_{12}) and hydroxocobalamin (vitamin B_{12b}) require treatment with strong mineral acid to yield the yellow protonated forms.

Aerobic photolysis of an aqueous solution of the sulphito complex gave hydroxocobalamin although under anaerobic conditions (10^{-6} mm) the complex was stable to photolysis. In this respect it resembled the simple alkyl analogues of the coenzyme[9] although the coenzyme itself undergoes fission when subjected to anaerobic photolysis[10]. Treatment of the sulphito complex with either potassium cyanide or hydrogen cyanide gives dicyanocobalamin. In this reaction the substitution at the cobalt atom occurs much easier, especially with potassium cyanide, than the similar reaction with the coenzyme and its alkyl analogues.

The course of the reaction of chloramine *T* with sulphitocobalamin also resembles the corresponding reactions with the coenzyme and the alkyl cobalamins, and differs in

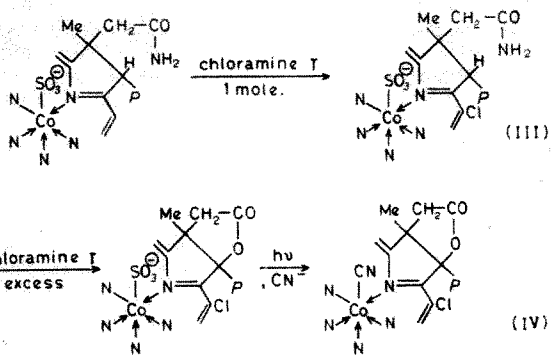
certain respects from the reaction of chloramine *T* with cyano- and hydroxo-cobalamins. In an earlier communication[11] it was shown that the action of the first equivalent of chloramine *T* with cyano- or hydroxo-cobalamin yielded a lactone (II; *R* = H) fused to ring *B*; further reaction caused substitution probably at C(10) of the chromophore to give the chloro-lactone (II; *R* = Cl).



(II)

(P = $CH_2 \cdot CH_2 \cdot CONH_2$)

The reaction of the sulphito complex with one mole of chloramine *T* caused substitution without cyclization and the product was the 10(?)-monochloro derivative (III). Reaction with excess chloramine *T* caused the formation of a monochloro-lactone (III) of the sulphito compound revealed by the appearance of the characteristic lactone carbonyl band at 1,778 cm^{-1}. Photolysis of this chloro-lactone (IV) in presence of air caused the formation of the corresponding hydroxocobalamin derivative and this with cyanide gave the same chloro-lactone (II; *R* = Cl) as had been obtained previously[11] by treatment of cyano-cobalamin with excess chloramine *T*.



The course of the chlorination reaction is thus similar to that observed[9] with methylcobalamin, but the order of reaction at different points of the chromophore differs in the chlorination of cyano- and hydroxo-cobalamins.

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Composition of Montan Wax

In a previous communication[1] we described the separation, identification and determination of the methyl esters of the long-chain fatty acids present in montan wax. This work was afterwards confirmed by Wollrab, Streibl and Sorm[2], who also used a flame ionization detector. They, however, used a chromatographic column containing 5 per cent 'Apiezon *L*' on ground tile and preconditioned by vacuum sublimation at temperatures of up to 350° C and operated at 335° C. These authors recorded the presence in montan wax of all the straight-chain normal fatty acids in the range C_{22}–C_{34} that we had previously identified, and in addition the C_{33} acid which we had not at that stage reported.

In further work we have used a 5-ft. length of column packed with polyethyleneglycol adipate and phosphoric acid[3] on 'Celite', fitted to a Pye 'Panchromatograph' with an argon ionization detector. This has enabled us to confirm the presence in montan wax of the C_{33} long-chain fatty acid, and also to detect a number of other fatty acids. We have also examined the hydrocarbon and alcohol fractions of montan wax, and confirmed the presence of long-chain carbon compounds.

For these separations an argon flow-rate of 37·5 ml./min was obtained with a supply pressure of 8 lb./in.^{2}. An anode potential of 1,000 V was used, and the column and detector were operated at 150° C for separation of the alcohol fraction and at 200° C for the hydrocarbon and fatty acid ester fraction.

The following summary of the composition of these three fractions of montan wax is based on a large number of separations of these materials, which were isolated as described by Tanner *et al.*[4] from the parent wax.

Hydrocarbon fraction. This fraction was found to be composed of straight-chain hydrocarbons, largely C_{23}–C_{33}, but with smaller amounts of C_{16}, C_{18} to C_{22}, C_{34} and C_{35}. These compounds were identified by the linear relation existing between log retention-time and carbon number. In addition to these straight-chain hydrocarbons, a second series of related compounds was also recorded, with a parallel log retention-time/carbon-number relation. These retention times did not correspond to those of unsaturated hydrocarbons, but are comparable with those expected for branch-chain hydrocarbons with carbon numbers similar to those of the straight-chain series observed.

An analysis of the hydrocarbon fraction, based on peak area measurements, is given in Table 1; the compounds are listed in order of elution, the straight-chain compounds being identified by carbon numbers. Compounds identified as present but not listed in Table 1 were present in trace amount only.

Table 1. Analysis of Hydrocarbon Fractions

Carbon No.	Wt. %	Carbon No.	Wt. %
C_{23}	2·3	C_{29}	10·3
C_{24}	3·7		1·6
	2·0	C_{30}	9·3
C_{25}	4·9		6·8
	0·9	C_{31}	7·1
C_{26}	7·4		1·3
	3·6	C_{32}	4·4
C_{27}	8·5		3·3
	1·9	C_{33}	3·1
C_{28}	10·4		1·0
	6·2		

Long-chain fatty acid fraction. All the normal straight-chain fatty acids in the range C_{16}–C_{35} were recorded as their methyl esters. An analysis reporting the major components of this fraction has already been published[1]: the additional compounds now identified as present occur in only trace amounts. In addition to the normal fatty acid esters, two other compounds were recorded in trace amounts but were not identified.

Alcohol fraction. The alcohols present as esters in the montan wax were converted to acid salts by heating with

soda lime and potassium hydroxide prior to esterification with methanol and sulphuric acid[4]. The normal, straight-chain compounds C_{10}–C_{21} were detected in amounts increasing from C_{10} to C_{18}, and decreasing from C_{18} to C_{21}. These compounds were also identified by the linear relation between log retention-time and carbon number.

Two further series of compounds were noted as present in the alcohol fraction, and from the close similarity of their retention times to those of the normal straight-chain series, it is concluded that these are related compounds.

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Temperature Dependence of Irradiation Damage to Polythene

In the course of work on radiation damage to polythene using the Woolwich 4·3 MeV linear accelerator, it was observed that a dose of 1,000 megarep raised the elastic modulus of 'Alkathene 7' by a factor of three without apparently affecting crystal content. This was confirmed both by an X-ray diffraction analysis of the material[1] and by a spherulite analysis. The density of the material was also found to have increased by 0·95 per cent.

These results are to be compared with those obtained by Charlesby and Hancock[2] using the B.E.P.O. reactor, where an equivalent dose[3] of 22 pile units was found to destroy all crystallinity and to create a product with elastic modulus lower than original. This was shown to be the net result of an initial sharp fall in modulus up to a dose of 7 units due to rapid destruction of crystallinity followed by partial recovery due to radiation cross-linking of the now fully amorphous material. Full recovery occurred at 30 units dose and continued irradiation then increased the modulus above original.

The difference between accelerator and reactor results is almost certainly not due to differences in the quality of the respective radiations. Charlesby[4] has considered the effect of ionizing radiation in some detail and concludes that the ionizing effect of direct collision of neutrons with atoms is relatively very small. He further shows that there is little difference in the effects produced by different radiations of equivalent absorbed energy.

This being so, the most likely factor to influence the nature of the radiation damage from accelerator and reactor appears to be the temperature at which irradiation is carried out. In the case of the accelerator, specimen temperature does not exceed 30° C whereas, with a nuclear reactor, heat from the unit can raise the specimen temperature to near its melting point. At these elevated temperatures, polythene is intrinsically more amorphous due to partial melting of the crystalline component, and it seems reasonable that radiation cross-linking of the amorphous increment at an elevated temperature will prevent it recrystallizing on return to normal temperature. This would result in an apparent destruction of the crystalline component although the mechanism would still be the simple one of cross-linking, common to both types of radiation.

Work is proceeding to examine the effect of X-rays on polythene at elevated temperatures. If the suggested temperature dependence is established, we have clearly a method of directly raising or lowering the elastic modulus of polythene by irradiation with the same dose but at the appropriate temperature.

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BIOCHEMISTRY

Total Synthesis of Adrenocorticotrophic Hormone

The total synthesis of β-corticotropin (ACTH), with the amino-acid sequence characteristic of the porcine species[1] (Fig. 1), has been achieved[2] using methods especially designed and developed for preparing large and complicated peptides[3]. They are: (*a*) formation of peptide bonds mainly by means of active esters[4] (for example, *p*-nitrophenyl esters[5,6]); (*b*) protection of amino and carboxyl functions with such blocking groups derived from *t*-butanol that easily can be removed in the last stage of the synthesis without destruction of sensitive peptides[3].

The latter method has proved to be essential for success, because ACTH is prone to severe alteration under conditions necessary for removing other, more common protecting groups such as carbobenzoxy[7], formyl[8], acetyl[8], and tosyl[9]. We also have successfully applied it to the syntheses of α-melanotropin (α-MSH) (ref. 10), β-melanotropin (β-MSH) (refs. 2, 11), and of β^{1-24}-corticotropin (the peptide comprising the *N*-terminal 24 amino-acids of ACTH) (ref. 12) as well as of other, similar peptides (β^{1-16}-corticotropin (ref. 13), β^{1-19}-corticotropin (ref. 14), Ac-Ser[1] β^{1-19}-corticotropin (ref. 15), etc.).

The synthetic route to ACTH starts from L-phenylalanine *t*-butyl ester (pos. No. 39) and proceeds in a stepwise manner by addition of one appropriately substituted amino-acid *p*-nitrophenyl ester after the other (removal of carbobenzoxy groups by hydrogenation, usually in 80 per cent acetic acid with palladium on charcoal; the resulting acetate salts may be reacted with the active ester to follow without addition of base) to the protected pentadecapeptide (pos. No. 15–39, Fig. 2). This compound, obtained in a pure state by counter-current distribution, was then reacted with the protected octapeptide (pos. No. 17–24) by the method of mixed anhydrides (mixed anhydride with trimethyl-acetic acid). The resulting derivative (pos. No. 17–39) was purified by counter-current distribution ($CHCl_3$, CCl_4, MeOH, 0·05 M NH_4OAc, pH = 6·5; 5 : 5 : 8 : 2 vol.; K = 2·3), hydrogenated and condensed with the azide of the protected hexapeptide (pos. No. 11–16) (ref. 16). The resulting protected nonacosapeptide (pos. No. 11–39) was extensively purified by counter-current distribution in the same solvent system (K = 0·8) and hydrogenated. Condensation with the decapeptide derivative (pos. No. 1–10) (ref. 17) by means of di*cyclo*hexyl carbodiimide gave a crude, protected nonatriacontapeptide which was purified by counter-current distribution ($CHCl_3$, CCl_4, MeOH, 0·1 M NH_4OAc, pH = 6·5; 8 : 8 : 17 : 7 vol.; K = 1·1). The purification by counter-current distribution was followed by chromatography on thin layers of alumina in the solvent system 100 (ref. 10) (Fig. 3). Material from the pure fractions was then treated with trifluoroacetic acid to remove all protecting groups and converted to the acetate salt. The

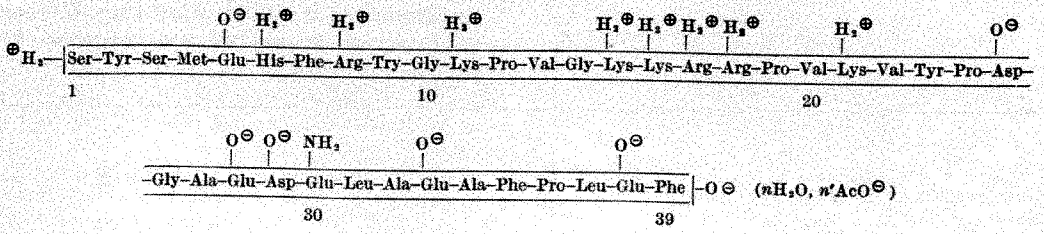


Fig. 1. Primary structure of porcine β-corticotropin (ACTH)[1]. (In natural material, water and acetate were estimated to be present in quantities of $n = 32$, $n' = 2$.) Abbreviations for amino-acids are those suggested by the Fifth European Peptide Symposium (Oxford, 1962); in particular: dashes extending above the symbols indicate loss of H— (on a nitrogenous group in the side-chains of basic amino-acids) or of —OH (from side-chain carboxyls) and replacement of these particles by groups symbolized in the formulæ

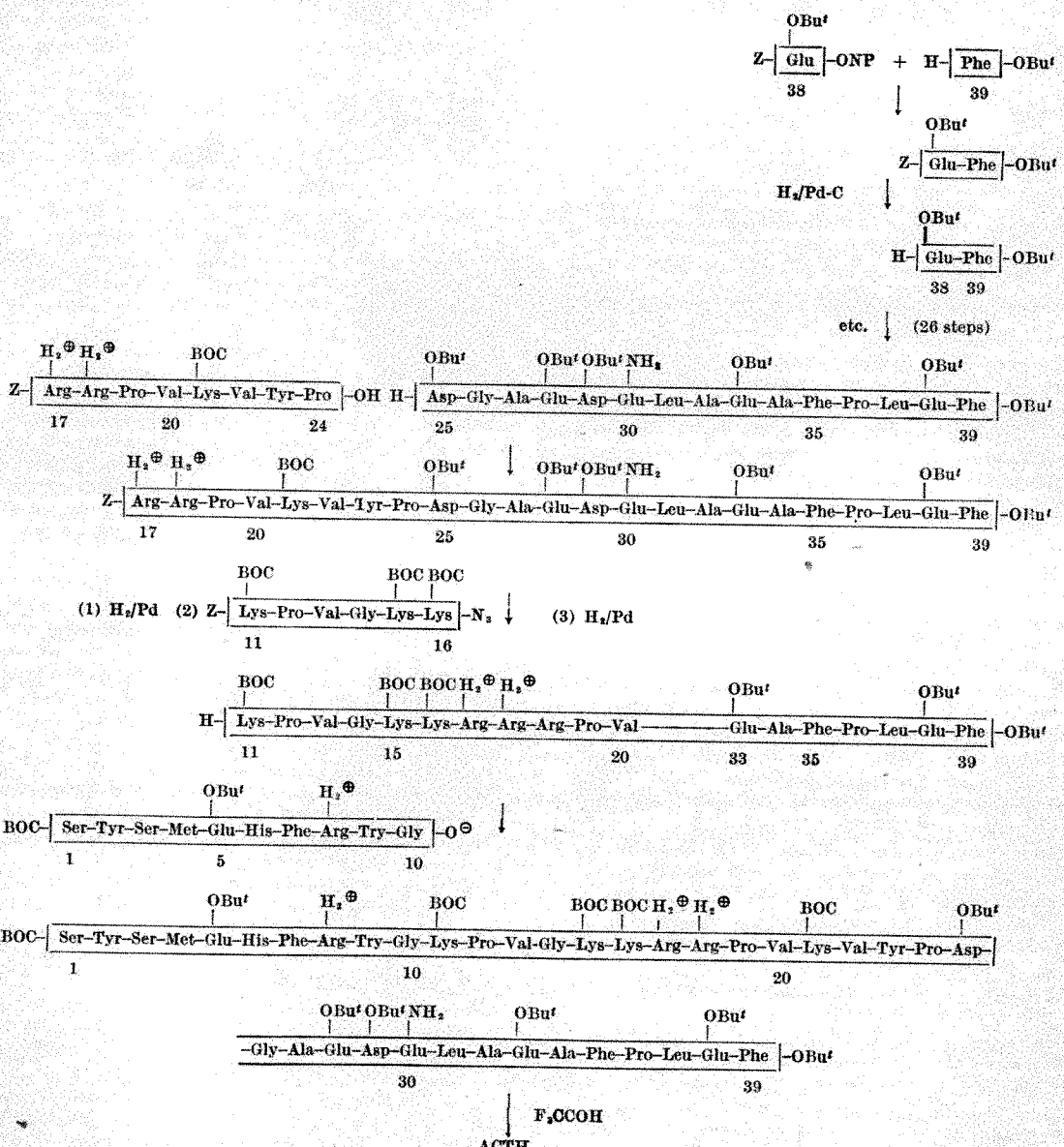


Fig. 2. Outline of synthesis of ACTH. Z- = carbobenzoxy-; Buᵗ = t-butyl-; BOC- = t-butoxy-carbonyl-. Positions referred to in text are indicated by numerals

resulting compound was found to be pure by thin-layer chromatography on silica gel, $R_F = 0{\cdot}4$, solvent system 101 (ref. 10) (Fig. 3). It was identical with natural ACTH derived from porcine sources with respect to R_F-values, colour reactions (Ehrlich, Pauly, Ninhydrin, Sakaguchi) and biological activity (110 U.S.P.-U per mg). Ratio Tyr/Try = 2 : 1 (ultra-violet). Amino-acid ratios in the total hydrolysate (calculated values in parentheses; method of Spackman, Stein and Moore[16]):

Ala 3·0 (3·0); Arg 3·3 (3·0); Asp 2·1 (2·0); Glu 5·2 (5·0); Gly 2·9 (3·0); His 1·0 (1·0); Leu 2·0 (2·0); Lys 4·0 (4·0); Met 1·1 (1·0); Phe 2·8 (3·0); Pro 3·8 (4·0); Ser 1·9 (2·0); Tyr 2·1 (2·0); Val 3·0 (3·0); NH_3 1·8 (1·0).

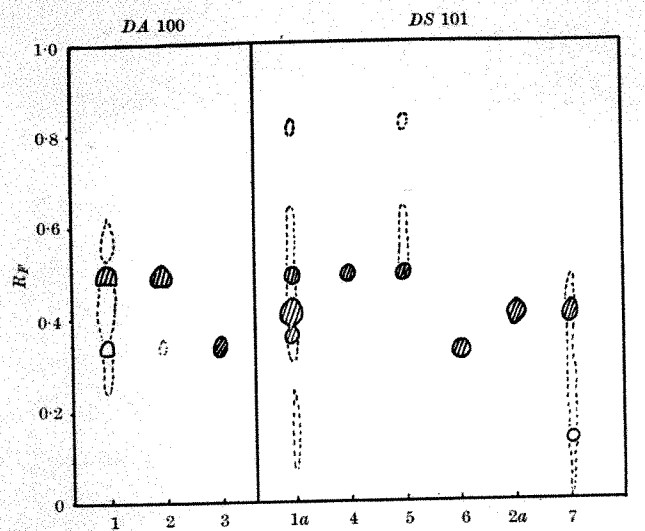


Fig. 3. Thin-layer chromatography of ACTH-peptides on alumina (*DA*) or silica gel (*DS*). Solvents Nos. 100 and 101 (ref. 10) stain according to Greig and Leaback (ref. 19). (1) Crude, protected nonatriacontapeptide; (2) protected nonatriacontapeptide after counter-current distribution; (3) sulphoxide of protected nonatriacontapeptide; (1*a*) crude, protected nonatriacontapeptide cleaved with F_3CCOOH; (4) β^{1-16}-corticotropin; (5) β^{1-16}-corticotropin treated with di*cyclo*hexyl carbodiimide; (6) β^{11-39}-corticotropin; (2*a*) synthetic ACTH: (2) treated with F_3CCOOH; (7) natural ACTH (Wilson Laboratories, Chicago), 95 U.S.P.-u/mg

Detailed physico-chemical, chemical, biological and immunological investigations are under way. A report on the details of the synthesis is being prepared[20].

The consequent development and application of new protecting groups has thus enabled the synthesis of all three types of hormone of the anterior and median lobes of the pituitary the primary structure of which is known: α-MSH, β-MSH and ACTH. For the synthesis of still longer chains, one may certainly rely on these protecting groups, but more effort will have to be put into the development of even better schemes and methods for the linking together of amino-acids and peptides.

This communication is dedicated to Prof. Hans Pallmann on the occasion of his sixtieth anniversary.

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Isolation of a Sialic Acid-rich Glycoprotein Fraction from Normal Human Urine

In a previous investigation[1], I subjected the non-ultrafilterable substances from normal urine to preparative zone electrophoresis at *p*H 8·6 and 4·5 and described a urinary glycoprotein fraction which had a high mobility towards the anode at both *p*H's. The mobility at *p*H 8·6 was similar to that of albumin, and the material was characterized by a relatively high sialic acid content and a relatively small peptide content[1]. It is the purpose of the present communication to report on the isolation and partial chemical characterization of this sialic acid-rich glycoprotein fraction.

Five-litre aliquots of pooled normal male urine were concentrated 700–1,000 times by ultra-filtration (cf. ref. 2). 'Visking' dialysis tubing of 23/32-in. inflated diameter was used as the filtering membrane. This membrane has been found to retain completely substances with molecular weights above 10,000–15,000[2]. One of the concentrated urines was separated by electrophoresis on a 'Pevikon' block at *p*H 8·6, and the distribution of protein and different carbohydrates following electrophoresis was determined[2].

The albumin zone obtained after such a separation contains the sialic acid-rich glycoprotein fraction and, in addition, a uronic acid-containing mucopolysaccharide fraction[1]. The eluates containing the maximum and the fast-moving part of the albumin peak were concentrated to a small volume by ultra-filtration and the concentrated material was subjected to zone electrophoresis at *p*H 4·5 (cf. ref. 1). At this *p*H the albumin migrated towards the cathode whereas the sialic acid-rich fraction and the mucopolysaccharide material moved towards the anode.

These two substances were almost completely separated, the mucopolysaccharide material having the higher anodal mobility. Small amounts of glycoprotein substances were distributed between the albumin and the sialic acid-rich glycoprotein fraction. The eluates containing the sialic acid-rich material were combined, concentrated, and subjected to electrophoresis at *p*H 8·6. The protein and carbohydrate curves obtained after the electrophoretic separation are shown in Fig. 1. It can be seen that the material gave a fairly symmetrical peak, and that the ratio sialic acid ('Bial') : hexose + methylpentose (orcinol) : protein ('Folin') was similar throughout the peak. The eluates containing the peak were pooled, dialysed against repeated changes of distilled water, and lyophilized. The yield of lyophilized material was about 1 mg/l. pooled urine. The dried material was analysed for sialic acid, hexose, fucose, hexosamine, and nitrogen with methods adopted earlier[2].

The composition is given in Table 1 (prep. No. I).

The two other preparations listed in Table 1 were isolated in somewhat different ways; prep. No. II by a series of three electrophoretic separations at *p*H's 4·5, 4·5, and 8·6, and prep. No. III by two successive separations

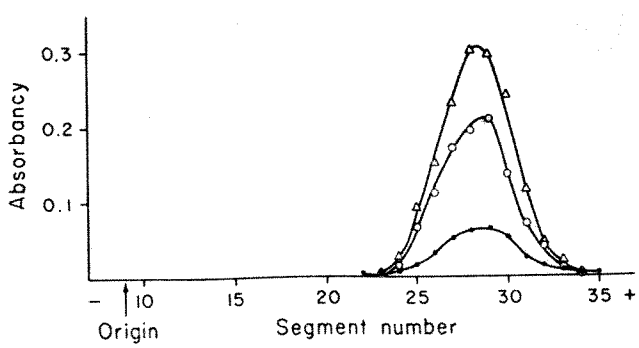


Fig. 1. Sialic acid-rich urinary glycoprotein fraction. Distribution of sialic acid ('Bial', Δ), hexose and methylpentose (orcinol, ○), and protein ('Folin', ●) after zone electrophoresis at *p*H 8·6

at *p*H 4·5. As can be seen, all three preparations had a similar composition. The neutral sugars present in prep. No. I were examined by one-dimensional paper chromatography, using the solvent system *n*-butanol–pyridine–water[2]. It was found to contain predominantly galactose with small amounts of mannose and fucose. Prep. No. I was also assayed for uronic acid with the carbazole method (cf. ref. 2) and was found to contain less than 1 per cent of this monosaccharide. A substance which was purified in the same way as prep. No. I was analysed in the Spinco ultracentrifuge model *E* at a protein concentration of 0·8 per cent in acetate buffer, *p*H 5·0. The sedimentation coefficient for the main part of the glycoprotein material was $s^{0.8\%}_{21,w} = 2{\cdot}5\ S$. The rest of the material sedimented more slowly as judged by the somewhat assymmetric appearance of the sedimentation patterns.

Table 1. Composition of Sialic Acid-rich Urinary Glycoprotein Fraction*

Preparation No.	Sialic acid	Hexose	Fucose	Hexosamine	Nitrogen
I	28·6	15·8	1·3	16·7	4·6
II	24·2	14·9	1·1	16·9	6·7
III	29·9	16·6	1·5	12·3	6·0

* Percentage dry weight (the materials were dried *in vacuo* at 80° for 48 h). The figures are uncorrected for ash and residual moisture content.

Urinary glycoprotein fractions that resemble the material reported in the present communication have recently been described by Bourrillon *et al.*[3]. Furthermore, Eylar[4] has given a preliminary account for the isolation from normal human serum of carbohydrate-rich glycoproteins with chemical compositions that coincide closely with that of the urinary glycoprotein fraction just described. It will be of interest to establish the relationships between these serum glycoproteins and the sialic acid-rich urinary glycoprotein fraction.

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Quantitative Relations between Ferritin and Siderin

Information about age-conditioned changes of the quantity of ferritin and total iron in human livers[11,12] has led to the conclusion that a strong quantitative correlation exists between ferritin and siderin. Several experimental investigations[1–8,14,16–19] support this conception. So far, however, there has been no special proof for the human liver.

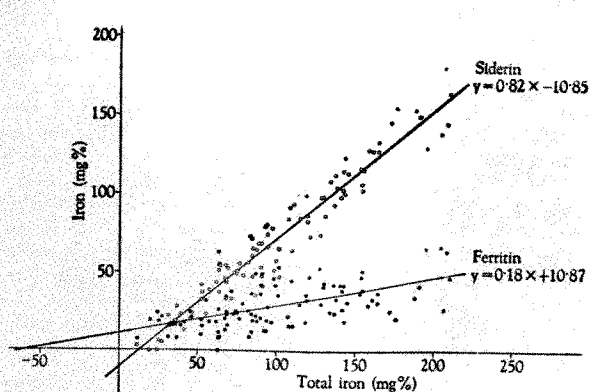


Fig. 1. Average value curve of siderin- and ferritin-iron (mg per cent) in relation to total iron of liver. All values are related to liver dry weight. Regression coefficient of single values:

$r_{calc.,\ ferritin} = 0{\cdot}59$; $r_{calc.,\ siderin} = 0{\cdot}95$
$r_{table,\ f=100} = 0{\cdot}25$; $r_{table}/r_{calc.}$ = significant

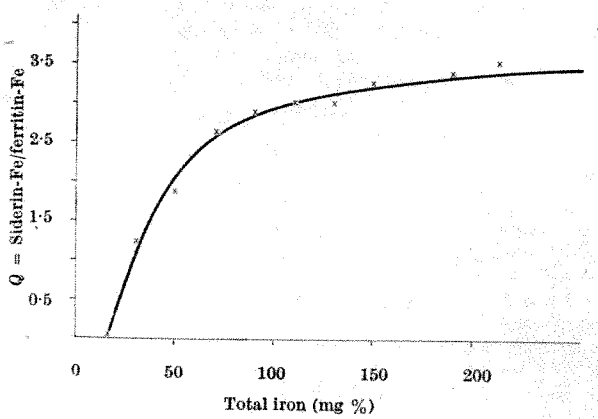


Fig. 2. Quotient of siderin-iron/ferritin-iron in relation to liver total iron

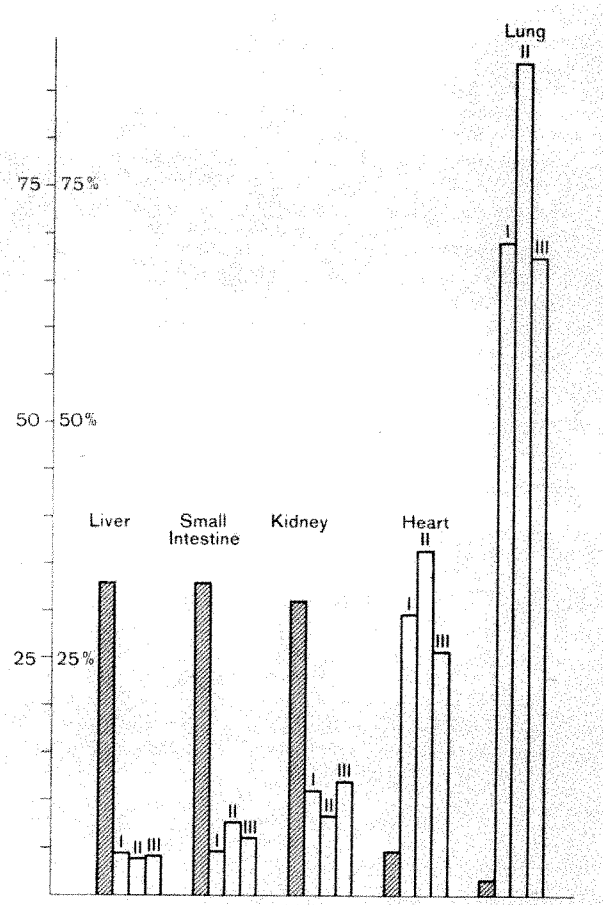


Fig. 3. Siderin-iron/ferritin-iron quotient (white columns) of different organs with transfusional hæmosiderosis in relation to protein metabolism (per cent) (ref. 13) (shaded columns) of these organs

We have examined 136 human livers with respect to quantitative relationships between ferritin and siderin. In preliminary experiments with eight livers from healthy men we compared the method for siderin estimation of Gabrio, Shoden and Finch[3] with the procedure of Wöhler, Otte and Schmidt[20]. We found no significant difference with $t_{calc.} = 0{\cdot}082$, $t_{tabl.} = 2{\cdot}260$. We performed siderin estimations according to the method of Wöhler[20]; ferritin, total-iron and hæmoglobin estimates were made following methods used in former investigations[11,12].

Our investigations of siderin content and ferritin content in 99 human livers from males with siderosis show that, with the elevation of total-iron, siderin- and

ferritin-iron rise in a similar way. It seems that a dynamic equilibrium exists between siderin and ferritin. Fig. 1 demonstrates that the effect on ferritin-iron is much smaller than on siderin-iron.

The view that there exists an equilibrium between siderin and ferritin is supported by Fig. 2. Here we have illustrated the quotient siderin-iron/ferritin-iron. It shows that in a range of about 100–150 mg per cent total iron the curve tends to approach asymptotically a constant value of the quotient. In this respect we do not agree completely with the results of the experiments of Shoden *et al.*[3,14,15].

A further confirmation of the supposition of a constant proportion siderin-iron/ferritin-iron resulted from the investigation of three cases with transfusional hæmosiderosis. We found that, although total iron rises to 1,400 mg per cent, the quotient of siderin-iron/ferritin-iron lies constant between 2·0 and 3·0. This fact demonstrates that even in the case of excessive iron storage the liver is able to produce in relation to siderin an aliquot part of ferritin-iron, if protein metabolism is normal.

With respect to protein metabolism, we observed by quantitative ferritin and siderin estimation of several organs in the three cases with transfusional siderosis that the quotient siderin-/ferritin-iron values change with the levels of protein metabolism. Fig. 3 shows these proportions in comparison with the height of protein metabolism.

Our results demonstrate that, under physiological conditions, ferritin-iron storage of liver has no absolute limit. In agreement with Keiderling and co-workers[7,8], Kettler[9], Wöhler and Zoll[21] and Zizza and Block[22], we see a limiting factor on one hand in an alteration of protein metabolism, and on the other in a change of oxidative metabolism in the liver, especially through changes in oxidative phosphorylation[10].

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Acid Ribonuclease in Liver Cell Sap

The acid ribonuclease of rat liver cytoplasm is located mainly in particles ('lysosomes'), and shows 'total' activity only if a treatment such as freezing and thawing is applied[1,2]. However, as much as one-quarter of the cytoplasmic activity is found in the supernatant fraction from liver homogenates, even with a centrifugal force as high as 105,000*g*. This supernatant-fraction activity, expressed per gm of tissue, is markedly increased in the case of hepatomas[3,4], precancerous liver from rats fed an azo dye[3], or liver from adrenalectomized rats[5]. De Duve[1] showed that autolysis likewise leads to increased supernatant-fraction activity of lysosomal enzymes, but remarked that the results "have been obtained on homogenates and it remains to be seen to what extent they apply to the cells themselves"; obviously an increase in supernatant-fraction activity could be attributed to increased fragility of lysosomes.

One cause of partial rupture of lysosomes might be exposure to an artificial medium as used for homogenizing; but it should be noted that the acid-ribonuclease activity of the supernatant fraction is of the same order with a dextran–raffinose medium as with the conventional 0·25 M sucrose medium[2]. Another cause might be mechanical damage to the lysosomes; but the following observations render this possibility unlikely.

Suppose that little activity is present in the cell sap *in vivo*. Then *mild* homogenization with a conventional Potter–Elvehjem homogenizer and subsequent removal of nuclei and unbroken cells at 600*g* should give, in somewhat low recovery, a cytoplasmic fraction containing lysosomes which had been little exposed to shearing; the supernatant fraction thereafter obtained by high-speed centrifugation might then show very low activity compared with the total activity of the cytoplasmic fraction. (Shearing during the centrifugation as distinct from the homogenization is probably negligible; supernatant-fraction activity was in fact undiminished when, in one experiment, the usual angle head was replaced by a swing-out head to reduce 'wall effects').

An initial experiment showed that the activity, in both the cytoplasmic and the supernatant fraction, was as high after one up-and-down stroke of the pestle (rotating at 2,000 rev./min) as after 16 strokes. Precancerous liver behaved similarly to normal liver. If, then, lysosomes are damaged in the homogenizing process, there must be a ceiling to the damage.

In further experiments, one-stroke ('mild') homogenizing at only 500 rev./min was compared with 5-stroke ('drastic') homogenizing at 2,000 rev./min, and found to give only about half as much cytoplasmic (total) activity. In both normal and experimental rats, the supernatant-fraction activity of acid ribonuclease, relative to the total activity in the cytoplasm, was no lower for mild than for drastic homogenizing (upper portion, Fig. 1). Some experiments included assays for glucose-6-phosphate dehydrogenase, an enzyme which is present in the supernatant-fraction but not in cytoplasmic particles[6]. The supernatant-fraction activity of acid ribonuclease, relative to that of this dehydrogenase, was again no lower for mild than for drastic homogenization; indeed, mild homogenization tended to give disproportionately high ribonuclease activity in the case of precancerous liver and liver tumours (lower portion, Fig. 1). In experiments such as these, there is the possible complication that cell constituents may have leaked, to an extent depending on the constituent, from the 'unbroken' cells which remain after mild homogenization, as has been found with liver cell suspensions[7].

It seems unlikely that limited release of ribonuclease from lysosomes would occur abruptly during homogenization. It would appear, therefore, that acid ribonuclease as found in supernatant fractions is indeed present in the cell sap *in vivo*. For normal rats as examined in this

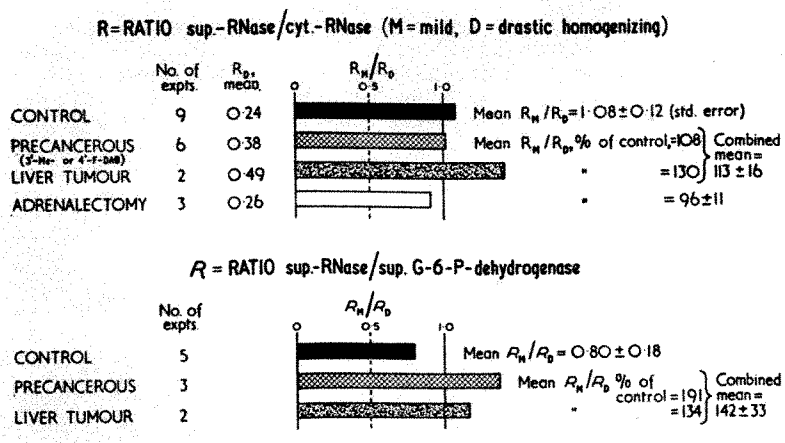


Fig. 1. Acid ribonuclease (RNase) activity in supernatant (sup.) fractions, compared with that in cytoplasmic (cyt.) fractions subjected to freezing and thawing, and with glucose-6-phosphate dehydrogenase (G-6-P-dehydrogenase) activity. Precancerous liver was obtained by feeding 3'-methyl- or 4'-fluoro-4-dimethylaminoazobenzene for 3–7 weeks, and primary tumours were obtained by prolonged feeding of the former compound. Adrenalectomized rats were maintained on saline and were killed 2–3 weeks after operation; cyt.-RNase as well as sup.-RNase is then increased[5], hence the value for R_D, mean is little above normal. All rats were male albinos, and were fasted overnight before killing. For preparation of homogenates, see text; in some experiments the 0·25 M sucrose medium was supplemented with *p*H 8·0 *tris* buffer (to 0·01 M) and with ethylenediamine tetraacetate (to 0·001 M), but no difference in the results was evident. Each experimental rat was compared with a control done side-by-side, and the R_M/R_D value calculated as percentage of that for the control; the mean values given for 'percentage of control' are therefore not based on the histogram representing all controls.

laboratory the ratio of supernatant-fraction activity to cytoplasmic (total) activity has consistently been, as illustrated elsewhere[2], somewhat higher for acid ribonuclease than for the classical lysosomal enzyme, acid phosphatase. Some preliminary evidence has been obtained (in collaboration with Mr. A. B. A. El-Aaser) that the activity in supernatant fractions may in part be due to an enzyme different from that in lysosomes.

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Further Separation of Iodine, Monoiodotyrosine and Diiodotyrosine by means of Modified Charcoal

A method for the separation of iodide from iodotyrosines extracted with Blau's alkaline reagent (4 N sodium hydroxide in 5 per cent sodium carbonate) from *n*-butanol extracts of thyroid hydrolysates by means of modified charcoal was previously described[1]. This method suffered from the following shortcomings: removal of traces of butanol from the alkali wash was quite difficult, and, since the butanol interfered with the adsorption of the iodotyrosines on the charcoal, repeated treatment of the alkali with the charcoal was required; the adsorbed iodotyrosines could be eluted from the charcoal *in toto*, but could not be separated into mono- and di-iodotyrosines. This communication deals with further developments of the method to overcome the foregoing handicaps.

The charcoal was modified with stearic acid as described before[1]. It was found, however, that the previously mentioned failure to elute the iodotyrosines from the stearic acid-modified charcoal was due to incomplete washing of the carbon to remove all the ethanol present. When the charcoal was washed carefully after being modified with stearic acid, no further treatment with tyrosine was necessary, and this step was therefore omitted. 5 g of the stearic acid-modified charcoal in 50 ml. Blau's reagent constituted the 'charcoal reagent'. The charcoal reagent was allowed to stand for at least overnight before use. The reagent could be kept unchanged for as long as a month.

5 ml. of *n*-butanol extract of thyroid hydrolysate, or of serum, was washed three times with 5 ml. of Blau's alkali reagent. The butanol carried over in the combined alkali washings was extracted with 15 ml. of chloroform. After allowing 1 h for the separation of the layers, an aliquot of 10 ml. of the upper, alkali layer was pipetted into a test-tube and thoroughly mixed with 12 drops of well-shaken charcoal reagent. The charcoal was precipitated by centrifugation and the alkali supernate was treated with additional charcoal reagent in a second test-tube. Up to 98 per cent of the iodotyrosines were thus adsorbed with no difficulty. The alkali supernate, containing the iodide, was quantitatively transferred into a volumetric flask and made to volume for radioactive or chemical analysis. A long-stem funnel of 7 cm diameter was then fixed in a Büchner-type filter tube with side-arm connected to a vacuum pump. A glass-wool plug was fitted into the top of the funnel stem, suction was applied and 'Celite', suspended in distilled water, was poured on to the plug to form an evenly distributed layer of about 2 mm in thickness. Next, the two precipitated carbons were suspended in alkali reagent and transferred to the funnel. This step was repeated as many times as necessary until the charcoal was quantitatively transferred to the funnel. When all the alkali passed through, the funnel with its contents was switched on to another filter tube.

Two solvents were prepared for eluting the mono- and di-iodotyrosines: a 1·25 per cent alcoholic–alkali solution—to 1·25 ml. of absolute ethanol was added Blau's alkali reagent to make a total volume of 100 ml.—and a 3 per cent alcoholic–alkali solution, prepared in a similar fashion. The monoiodotyrosine (MIT) was eluted with suction by 3 volumes of 10 ml. each of the 1·25 per cent alcoholic–alkali solution. The funnel was then set in another filter tube and the diiodotyrosine (DIT) was eluted with three 10 ml. volumes of 3 per cent alcoholic–alkali. The eluting solvents had to be thoroughy shaken immediately prior to elution, otherwise an uneven mixture of alcohol and alkali resulted.

Pure solutions containing a mixture of MIT and DIT in Blau's reagent were treated with charcoal reagent as described here, and the two iodotyrosines were separated by elution. Each eluent was neutralized in the cold with 10 per cent sulphuric acid and small volumes were chromatographed on paper using *n*-butanol/acetic/water (78 : 5 : 17) as solvent[2]. The results of the chromatographic analysis showed that a complete separation of MIT and DIT was effected. The spots on the paper were revealed as recommended by Bowden *et al.*[3] with the following exceptions: 0·1 N Ce^{+4} in 5 N sulphuric acid and 0·3 N $H_2AsO_3^-$ in N sulphuric acid[4] in a ratio of 1 : 1 was used to detect as little as 0·05 I^- per spot. Sheets of

'Parafilm' were placed between the papers and the glass plates to form a 'double-decker sandwich'. The spots could then be cut out, paper and 'Parafilm', with no contamination or losses, and placed in test-tubes for radioactive counting, or for chemical analysis. The chemical analysis of the alcoholic–alkali eluents, by means of an arsenious–cerate method to be described elsewhere, showed that the iodothyrosines were recovered quantitatively in comparison to standard solutions treated in an identical manner.

A stand which could hold a series of 6 filter tubes and funnels was constructed, and this permitted the handling of 6 samples at a time.

Thyroids of rats injected with iodine-131 were digested with alkali as before[1]. It was noticed that a large percentage of the adsorbed activity remained on the charcoal after elution. Longer alkaline digestion of the glands resulted in a decrease of the uneluted fraction, suggesting that the uneluted fraction was probably undigested peptides and polypeptides and indicating that the time of hydrolysis, namely 4 h, was inadequate. Longer periods of hydrolysis caused much deiodination. Thyroid glands of rats injected with iodine-131 and hydrolysed enzymatically[2] were analysed by the method described here and found to contain: 12 per cent I^-; 10 per cent iodothyronines; 20 per cent MIT; 38 per cent DIT; 15 per cent unhydrolysed peptides (that is, a fraction which remained uneluted). These results are comparable with those commonly obtained by paper chromatography.

The use of the method described here offers the following advantages: it saves time; very low iodine-131 activities need to be injected (0·5 μc./rat); it is fairly simple and requires no specialized equipment; it yields results which are easy to come by. The ethanol present in the alcoholic–alkali eluent can be evaporated by bubbling nitrogen through, and the resulting Blau's solution can be treated again with the charcoal reagent. The adsorbed MIT, or DIT, can then be eluted with water. This is of particular interest for preparative purposes of pure MIT or DIT.

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Free Amino-acid Content of Tobacco-seeds

Although much has been published about the various aspects of tobacco leaves, the same cannot be said about tobacco seeds. As regards organic nitrogen compounds in tobacco seeds, Abdoh and Taufel[1] have produced evidence for the existence of pyridine derivations in mature tobacco seeds.

Il'in and Lovkova[2] have found a high level of free amino-acids in immature tobacco seeds, but supposedly have found only bound amino-acids in the mature seeds. These authors assume that a direct relation exists between the biosynthesis and decomposition processes of nicotine and nitrogen metabolism in tobacco plants.

This communication attempts to show the existence of a number of free amino-acids in mature tobacco seeds, especially in seeds of tobacco grown in Iran.

The mature seeds (which were 1·5 years old) from three species of Iranian tobacco plants—*Nicotiniana basmacerus*, *N. trabzant*, *N. tikolak*—acclimatized to the northern areas of Iran were used in this work.

The seeds were first ground in a porcelain pestle and mortar, then extracted with 80 per cent ethanol in the mortar. Chloroform was added to the filtrate and shaken in a separatory funnel. The chloroform layer was discarded. This chloroform washing was repeated three times. The resultant water solution was then examined for the presence of amino-acids by paper chromatography. To the top layer 1/10 volume of isopropanol was added and samples of the resultant were applied to Whatman

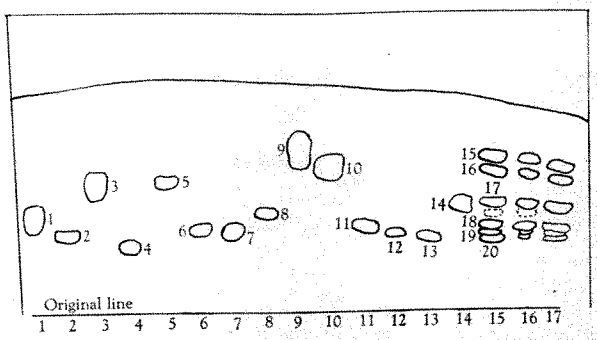


Fig. 1. First test: 1, tyrosine; 2, glycine; 3, methionine; 4, histidine; 5, proline; 6, serine; 7, arginine; 8, threonine; 9, leucine; 10, valine; 11, glutamic acid; 12, aspartic acid; 13, lysine; 14, alanine; 15, leucine; 16, valine; 17, alanine; 18, glutamic acid; 19, aspartic acid; 20, arginine

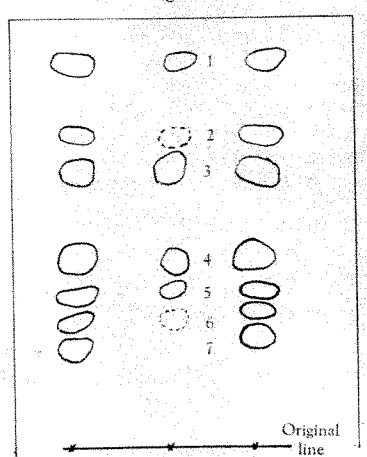


Fig. 2. Second test: 1, leucine; 2, valine; 3, alanine; 4, glutamic acid; 5, aspartic acid; 6, arginine; 7, lysine. Solvent—*n*-butanol/acetic acid/water = 250 : 60 : 250 v/v/v—upper layer is used

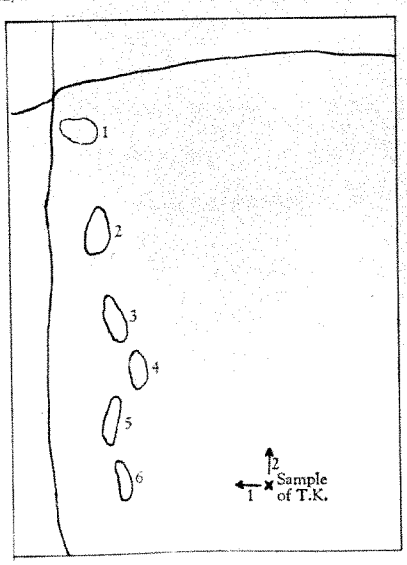


Fig. 3. Third test: 1, leucine; 2, valine; 3, alanine; 4, arginine; 5, glutamic acid; 6, aspartic acid. Solvent 1—*tert.*butanol/formic acid/water = 70 : 15 : 15 v/v/v for 29 h. Solvent 2—phenol/water = 400 : 100 w/v in atmosphere of ammonia for 20 h

er. Concurrently, 14 amino-acids (Merck) were on the same paper. For these Merck amino- solvent was 10 c.c. *tert.*butanol, 15 c.c. 98 per nic acid, 15 c.c. water. The chromatogram was l with ninhydrin reagent after 20 h.

ve found for each of the three species of tobacco x amino-acids, characterized as leucine, valine, glutamic acid, aspartic acid and arginine. An in spot appeared in the place for lysine, but to be this was repeated using a solvent of acetic acid, nol and water. The seventh point repeated itself ine.

lysine and valine spot for the *N. trabzant* seed was weak. The lysine spots for *N. tikolak* and *N. bas-us* were also weak.

confirm the foregoing results, a two-dimensional natogram was run. In the first dimension, the nt was *tert.*butanol, formic acid and water, and in the nd dimension the solvent was 400 g phenol, 100 c.c. er in an atmosphere of ammonia 20 c.c. 0/3 per cent nonia solution. The result of the two-dimensional omatogram showed the existence of six amino-acids: cine, valine, arginine, glutamic acid, aspartic acid and anine. Lysine did not appear in this chromatogram erhaps because its quantity was too scarce to be stained fficiently for detection.

The results show that at least 6 amino-acids do exist in he free state in mature tobacco seeds of some species. Further, 4 or 5 of these free amino-acids are among the essential amino-acids for animals, for example, rats.

We thank the Agricultural Tobacco Experimental Farm, Tirtash, of the Iranian Tobacco Monopoly, especially Engineer Saphir Paulus, director of the Experimental Farm, for the supply of tobacco seeds.

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Intermediary Metabolism in Bull Semen

We have examined the incorporation of radiocarbon from [^{14}C] glucose, [2-^{14}C] acetate and [1 : 4-$^{14}C_2$] succinate into the soluble metabolic intermediates of bull semen. Samples (50 μl.) of the semen, used within 6 h of collection, were mixed with 50 μl. of a solution containing 0·1 mM adenosine triphosphate, 2 mM magnesium sulphate, 10 mM potassium chloride and 1 μc. of the labelled substrate dissolved in 0·1 M potassium phosphate buffer, *p*H 7·4. The mixtures were incubated aerobically at 37° C for 30 min. At the end of the incubation period the enzyme systems were inactivated by the addition of 400 μl. of boiling ethanol and the mixtures centrifuged at 500*g*. The radioactive substances present in the supernatants were separated by two-dimensional paper chromatography, visualized by autoradiography and the carbon-14 measured by the techniques described by Smith and Moses[1].

The results (Table 1) show that whole bull semen utilized 99 per cent of the labelled glucose and that radiocarbon was incorporated mainly into hexose phosphates and lactate. Redenz[2] has shown that bull spermatozoa are able to glycolyse various sugars, including glucose, to lactate, and it has become an established fact that the metabolism of spermatozoa in several mammalian species is predominantly of a glycolytic character[3]. The presence of radioactivity in fructose is explicable because the initial product of glycolysis, glucose-6-phosphate, is readily converted by spermatozoal phosphohexose isomerase to fructose-6-phosphate, which may form fructose as a result of the action of phosphatase enzymes in the seminal plasma.

Table 1. Metabolism of Labelled Substrates by Bull Semen
Results given as ^{14}C (counts per min × 10^{-2}) incorporated into each soluble intermediate

[^{14}C] Glucose		[2-^{14}C] Acetate		[1:4-$^{14}C_2$] Succinate	
Intermediate	^{14}C	Intermediate	^{14}C	Intermediate	^{14}C
Hexose phosphates	29	Lactate	90	Fumarate	113
Fructose	2	Alanine	1	Malate	330
Lactate	16	Glutamate	3	Citrate	1
% of labelled glucose utilized	99	Glutamine	9	Aspartate	1
		Citrate	4	Glutamate	1
		Succinate	1	% of labelled succinate utilized	67
		Fumarate	2		
		Malate	5		

Most of the incorporated isotope from the labelled acetate was found in lactate which was presumably formed via pyruvate and acetylcoenzyme *A*. The preponderance of labelled lactate over labelled alanine in both the radioactive glucose and acetate experiments suggested that transamination of pyruvate does not occur to any appreciable extent in bull semen. In the acetate and succinate experiments only small amounts of radiocarbon were incorporated into aspartate and glutamate, and Bhargava, Bishop and Work[4] have shown that the intracellular amino-acid pools of bull spermatozoa are low compared with those in other mammalian tissues. However, the presence of radioactive glutamine in the acetate experiments shows that bull semen possesses glutamate synthetase activity. Succinic acid has been reported to have little effect on the oxygen uptake of intact mammalian spermatozoa[3]. In the work recorded here, the bull semen was found to utilize 67 per cent of the labelled succinate; but the incorporated radiocarbon accumulated in fumarate and malate, with only trace amounts in aspartate, citrate and glutamate. These results suggest that bull semen possesses appreciable succinate dehydrogenase activity but only a very limited capacity to metabolize malate.

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A Rapid Method of Disk Electrophoresis

Disk electrophoresis, as originated by Ornstein and Davis[1], provides a delicate analytical technique for the resolution of protein mixtures, but it requires nearly 2 h to complete a run. The formation of two acrylamide gels which merely prevent convection and take no direct part in the separation occupies some forty minutes. Barka avoided this step by simply layering the proteins in an appropriate buffer over the running gel[2]. Reisfeld[3] added sucrose to the sample mixture so that the density gradient diminished diffusion, but it has been reported that this manœuvre necessitates a reduced voltage during electrophoresis[4].

This communication describes a simple general procedure which gives a result in about 70 min. This same procedure enables electropherograms of the plasma proteins (as anions) to be obtained from capillary blood and, finally, some other applications are outlined.

A typical plasma protein electropherogram from equine whole blood is illustrated in Fig. 1*a*. To achieve this, the 'running gel' (*p*H 9·1) was formed in a glass tube exactly as described by Ornstein and Davis, but immediately before use it was overlaid with a 1-cm column of a buffer–

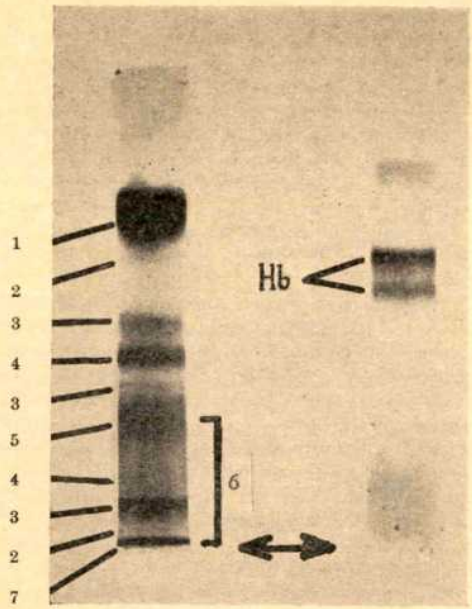


Fig. 1. Disk electropherograms obtained by the technique described in the text. The origins are indicated by ←→. *a*, Equine plasma; the numbered bands move with the following paper electrophoretic fractions: (1) Albumin, (2) α_1, (3) α_2, (4) β_1, (5) β_2 (6) region of γ-globulins and (7) fibrinogen (obscuring the β lipoprotein band); *b* An equine hæmolysate showing the typical hæmoglobin doublet (Hb) of this species

sucrose–'Sephadex' (Pharmacia, Uppsala, Sweden) mixture in place of the large pore acrylamide spacer gel.

The 'Sephadex' (grade *G*-200) had previously been washed with water, decanted and finally suspended in a 20 per cent w/v sucrose solution. The working mixture was prepared by diluting two volumes of the sedimented 'Sephadex' with one of *tris*–chloride buffer (*p*H 6·7, 180 mM with respect to chloride ions). Meanwhile, 30 μl. of the whole blood had been diluted with 0·5 ml. of the same *tris*–chloride buffer which is sufficiently near isotonicity to prevent lysis and the hæmic cells deposited by a brief centrifugation. To an aliquot of the supernatant was added two volumes of the sucrose–'Sephadex' suspension and a quantity of the resulting mixture was introduced into the gel tube to give a column 8 mm high. Although these 'Sephadex' mixtures may be dispensed like a liquid, the gel tubes can be safely inverted without mixing or loss, so that further manipulations followed the published technique. However, care was exercised in pouring the *tris*–glycine buffer into the upper tank lest the sample be dislodged; also, a hypodermic needle was used for the removal of the gel after running[3]. The gel was stained for 10 min in the amidoschwarz–acetic acid mixture and finally electrically cleared. The short staining time has been found to affect the electrophoretic pattern to a negligible extent, providing a permanent result is not required.

The use of 'Sephadex *G*-200'–sucrose mixtures instead of the large-pore acrylamide gels offers advantages in other fields. Equine hæmolysates inhibit polymerization, so preventing hæmoglobin separations. No such difficulty arises with 'Sephadex'. An appropriate volume of the hæmolysate is mixed with the 'Sephadex'–sucrose–buffer fluid, which is then processed as for plasma. Excellent resolution is attainable (Fig. 1*b*). The result is typical for the horse and confirms that equine hæmoglobin consists of at least two major components[5]. Nor is it necessary for the protein to be in solution, for disks punched from air-dried paper electrophoresis strips may be inserted in the 'Sephadex' column. The Kohlrausch boundary automatically elutes the protein so that normal fractionation follows. The relationship between paper and di
phoresis given in the legend to Fig. 1*a* was deriv
means.

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Electron Microscope Examination of Myog

THE structure of myoglobin has been examin
means of X-ray analysis; I have now made an ele
microscopical investigation of this protein. Ther
however, a special reason why I have embarked o
work. Some authors report that they have occasio
encountered annular structures on electron micrograph
of larger globular proteins[1–4]. These findings, at lea
the case of hæmoglobin, do not agree with those of X
analysis. According to X-ray investigations the hæ
globin molecules should be represented on electron mic
graphs as nearly circular-shaped dots. The same ho
also for myoglobin, so I thought it should be of so
interest to find out whether annular structures are als
found on electron micrographs of a comparatively sma
protein, such as myoglobin, which consists only of on
Svedberg unit.

For the investigation of myoglobin in the electron
microscope a small droplet of an aqueous solution of the protein was deposited on a collodion-covered specimen grid, and shadowed with a very thin layer of tantalum. For another series of experiments osmium tetroxide was added to an aqueous solution of the protein. By careful

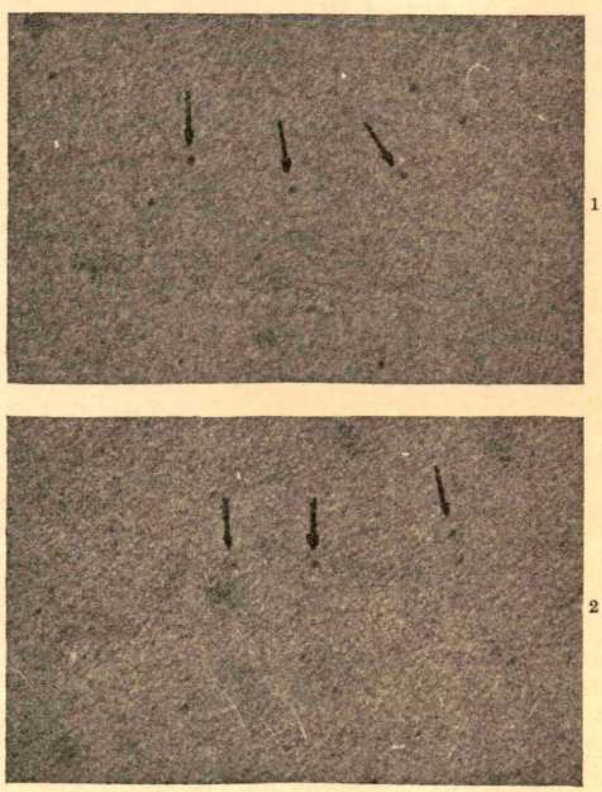


Fig. 1. Myoglobin molecules, shadowed. (× 200,000)
Fig. 2. Myoglobin molecules, osmium stained. (× 200,000.) Some molecules are indicated by arrows

dialysis all excess of osmium was removed, and again a droplet was deposited on a specimen grid. (For a more detailed description of this method see ref. 5.)

Figs. 1 and 2 show the results. The molecules are represented as small circular dots (diameter about 40 Å) and no annular structures were detected. These findings are in agreement with the results of X-ray analysis. Biological specimens are very delicate structures, and in the interpretation of electron micrographs the possibility of structural changes has always to be considered[6]. It is quite feasible that in some cases the Svedberg-units of some larger protein molecules (for example, hæmoglobin and albumin) are somewhat displaced as the result of specimen preparation and/or by the effect of the vacuum and of the electron beam. A small hole may thus be formed, giving rise to annular structures on electron micrographs. This concept seems to be supported by certain findings of Hall, who reports in an electron microscopical investigation that he found occasionally disintegration of protein molecules[7]. In view of the investigation reported here, and in view of the results of X-ray analysis, this interpretation appears to be more tenable than a tentative one put forward in a previous paper[1], which was based on the assumption that the 'hole' might be an intrinsic property of the Svedberg-unit. But I feel that electron microscopical investigations alone cannot completely prove or disprove a theory on the structure of proteins.

I thank Dr. John C. Kendrew, Laboratory of Molecular Biology, Cambridge, for providing the sample of myoglobin.

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PHYSIOLOGY

Influence of Diet on Urine Concentration in *Aplodontia rufa* and the Rabbit

Urea plays an important part in the production of a concentrated urine in mammals[1]. Berliner *et al.*[2] postulate that urea contributes to the renal concentrating ability because it diffuses from the collecting ducts into the medullary interstitium and is trapped by capillary countercurrent exchangers, called vasa recta. Urea, therefore, increases the osmotic pressure of the medullary interstitial fluid with which the collecting duct fluid equilibrates during the formation of a hypertonic urine. Thus, increasing the available urea enhances maximum urine concentrating ability in most species which have been investigated (man, dog, rat, cat, kangaroo rat, and jerboa[3])

However, Schmidt-Nielsen *et al.*[3] showed that this is not true for certain species: pig, beaver, and gerbil. These workers could not correlate the difference in function with anatomical differences in the nephron populations of these three species. They expressed the hope that more work would be done to explain why diet does not affect the urine concentration ceiling in certain species.

The specialized capillaries of the renal medulla (vasa recta) are an important factor in the urine concentration process[4]. Those mammals with a fixed osmotic ceiling of the urine may possess vasa recta with a morphology different from those species the urine osmotic ceiling of which does change with diet. We therefore determined the effects of high and low nitrogen intake on urine concentration in two species with differing morphology of the vasa recta: *Aplodontia rufa* and the domestic rabbit.

In *Aplodontia* the vasa recta do not form loops[5]; but such loops are present in the medulla of the rabbit[6]. Both these species are similar in size and dietary habits, and can, therefore, be treated in a uniform manner. This is an important consideration because the three species investigated by Schmidt-Nielsen had such diverse feeding habits that uniform procedures could not be applied.

Eight *Aplodontia* and ten rabbits were examined. In general, several *Aplodontia* and rabbits were examined simultaneously. Osmolality of urine and plasma was determined in both species following vasopressin injection during water diuresis according to the method of Dolph *et al.*[7]. Control animals received subcutaneous injection of 1 ml. water.

For a period of at least 13 days prior to any experimental work each animal was given a high or low protein diet. The high-protein diet consisted of pellets containing 15–17 per cent protein and 0·2 per cent sodium. The low-protein diet consisted of lettuce and carrots containing 1·2 per cent protein and 0·0012–0·0031 per cent sodium. Water was given freely with both diets. On a pellet diet the daily protein intake of *Aplodontia* was approximately 7 gm and on a lettuce and carrot diet it was approximately 3·6 gm. Six high-protein-fed *Aplodontia* also received 1 per cent urea in the drinking water *ad libitum* for 36 h prior to any experimental work to ensure a high plasma urea concentration.

The urea-nitrogen and sodium excreted in 24 h were determined from urine samples collected from each animal after it had been fed one or the other diet for at least 13 days. The sodium concentration was determined on a Coleman flame photometer and the urea-nitrogen concentration by colorimetric analysis. No urine urea-nitrogen or sodium concentrations were determined for urea-loaded animals. Prior to any experimental work, plasma urea-nitrogen and sodium concentrations were also determined.

The ratio of urine osmolality to plasma osmolality (U/P ratio) was determined during the vasopressin-injection procedure at the time of maximum urine concentration. Table 1 presents the maximum U/P ratios achieved by *Aplodontia* and the rabbit when fed different diets. *Aplodontia* can concentrate no better when eating a pellet diet ($U/P = 1{\cdot}47$) or urea loaded ($U/P = 1{\cdot}57$) than when eating lettuce and carrots ($U/P = 1{\cdot}48$). However, the rabbit can raise its urine concentration when fed a high-protein diet ($U/P = 3{\cdot}28$) over that achieved when fed a low-protein diet ($U/P = 2{\cdot}59$).

Table 1. Urine and Plasma Osmolality at Time of Peak Urine Concentration following Hydration and Vasopressin Injection

Animals and diet	Plasma (mOsm)	Urine (mOsm)	U/P
A. rufa, *H.P.*†(11)	292	429	1·47
A. rufa, *L.P.*‡ (7)	282	421	1·48
A. rufa, *H.P.* (6) (plus urea)	290	456	1·57
		*P	> 0·05
Rabbit, *H.P.* (20)	285	883	3·28
Rabbit, *L.P.* (9)	288	715	2·59
		*P	< 0·05

Number of observations in brackets.
* Probability calculated at the 0·05 level of significance using the Mann-Whitney U test.
† High protein diet.
‡ Low protein diet.

The urine osmolality of all control animals declined during the experimental procedure while the urine osmolalities of all vasopressin-injected animals rose.

Table 2 presents the mean urine and plasma urea-nitrogen and urine sodium concentrations in *Aplodontia* and rabbits fed the different diets. The plasma urea-nitrogen concentrations of *Aplodontia* (11·4 mg/100 ml.) and the rabbit (16·4 mg/100 ml.) on a high-protein diet

Table 2. Mean Urine and Plasma Urea-nitrogen and Urine Sodium Concentration on Different Diets

Animals and diet		24-h urine samples Na (m.equiv./kg per 24 h)	24-h urine samples U–N (mg/kg per 24 h)	Plasma U–N (mg/100ml.)
A. rufa, *H.P.* †(15)		3·15	498	11·4
A. rufa, *L.P.* ‡(7)		12·96	477	7·7
	P	< 0·05	> 0·05	< 0·05
A. rufa, *H.P.* (7) (plus urea)		—	—	20·5
Rabbit, *H.P.* (14)		5·30	345	16·4
Rabbit, *L.P.* (12)		7·13	319	12·6
	P	> 0·05	> 0·05	> 0·05 but < 0·10

Legend same as for Table 1.

were significantly higher than those obtained from animals on a low-protein diet (7·7 mg/100 ml. and 12·6 mg/100 ml. respectively). The highest plasma urea-nitrogen concentrations (20·5 mg/100 ml.) were from *Aplodontia* fed a high-protein diet and 1 per cent urea in the drinking water. The plasma sodium concentration did not vary with dietary changes in either species.

Aplodontia excreted much more urea per unit of body-weight than did the rabbit regardless of diet. It also excreted more sodium per unit of body-weight when fed a low-protein diet than when fed a high-protein diet, but the rabbit did not demonstrate this difference in sodium excretion. Thus, in *Aplodontia*, sodium excretion increased when urea excretion decreased and vice versa. This interdependence of urea and sodium in producing a concentrated urine was described by Schmidt-Nielsen *et al.*[3] in beaver, pig, and gerbil, and appears to occur in *Aplodontia* but not in the rabbit.

These experiments show that when intake of nitrogen is increased in *Aplodontia* there is no increase in maximum urine concentration following vasopressin injection. The response of *Aplodontia* to a high-protein diet is, therefore, similar to that of the pig, gerbil, and beaver. Increased nitrogen intake in the rabbit, however, results in increased maximum urine concentration.

Because urine-concentrating capacity is dependent on the concentration of the renal medullary interstitial fluid, our results suggest that the absence of looped vasa recta in *Aplodontia* may prevent the efficient trapping of solute such as urea in the medullary interstitium. This is supported by our results, which show higher plasma urea concentrations in the rabbit than in *Aplodontia* with greater urinary excretion of urea in *Aplodontia*.

It would be valuable to know if the pig, beaver, and gerbil lack looped vasa recta, as is known to be the case for *Aplodontia*. We suggest that a comparative examination of vertebrate renal medullary blood supply will be as fruitful as previous comparative examinations of the nephron[8] in explaining certain differences in renal function.

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Effects of *d,l*-Aldosterone on Renal Excretion of Water, Sodium, and Potassium induced by Renin

Both renin and oxytocin augment the renal excretion of water, sodium, and potassium in rats. When these substances are injected simultaneously, the increase of diuresis is approximately equal to the sum of the amounts excreted when they are given separately. This effect is the same, even when the dose of renin is large enough to elicit a maximum excretory response within the first 2 h (ref. 1). Besides showing that oxytocin affects the output of electrolytes by a mechanism different from renin, these experiments would appear to support the assumption that oxytocin enhances the elimination of sodium by counteracting the effect of aldosterone. It has been shown that a dose range of 2·5–5 mU of oxytocin neutralizes the sodium-retaining activity of 1 μg of aldosterone[2]. Moreover, recent investigations by Bartter *et al.*[3], Mulrow and Ganong[4], and Kaplan and Bartter[5] have demonstrated that renin, through its ability to release angiotensin, stimulates aldosterone secretion. In view of aldosterone's sodium-retaining potency, it was expected that this hormone would counteract the effect of either renin or oxytocin on sodium excretion. But contrary to the expectation, aldosterone was shown to facilitate the action of renin in the experiments here described.

Four groups of 40 adult female rats of the same weight and submitted to the same conditions were used. On the day of the experiment, after fasting 12 h, they were placed in metabolic cages to collect the urine. In each cage there were four rats with free access to a drinking solution containing 250 mg of sodium and 25 mg of potassium per litre.

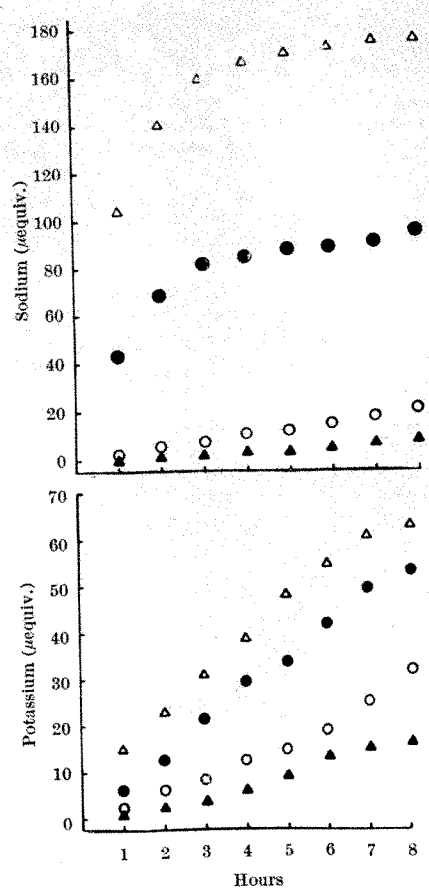


Fig. 1. Excretion of sodium and potassium in μequiv./100 g/body-wt. during 8 h with four groups of 40 rats each: aldosterone group injected with 5 μg aldosterone; renin group with 10 G.U. renin from rat kidney; renin–aldosterone group with 5 μg of aldosterone and 10 G.U. renin; control group with solvent solution. △, Renin–aldosterone; ●, renin; ○, saline; ▲, aldosterone

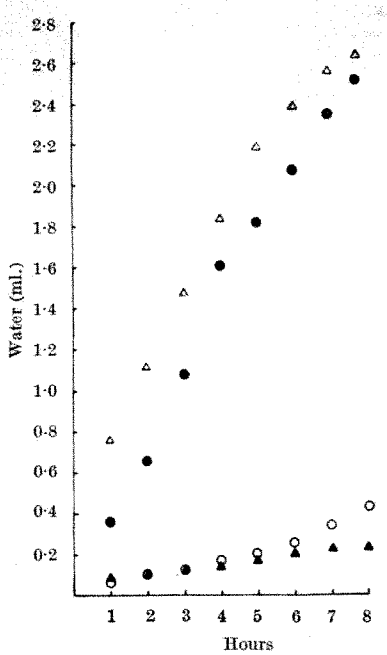


Fig. 2. Excretion of urine in ml./100 g/body-wt. by the four groups of rats indicated in Fig. 1

The first group of 40 rats was injected subcutaneously with 5 μg of aldosterone per 100 g body-weight; the second group received intraperitoneally 10 Goldblatt units (G.U.) per 100 g/body-wt. of renin from rat kidney purified by the method of Braun-Menendez *et al.*[6]; the third received a subcutaneous injection of 5 μg of aldosterone and 10 G.U. of renin; and the control group was injected subcutaneously and intraperitoneally with solvent solution (Tyrode). During 8 h the volume of urine collected in each cage and its sodium and potassium content were measured half-hourly; the Na/K ratio was calculated.

In the first group aldosterone clearly inhibited excretion of sodium throughout the entire experiment (Fig. 1), but the output of potassium was also less than that of the control (Fig. 1). The reduced sodium elimination was reflected in the very low Na/K ratios (0·22) in the third and fourth hours of the experiment.

Renin given with aldosterone to the third group strikingly increased the excretion of water, sodium, and potassium during the first 5 h, but particularly in the first 60 min. The amount of sodium and potassium eliminated at the end of the second hour was approximately twice as much as that excreted by the group given renin alone (Fig. 1). Moreover, excretion of water and electrolytes began much sooner in the renin–aldosterone-injected rats than in the renin group (Fig. 2).

In a second experiment using a different batch of aldosterone the results were very similar.

Obviously the 5 μg dose of aldosterone per 100 g/body-wt. facilitated the characteristic effect of renin by intensifying its influence over the excretion of all the constituents. The Na/K ratios of the renin–aldosterone and renin groups rose to 7·3 and 7·1, respectively, in the first hour and afterwards fell in parallel, while in the control group the ratio was close to unity for the first three hours.

Related experiments utilizing the same renin demonstrated that the intraperitoneal injections of 2·5, 5, and 10 G.U. per 100 g/body-wt. produced adrenal ascorbic acid depletion of 21·5, 34·3, and 49·6 per cent respectively in glands removed 1 h after the injection[7]. Although no direct measurement was made to determine aldosterone content in the blood, activation of the adrenal gland was evident and presumably the diuretic action of renin coincided with increased endogenous secretion of aldosterone.

It remains to be explained how aldosterone, usually an anti-natriuretic factor, facilitates the output of sodium induced by renin. Perhaps the work of Croxatto and Foradori[8] concerning the effect of aldosterone on the intra/extracellular sodium ratio could explain the phenomenon. Under the influence of aldosterone, the intracellular sodium moves out when the intra/extracellular sodium ratio is high. If this were the case, aldosterone could enhance the escape of sodium through the kidney.

It is interesting to note that the injection of angiotensin elicits increased sodium and chloride excretion in hypertensive human beings[9] and that higher aldosterone elimination has been found in severe hypertensive cases[10].

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Perfusion of the Viable Sheep Fœtus

The perfusion of the isolated fœtus allows aspects of fœtal metabolism to be investigated in the absence of placental transfer or synthesis of metabolites. A future extension of this technique may find application in the management and treatment of the premature or hypoxic human infant.

A simple technique has been developed for the perfusion of the sheep fœtus, and an attempt has been made to validate the method by perfusing animals of a viable age and allowing them to survive after the perfusion. Three such experiments have been carried out and in each case the animal showed normal behaviour for the following 24 h. Although techniques for fœtal perfusion have been described previously (human[1], pig[2] and sheep[3]), only recently, while this investigation was in progress, has survival after perfusion been reported[4].

Three ewes bearing fœtuses of a viable age (over about 140 days' gestation) were anæsthetized under procaine spinal anæsthesia, supplemented with intravenous thiopentone to maintain light general anæsthesia. After withdrawing between 150–200 ml. of blood from the dorsalis pedis artery to prime the perfusion circuit the fœtuses were exposed by Caesarean section. The fœtal head was enclosed in a bag of 0·9 per cent sodium chloride to prevent the onset of breathing. Following a local subcutaneous injection of procaine the fœtal femoral artery was catheterized for blood pressure determinations and the subsequent removal of blood samples. The umbilical vessels were prepared for cannulation under the

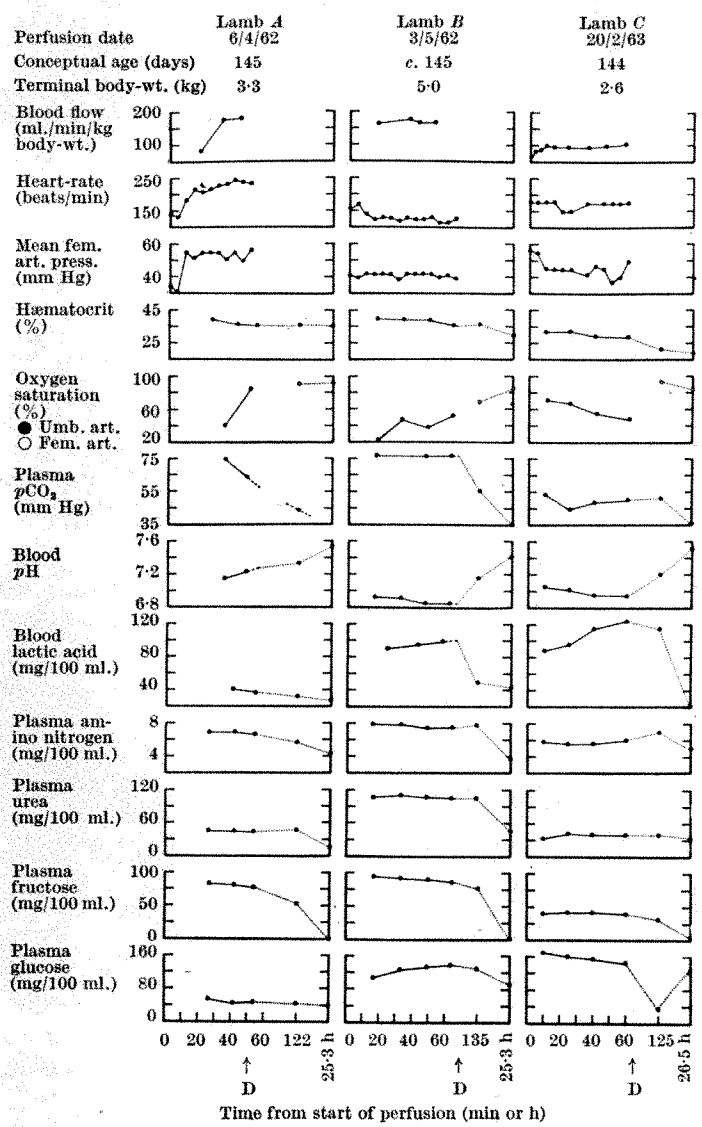


Fig. 1. Data obtained from the lambs during perfusion (●——●) and after perfusion (●·····●) plotted against time from the start of perfusion. Detachment from the perfusion circuit took place at *D*

local application of formol–saline. After injecting 50 mg heparin into an umbilical vein an arterio-venous anastomosis was established in one of the two pairs of arteries and veins of the umbilical cord. The remaining artery and vein were then cannulated and the umbilical cord cut. The latter vessels were connected to the perfusion circuit which had been primed with heparinized maternal blood, and finally the umbilical arterio-venous anastomosis was severed and its connexions incorporated into the circuit. The perfusate volume was approximately 150 ml. The function of the anastomosis was to maintain the cardiac output by ensuring that at no time was there stasis of flow of blood in the fœtal-umbilical circulation. Preliminary experiments had shown that such an anastomosis was well tolerated by the fœtus.

In the perfusion circuit blood was pumped by the fœtal heart along the cannulated umbilical arteries into a spinning disk oxygenator through which passed a warm-moist 97 per cent oxygen + 3 per cent carbon dioxide gas mixture. The outflow into the oxygenator was controlled either by a screw clip or by altering the height of the oxygenator with respect to the level of the fœtal heart. Oxygenated blood accumulated in a reservoir below the oxygenator from which a variable speed rotary pump conveyed the blood through a filter to the umbilical vein. The animal, wrapped in polythene sheeting and cotton wool, lay on an electric blanket; the oxygenator was wrapped with an electric heating mantle and the rest of the perfusion circuit lagged to prevent heat loss. The inflowing blood temperature lay between 34·5° and 37° C. The speed of the pump was adjusted manually such that the volume of blood in the fœtus, as judged by the level in the reservoir, remained constant. Blood flow was computed from knowledge of the output per turn of pump and the number of turns per minute.

Perfusion was allowed to proceed for about 60 min; during this period, samples of blood were removed from the circuit for analysis. Occasional limb and abdomino-thoracic movements were made by the fœtus. The bag covering the fœtal head was then removed and the umbilical cord stretched and tied. The animal was dried and stimulated generally by rough towelling. The catheter in the femoral artery was bandaged against the thigh and blood was removed for analysis approximately 1 and 24 h after detachment from the perfusion circuit.

The changes in concentration of various blood components during and after perfusion in the three lambs are shown in Fig. 1.

The heart-rate, femoral arterial pressure, blood flow and umbilical arterial oxygen saturation, although variable, were of the same order as those encountered by other workers[5,6]. The hæmatocrit values remained fairly steady, falling in the post-perfusion period in lambs *B* and *C*. The very low value found in lamb *C* is probably due to fluid transfer associated with a hæmorrhage sustained prior to the start of the perfusion.

Considerable differences in the concentration of plasma constituents were observed in the three animals during the perfusion period; nevertheless, these did not appear to affect the well-being of the animal in the post-perfusion period. Thus in lambs *B* and *C* the blood lactic acid was very high and was associated with acidotic *p*H values. The high lactic acid concentrations may have been due, at least in part, to hypoxia before attachment to the circuit; they were not general in perfusions of younger fœtuses, which will be reported later. The plasma partial pressure of carbon dioxide was high in lambs *A* and *B* but fell to normal values in the post-perfusion period. These variations in plasma pCO_2 illustrate the difficulty in constructing a suitable oxygenator of low priming volume. The concentration of α-amino nitrogen and urea in the plasma remained steady during the perfusion but declined in the post-perfusion period. Hyperglycæmia (for the sheep) was present in lambs *B* and *C*. In all lambs the fructose concentration was seen to fall to zero within 24 h of their detachment from the placental site of synthesis[7]. This decline can be attributed to urinary loss[8,9].

Within 3 h of tying the umbilical cord all the lambs were capable of standing voluntarily. They were placed in a pen at room temperature and maintained on a 3–4 h bottle-feeding regimen with cow's milk. After some 24 h the animals were killed by intraperitoneal 'Nembutal'. At post-mortem no macroscopic changes were observed. The behaviour of the animals observed in the post-perfusion period, for example, suckling, walking, bleating, response to noise and light, interest in environment, etc., appeared to be unaffected by the perfusion as judged by our previous experience with non-perfused premature lambs[10]; and this was also clearly seen in lamb *C*, the twin of which, subsequent to the termination of perfusion, was removed by Caesarean section and maintained in the same pen.

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Application of the Arrhenius Equation to Rat Tail Tendon Collagen

An important and widely investigated property of collagen is its hydrothermal shrinkage. The concept of shrinkage temperature (T_s) has been developed as a manner of characterization of the hydrothermal stability of the fibre. Shrinkage temperature is determined by heating the fibre in water, the temperature of which is increased at the rate of 2 deg./min. The fibre is kept under observation, and the temperature at which contraction occurs is noted as the T_s of the fibre in question. Usually a range of temperature is recorded because the end point of reaction is not precise. Although useful, the concept of T_s is not a strictly valid one. Shrinkage curves constructed from data on length of fibres plotted against time at various temperatures clearly establish that the process of shrinkage of collagen is a rate phenomenon and that a true shrinkage temperature does not exist from a thermodynamic point of view. For this reason, Weir and Carter[1,2] examined the hydrothermal stability of collagen as a rate process. They stated that the reaction appeared to be of the first order and applied the theory of absolute reaction rates to the process. The half-time of the fibre shrinkage was taken as an index of the reaction rate at each of several temperatures and these $t_{1/2}$ values were substituted for rate constants in the Arrhenius equation.

ΔH, the heat of activation, was substituted for ΔE, the energy of activation in the equation, since ΔH is within 1 kcal/mole of being numerically equal to ΔE. A graph of log $0{\cdot}6932h/kTt_{1/2}$ against $1/T$, where k is Boltzmann's constant, h is Planck's constant, T is the absolute temperature and $t_{1/2}$ is the half-time of collagen shrinkage, yields a line that has a slope and intercept proportional to ΔH and ΔS respectively. In this manner, it was possible to determine values of ΔH and ΔS for kangaroo tail tendon and from the relationship of $\Delta F = \Delta H - T\Delta S$ to determine ΔF. Weir used this method to describe in thermodynamic terms the changes which occur in collagen as a result of the tanning of collagen by various chemicals, such as formaldehyde and non-ionic chromium complexes.

Surprisingly, although hydrothermal shrinkage of rat tail tendon has been investigated from many points of view, Weir's conception of the reaction as a temperature-dependent rate process has not been used in any of the investigations so far described. Kohn and Rollerson[3], using human diaphragm tendon, attempted to produce an Arrhenius plot at elevated temperatures but stated that the results were not sufficiently accurate to produce a linear relationship.

If such a plot could be satisfactorily produced for rat tail tendon collagen, a valuable tool would be made available for the examination of various pathological problems in which alterations of connective tissue become manifest. The values of ΔF, ΔH and ΔS could be compared in control and experimental animals, and if changes are noted these could be further compared with the reaction of the fibres in other situations such as ageing or cross-linking by exposure of collagen to physiologically occurring aldehydes.

The work reported here was undertaken to determine whether Weir and Carter's formulation could be applied to rat tail tendon collagen. This collagen was taken from Long–Evans strain rats of 60 ± 2 days of age. Collagen fibres removed from the tails were placed in distilled water in an ice bath and were divided into five groups of tendons about 10 cm long and 0·15 mm in diameter. These tendons were weighed before use in order to ensure uniformity of the group. The tendons were allowed to stand in distilled water at 4° C overnight and the following day shrinkage rates were determined in a test-tube in distilled water using a full visibility water bath at step-wise temperature gradients between 63°–67° C. A 50-mg lead weight was suspended from the end of the collagen fibre to keep slight tension on the tendon so that it should not coil, and its upper extremity was held in a slit rubber stopper. Especially prepared test-tubes graduated in 1-mm steps were used for determining length of the collagen fibre and time elapsed was determined by a direct-reading timer which was graduated in 0·01 min. Values of $t_{1/2}$ were obtained by interpolating the time corresponding to the fibre-length given by the equation $(l_0 + l_c)/2$, where l_0 is the original length and l_c is the fully contracted length.

Although there is some scatter of the $t_{1/2}$ values at any given temperature, the group means of six determinations (expressed in terms of log $0{\cdot}6932h/kTt_{1/2}$) at each of five temperatures usually produce linear relationship when plotted against the reciprocal of the absolute temperature. The best line through the 30 points was determined by the method of least squares and the slope and intercept were used to calculate the heat of activation, ΔH, and the entropy of activation, ΔS. From these values, the free energy of activation at 60° C, ΔF_{60}, was calculated as already described.

The linearity of the regression line was tested by comparing the variance within groups with the variance of the deviations of the group means from the estimated regression line. By this test, linearity was not rejected in 10 of the 13 animals examined. Table 1 gives the values found. The values calculated in the statistically non-linear group are not included, but all values in those animals were within two standard deviations of the values in Table 1.

Weir and Carter were of the opinion that the regression line constructed in this manner could be used to calculate a shrinkage temperature which would be of more significance than the T_s as ordinarily determined. The temperature at which half-shrinkage occurs in 1 min for any animal is noted in Table 1.

Table 1. Eight to Nine Week Age-group (60 ± 2 Days)

Animal	ΔH (kcal/mole)	ΔS (cal/mole deg.)	ΔF_{60} (kcal/mole)	T_s (1 min)	$Sy{\cdot}x$*
2 *BH*	81·597	174·90	23·328	63·3	0·093
4 *BH*	90·531	201·50	23·398	63·3	0·073
6 *W*	103·19	239·04	23·555	63·4	0·072
7414 *BH*	91·828	205·86	23·245	62·7	0·125
7433 *BH*	88·191	195·37	23·101	62·1	0·128
7464 *BH*	87·040	191·82	23·134	62·4	0·107
7468 *BH*	98·771	226·53	23·299	62·7	0·115
7845 *BH*	87·550	192·53	23·406	63·4	0·084
7848 *BH*	79·620	168·99	23·317	63·3	0·102
7849 *G*	89·496	198·26	23·445	63·5	0·073
Sum	897·814	1,994·80	233·228	630·1	
Average	89·781	199·48	23·323	63·0	
Standard deviation	7·08	21·1	0·139	0·493	

* Standard error of the estimate: The mean deviation of the thirty points of the plot of log $0{\cdot}6932h/kTt_{1/2}$ against $1/T$ from the estimated regression line.

Average values for activation constants of kangaroo tail tendon given by Weir are as follows: ΔH: 141 kcal/mole, *S.D.*, 15; ΔS: 349 cal/mole deg., *S.D.*, 43; and ΔF_{60}: 24·7 kcal/mole, *S.D.*, 0·6. The results given in Table 1 are lower than those given by Weir for kangaroo tail tendon. Preliminary investigations in this laboratory suggest that older animals give higher values for these activation constants than 60-day-old animals, which may indicate these values are dependent on age. The results for rat tail tendon of young rats are, however, clearly of the same order of magnitude as described for kangaroo tendon.

The extrapolation of rat tail tendon data to other mammalian collagen has been criticized by Klein and Curtis[4] because of greater solubility of rat tail tendon collagen than most other mammalian collagen in cold distilled water, dilute acetic acid, and following heat treatment in water at 60° C. While rat tail tendon collagen may have unique properties, it continues to be an extremely useful tool in researches on various aspects of the reactivity of collagen.

The preliminary results described here suggest that this technique may be of value in providing another parameter for comparison of rat tail tendon collagen with other mammalian collagen and may be of value in investigations of processes such as ageing of collagen and changes of collagen in various pathological states in the rat.

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Differences between Cortical Recruiting Responses observed during Wakefulness and Natural Sleep

In 1942, Morison and Dempsey[1] demonstrated that low-frequency stimulation (8–12 c/s) of the intralaminar thalamic nuclei of the anæsthetized cat diffusely evoked recruiting responses on the cerebral cortex. These long-latency, large surface-negative responses are distributed widely over the hemispheres and reach their maximal size after repetitive shocks. Long-continued stimulation at appropriate frequencies leads to a periodic waxing and waning of these potentials.

The intravenous administration of 5–20 mg/kg of either thiopental or pentobarbital can enhance the amplitude of recruiting responses and lower their thresholds[2]. Further, since stimulation of the brain stem reticular formation prevented their occurrence[3], it was assumed that recruiting responses could not be obtained in the unanæsthetized, non-immobilized animal. However, Evarts and Magoun[4] have demonstrated that stimulation of the intralaminar nuclei may evoke recruiting responses in normally awake animals. The relationship between the characteristics of recruiting responses and the various levels of consciousness still remains to be determined.

In the present investigation, low-frequency stimuli were delivered to non-specific thalamic system during the transition from wakefulness to sleep in 5 unanæsthetized and unrestrained cats with chronically implanted electrodes. The stimulating electrodes were implanted either into the n. centralis lateralis (CL), the n. centralis medialis (NCM) or n. centrum medianum (CM). Recording electrodes were placed on the cranial dura mater and into the dorsal hippocampus (DHIPP), and the mesencephalic reticular formation (MRF). EKG and EMG (neck muscles) were recorded simultaneously.

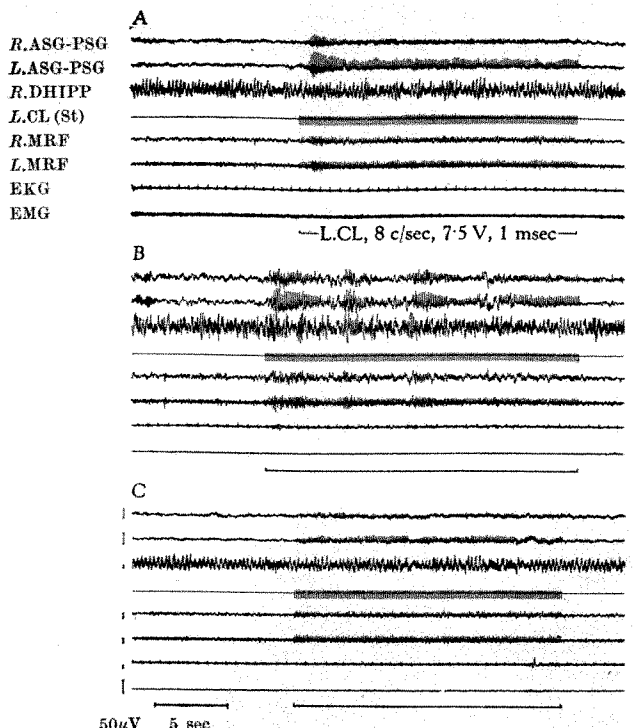


Fig. 1. Recruiting responses observed in the same animal following low-frequency electrical stimulation (8 c/s, 7·5 V, 1 msec pulse) of the left CL during wakefulness (*A*), slow-wave sleep stage (*B*) and fast-wave sleep stage (*C*). *B* recorded 20 min after *A* and *C* recorded 30 min after *B*. In all figures, recordings are bipolar. *R*, right; *L*, left; ASG, anterior sigmoid gyrus; PSG, posterior sigmoid gyrus; St, stimulating electrode

During wakefulness, stimulation was begun only when rapid low-voltage electrocortical activity and hippocampal rhythmic theta waves were present. In such instances recruiting responses were induced by 8-c/s stimulation of the non-specific thalamic nuclei (for example, stimulation of left CL, Fig. 1*A*). If the animal became attentive or moved, these recruiting responses were depressed.

If the animal spontaneously became more drowsy and entered the slow-wave stage of sleep successive thalamic stimulations produced clear-cut recruiting responses characterized by unmistakable waxing and waning of potentials (Fig. 1*B*). At the same time heart rate, respiratory rate and amplitude of EMG were decreased. In the intervals between stimulations both high-voltage slow-waves and sleep spindles are prominent.

After a prolonged period (usually more than one hour) of complete isolation in a sound-proofed environment, low-voltage fast cortical activity, hippocampal rhythmic theta waves, absence of EMG activity of the neck muscles, increased respiratory rate and sporadic twitching movements of the paws, ears, tail and vibrissæ are observed simultaneously in the sleeping animal. This fast-wave sleep stage has also been described as activated sleep[5], rhombencephalic sleep[6] or deep sleep[7]. Recruiting response elicited under these conditions following identical thalamic stimulations were of lower amplitudes and did not exhibit clear-cut waxing and waning of potentials (Fig. 1*C*).

Although the EEG pattern of the fast-wave sleep stage is similar to that of the awake, alert animal, dissimilar recruiting responses are elicited in these two circumstances. It has been reported that the arousal threshold for reticular stimulation increases during the fast-wave sleep stage[8,9]. Anæsthetic doses of barbiturates also increase this threshold, but at the same time they also enhance recruiting responses[2].

The phenomenon of the recruiting response has been regarded as synchronization of activity of the diffuse thalamic projection system[10]. It is suggested that depression of the mechanisms responsible for inducing

the recruiting response may be greater during the fast-wave sleep stage than during wakefulness or the stage of sleep associated with high-voltage, slow-wave electrocortical activity.

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HÆMATOLOGY

Antihæmophilic Globulin in Frozen Plasma

IN a recent communication[1], I described the effect of certain conditions on the stability of freeze-dried factor VIII (antihæmophilic globulin). Owing to the antigenicity of animal material and the restricted supply of human factor[2], other sources of this labile factor have been sought. Plasma, kept frozen after being separated from fresh blood, is now being widely utilized in the management of hæmophilia. This raises for consideration the effect of different methods of freezing on factor VIII and other blood-clotting factors.

Plasmas collected by the same person on the same day showed unequal loss of factor VIII activity after being stored in a deep freeze at −30° C for 48 h. In some cases this amounted to 50 per cent. The following two conditions were thought to contribute to this unexpected finding.

(1) *Variations in the composition of plasma.* These arise mainly from differences in: (*a*) the concentrations of protein, lipoids, minerals, etc., in the donor's plasma; (*b*) the packed cell volumes; (*c*) the ratio of acid-citrate-dextrose solution to the amount of blood collected in each bottle.

(2) *Rate and uniformity of cooling and thawing.* The rate of freezing varied according to whether the M.R.C. bottle was surrounded by air or liquid (Table 1). Frozen plasma (400 ml.) thawed in 4–4½ h at room temperature (18°–20° C). Under running tap-water (18° C), thawing occurred in 1 h. This was shortened to 30 min in a thermostatically controlled water-bath at 37° C. On lowering the temperature, the composition of a solid–liquid system changes by the deposition of solid(s). The separation of solid is accompanied by the evolution of heat which diminishes the rate of cooling. The temperature remains constant until the system is completely solid whereupon cooling proceeds further. The reverse occurs during thawing. These changes may lead to the development of *p*H, salt concentrations or other conditions which, depending on the temperature and the period (reduced by freezing and thawing in liquid, as mentioned here) during which they operate, adversely affect the blood-clotting factors. Some of these changes are given in Table 2.

Table 1. TIME REQUIRED FOR FREEZING 400 ML. PLASMA IN M.R.C. BOTTLE

Temperature (° C)	Surrounding medium: Air	Liquid
	Freezing time (min)*	
−20	180–240	60–70
−30	150–200	45–55
−40	150–170	30–40
−65	—	8–10†

* Complete solidification of contents. Longer periods are required for the contents to reach a homogeneous temperature equal to that of the surrounding medium.
† In order to avoid breakage of bottle, contents were mixed by rotating the bottle in the freezing liquid. In all other instances the bottle was not handled (usual procedure for freezing plasma in deep freeze in blood banks).

Table 2. LOCAL VARIATIONS IN PLASMA COMPOSITION DURING THAWING

Test	Sample* 1	2	3	4 (control)
*p*H	6·75	6·72	6·69	6·70
Total protein (g/100 ml.)	9·8	4·6	2·5	7·2
Blood-clotting factors (% of normal) †:				
Factor II, V	150	70	45	100
VII	150	80	45	110
VIII	120	45	8	80
IX	160	65	35	95
X	150	75	40	100
XII	170	80	40	100

* Freshly collected plasma frozen in a deep freeze at −30° C for 24 h. Liquid samples were obtained at intervals without mixing contents. The control (sample 4), however, was obtained after complete thawing and mixing of contents.
† For 100 per cent standard, fresh plasmas (collected in A.C.D. mixture) from 5 normal subjects were pooled.

That the foregoing two conditions affect the stability of factor VIII in frozen plasma was deduced from the consistency achieved and the negligible loss occurring on rapid pre-freezing of plasma in liquid and thawing in a bath at 37° C.

The effect of storage for longer periods was also investigated. At −30° C factor VIII activity showed 20 per cent and 50 per cent loss after 3 and 6 months respectively. Preservation at −40° C reduced this by approximately 20 per cent. Plasma factor IX activity, as measured by the thromboplastin generation test, was similarly affected. On the other hand factor IX, evolved after coagulation[3], was comparatively stable, less than 10 per cent being lost after 6 months at −30° C. Repeated thawing and re-freezing caused marked reduction in the activity of plasma factors V, VIII and IX, but had no significant effect on other blood-clotting factors. The latter were also highly stable at −30° C.

It would now appear that for maximal activity of factor VIII, fresh plasma should be pooled, rapidly frozen in liquid, and stored at −30° C or at −40° C for a period not exceeding 3 and 4 months respectively. Thawing with continuous mixing of contents at 37° C is desirable. Re-freezing of thawed material should be avoided.

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Oxidation of Reduced Glutathione by Acetylphenylhydrazine, Hæmoglobin and Erythrocytes

INCUBATION of erythrocytes, from individuals having the sex-linked, inherited deficiency of glucose-6-phosphate dehydrogenase, with oxidant drugs such as acetylphenylhydrazine (APH) produces a drop in the level of intracellular reduced glutathione (GSH)[1]. Beutler *et al.*[2] consider that APH itself has no effect, but modifies cell hæmoglobin to a complex, not methæmoglobin, which oxidizes GSH. Allen and Jandl[3], on the other hand, have indicated that these two compounds oxidize one another, and that the APH oxidation products aid GSH oxidation.

This communication describes the effects of APH, hæmoglobin and red cells on GSH in air and nitrogen. Heparinized human blood was centrifuged, the red cells washed in 0·9 per cent sodium chloride and used to prepare saline suspensions, 0·1 per cent saponin hæmolysates and

Table 1. Effects of APH, Hæmoglobin, Hæmolysates and Intact Erythrocytes on GSH-Levels

	Pre-incubation mixture	Additions	Dialysis period (h)	% original GSH Air	% original GSH Nitrogen
A	—	APH + buffer + GSH	0	85	96
		Hb(0·17 mM) + GSH	0	100	—
		Hb(0·34 mM) + GSH	0	93	—
		Hb(0·76 mM) + GSH	0	83	—
		APH + Hb(0·17 mM) + GSH	0	91	—
		APH + Hb(0·34 mM) + GSH	0	73	—
		APH + Hb(0·76 mM) + GSH	0	53	—
		APH + Hb(1·0 mM) + GSH	0	39	88
		APH + hæmolysate	0	69	113
		APH + erythrocytes	0	67	108
B	APH + buffer + air	GSH	0	36	70
		,,	2	70	—
		,,	24	97	—
	APH + buffer + N_2	,,	0	90	100
	APH + Hb(mM) + air	,,	0	40	71
		,,	2	44	74
		,,	48	—	74
	APH + Hb + N_2	,,	0	53	81

Flasks containing 50 mM potassium phosphate buffer, *p*H 7·4, mM APH and hæmoglobin (Hb) as indicated were pre-incubated for 3 h at 37° C with shaking (Exp. *B*), then incubated 30 min at 37° C with mM GSH (Exps. *A* and *B*). Flasks incubated with nitrogen were flushed for 5 min with the gas at 0° C prior to incubation and stoppered. 1 ml. aliquots were deproteinized with 1 ml. 10 per cent trichloroacetic acid, the supernatants filtered after centrifuging and assayed for GSH[4].

hæmoglobin solutions from hæmoglobin crystals[4]. All preparations were adjusted to mM hæmoglobin concentration.

Experimental details and results are given in Table 1. APH under nitrogen had little effect on GSH but in air some GSH was oxidized, even in the presence of 'Versene' (contrary to Allen and Jandl's[3] findings). The effect was markedly increased in the presence of hæmoglobin, indicating some interaction between APH and hæmoglobin to form an oxidant. There was no evidence for an additional cellular GSH-oxidizing mechanism other than hæmoglobin, such as glutathione peroxidase[5]. In fact, hæmoglobin had a stronger oxidant action than hæmolysates or intact red cells, possibly due to the presence of oxidizable substances in the latter. Hæmoglobin itself could oxidize GSH to some extent, the degree of oxidation increasing as the hæmoglobin concentration was raised from 0·2 to 1·0 mM; this effect was greatly enhanced if APH was also present. If APH in buffer was oxidized by pre-incubation in air, its ability to oxidize GSH on subsequent incubation was much greater and independent of the presence of hæmoglobin. However, while the yellow-coloured oxidation products of APH in air were dialysable, the products of interaction of APH with hæmoglobin in air were not. This may be due to the attachment of APH oxidation products to the protein molecule. On addition of trichloroacetic acid to the dialysed APH–hæmoglobin complex, the typical yellow colour of APH oxidation products was obtained in the supernatant. The hæmoglobin itself was partially oxidized to methæmoglobin under these conditions, since the methæmoglobin content increased from 8 per cent (present in freshly prepared solutions) to 23 per cent after 3 h incubation in air with APH.

These results, while indicating the formation of an APH-modified hæmoglobin[2], also confirm that APH oxidation products oxidize GSH. No evidence was obtained for the existence of a cellular GSH-oxidizing mechanism other than hæmoglobin.

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PATHOLOGY

Infiltration of Intra-abdominal Organs by Ascites Tumours

The ability of tumour tissue to invade 'normal' tissues, to enter circulatory systems of the body and to grow at sites near and distant from the primary source is one of the basic characteristics of malignant growth. However, this property is not diagnostic, for certain non-malignant (normal) tissues can permeate other normal tissues, for example, trophoblasts, macrophages, polymorphonuclear leucocytes. These invasive cell types are under the control of the body and although they may be actively destroying host tissues at times, their service to the organism is strictly regulated and made beneficial ultimately while malignant neoplasms are destructive ultimately. The capacity to infiltrate may be associated with behaviouristic changes in cells of tumours, shown *in vitro* (loss of contact inhibition)[1], changes in the surface properties[2] and possibly with enzymatic activity[3]. However, it is desirable to be able to study invasiveness of neoplastic tissue *in vivo*.

There have been several reports of the invasiveness of ascites tumours[4–6], reports of the infiltrative properties of ascites tumours following injury[7] and of solid tumour infiltration due to mechanical injury[8]. It appears that: (1) certain organs may be infiltrated more rapidly than others; (2) various treatments of the peritoneal surface render it more liable to be infiltrated. The work described here is concerned with ascites tumours since these transplantable tumours provide a reasonably reproducible system of cell invasion where tumour mass can be assessed easily and accurately. With this system it is possible to examine the relationship between tumour growth and the commencement of tumour cell infiltration and also the role of factors other than tumour growth in infiltration.

The rates of infiltration into eleven different regions of the abdominal viscera and peritoneum were found by inoculating 20 × 10^6 Ehrlich's ascites carcinoma cells into female *BALB/c* mice. The amount of tumour was estimated by total free cell counts after killing the animals (Table 1).

It is evident that host response, adhesion and infiltration of tumour cells go hand-in-hand, although in the case of the mesenteries only the thickest regions show host defence cells present along with adhering tumour cells. Tumour cells appear to adhere virtually all over the mesenteries within a very short time. The duodenum, uterus, ovary, kidney, liver and spleen appear to have almost entirely refractory capsules. At the very last stages of tumour development prior to host death (about 20 days after injection of tumour), there are still no tumour cells attached to the duodenum; the liver may have one or two clumps of tumour cells adhering to a very vascularized periphery and likewise the spleen.

The body wall has been studied most carefully. When tumour development has reached a total free ascites cell count of 400–500 × 10^6 cells, host response is seen by the infiltration of lymphocytes and leucocytes near the peritoneal mesothelium. The submesothelial region

Table 1

Days after inoculation	3	4	5	6	7	8	9
Total No. of *t* cells (× 10^6)	to 50	to 105	to 135	300–420	470–660	800–1,000	to 1,500
Body wall	–	–	–	–	*r*	*RA* (*r*)	*RAI* (*r*)
Liver	–	–	–	–	–	–	–
Kidney	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	– –	– –
Pancreas	*r*	*ra*	*RA*	*RAI* →			
Adipose tissue	*r*	*ra*	*RA*	*RAI* →			
Mesentery	*A* →						
Ovary	–	–	–	–	–	–	–
Uterus	–	–	–	–	–	–	–
Duodenum	–	–	–	–	–	–	–
Areolar tail of pancreas (containing lymph node and vessels)	*RAI* →						

(*r*), *r*, *R*, Weak, moderate and heavy host response (leucocytes). *a* and *A*, Few and many adhesions of tumour cells. *I*, Infiltration.

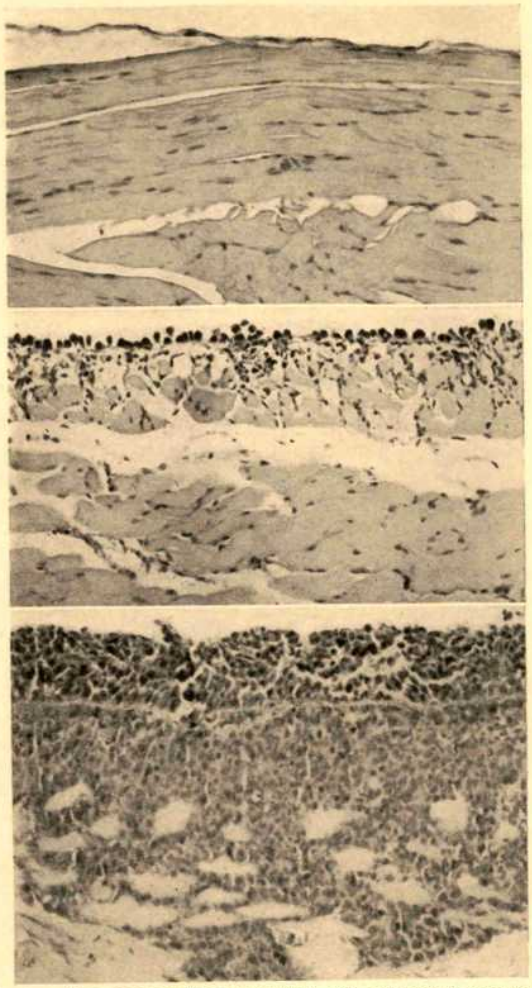


Fig. 1. Top, body-wall musculature and peritoneal mesothelium (detached at left) of normal animal and up to six days after intraperitoneal injection of 20 × 10^6 Ehrlich's ascites cells. Middle, layer of tumour cells adhering to the peritoneal mesothelium or subserosa. Eight days after tumour injection. Note œdema, vascularization and presence of host defence cells. Infiltration just beginning. Bottom, heavy invasion of ascites cells forming solid tumour around the body-wall musculature; 21 days after injection of tumour. Note the presence of delineation between peritoneal cavity and body wall

enlarges with apparent proliferation of the connective tissues, and also with œdema. The peritoneal mesothelium itself is hypertrophied. These changes occur very suddenly, not being evident on the 6th day (300–420 × 10^6 tumour cells) in light microscopic examination of histological sections, evident on the 7th day (400–660 × 10^6 cells) and pronounced with adhesions and infiltration of tumour cells after the 8th day (to 1,000 × 10^6 cells) (see Fig. 1).

The adhesions of tumour cells have been confirmed by electron microscopical and histochemical studies. Previously the difficulty had been to distinguish hypertrophied mesothelial cells from tumour cells in the light microscope. The electron microscopical study of this process of cell infiltration has confirmed the light microscope observations of the changes occurring in the subserosal connective tissue and has shown that the mesothelium is sometimes removed where tumour cells are adhering.

The presence of infiltrated tumour cells below the peritoneal mesothelium has been confirmed by subcutaneous implantation of pieces of body wall stripped of the peritoneal mesothelium from nine-day ascites tumour-bearing mice. The grafts grew up into typically subcutaneous Ehrlich carcinomata in the majority of hosts.

The same pattern of adhesion and infiltration has been demonstrated in other ascites tumours (Landschütz ascites in *BALB/c* mice, *MClM*, M_{AA} in *C3Ha* mice, Krebs's in *CBA/H* and *NK/Ly* in *C3H* mice).

The *MClM*,M_{AA} tumour became very hæmorrhagic during the course of transplantation into different strains of mice (though closely related strains). This hæmorrhagic nature has been correlated with the natural resistance of the host to the tumour[9]. It was found that development of the tumour was slower when the tumour induced hæmorrhage than when it was non-hæmorrhagic, but there was a stronger host reaction developing earlier after an inoculation of 20 × 10^6 tumour cells. Adhesions of tumour cells occurred earlier in hæmorrhagic tumours and infiltration began about the eighth day (as for non-hæmorrhagic tumours) when the total free cell count was about 200 × 10^6 cells. This is only about a third of the total tumour cell count in the non-hæmorrhagic tumours when infiltration begins.

The adhesion of ascites tumour cells to the peritoneum and their subsequent infiltration can be enhanced by injecting mechanical irritants into the peritoneum. When kieselguhr was injected in small doses intraperitoneally ascites tumour cells adhered to the peritoneum at points of injury within a short time (evident in sections taken at 1 day after inoculation). With larger doses of kieselguhr a more general adhesion of tumour cells was apparent with less localization at points where kieselguhr was actually wounding the mesothelium. This treatment of the peritoneum enhancing implantation of tumour tissue was shown in 1914 by Jones and Rous[8], who used fragments of solid tumours.

In conclusion, it has been shown that certain regions of the viscera are infiltrated earlier than others and infiltration seems to follow the development of host response to the tumour. Although ascites tumour cells may possess the surface characteristics and motility to enable them to invade, this process can only occur in the rather refractory tissues of the body wall[8] after the initiation of this host response. By altering the nature of the peritoneal surface and sub-mesothelial connective tissue[10] by immunological, chemical and mechanical means, it is possible to enhance the infiltration of tumours. Our more recent experiments have suggested the importance of the role of the immunological responses in the invasiveness of tumours in that, within certain limits, the early stages of invasion would appear to be facilitated by an increase in the immune responses of the host and retarded by a decreased response. These experiments will be reported later.

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Induction of Tumours of the Stomach in Rats and Mice by *N*-nitroso-*N*-alkylurethanes

The induction of squamous carcinoma of the stomach and of the œsophagus in the rat by *N*-nitroso-*N*-methylurethane (NMU)[1] and by some other nitrosamines[2] has been reported. Squamous tumours of the stomach have been known to develop following treatment with various other carcinogens; but very few instances have been reported of the induction of tumours of the glandular part of the stomach in rodents. Such tumours have usually been produced by direct intramural injection of carcinogenic polycyclic aromatic hydrocarbons[3]. One adenocarcinoma resulted after administration of 4-nitroquinoline-*N*-oxide through a stomach fistula to 16 rats which had in addition percutaneous applications of 20-methylcholanthrene[4]. Recently, Stewart *et al.*[5] induced adenocarcinomata of the glandular stomach in four out of 47 rats, given 2,7-*bis*(acetamido)fluorene in diet.

It is therefore of interest that in the course of further experiments using NMU under conditions similar to those described previously[1] a rat, which survived 2 years after the first of two oral doses (2·5 mg per dose, at 1-month interval), was found to have a large papillary growth on the glandular part, projecting into the lumen of the stomach (Fig. 1). The squamous part was very small, and the duodenum greatly distended. Microscopically, this lesion had characteristics similar to those described as adenocarcinoma by Baba *et al.*[4]; but no definite evidence of invasiveness was seen in the section. Other animals of the same series had squamous carcinomata, sometimes accompanied by foci of hyperplasia in the glandular part of the stomach. Various degrees of hyperplasia of the glandular part of the stomach have been noted already among rats in the previous series[1].

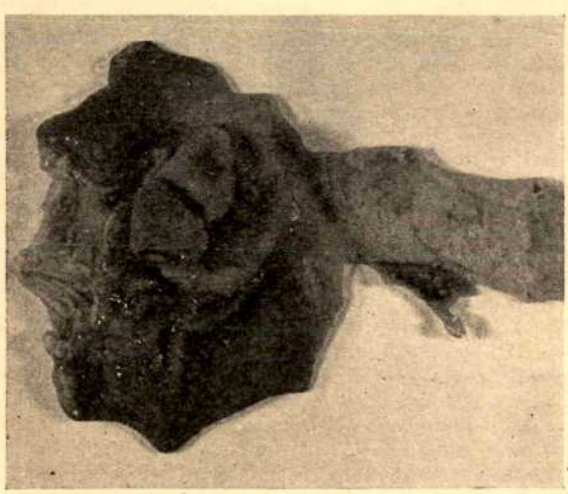


Fig. 1. Stomach and duodenum of a female rat which was given two doses of *N*-nitroso-*N*-methylurethane by stomach tube (2·5 mg per dose at 1-month interval). Killed 2 years after the first dose. Note the small size of the stomach, especially of its squamous part, the distended duodenum and the large papillary growth in the middle of the glandular part (× *c.* 1·3)

In view of the striking carcinogenic properties of NMU it was of interest to test its homologue, *N*-nitroso-*N*-ethylurethane (NEU). Among seven male white mice which survived 11–13 months after the first of four oral doses of NEU (1–2 mg per dose in aqueous ethanol, given in the course of nine months), three developed squamous carcinomata, one had in addition a sarcoma and one mouse had a papillary growth, with features suggestive of neoplasia, on the glandular part of the stomach, but again no evidence of invasion was seen.

Thus NEU induces in mice stomach tumours and lesions similar to those seen in rats given NMU. The type of tumour and the site of its appearance may depend on the site of the deposition of the *N*-nitroso-*N*-alkylurethane from the stomach tube. This can be introduced deep into the glandular part, or only as far as the squamous part of the stomach or the œsophagus. Thus, even a slight variation in the conditions of administration may determine the type and the site of tumours induced by nitroso-alkylurethanes. Like NMU[6], NEU undergoes decomposition with evolution of nitrogen in contact with —SH containing compounds or tissues, at neutral *p*H.

The induction of proliferative lesions of the glandular part of the stomach of mice and rats by these *N*-nitroso-*N*-alkylurethanes is of particular interest, and suggests that in time unequivocally malignant tumours might develop among the surviving animals.

I thank Dr. P. N. Magee for the microscopic evaluation of the lesions.

Note added in proof. Since this communication was submitted an undoubtedly malignant adenocarcinoma of the glandular stomach, with local spread, was found in a rat treated with NMU.

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Pinealoma : a Variety of Argentaffinoma?

Serotonin (5-hydroxytryptamine) contained in the gastrointestinal tract is intimately associated with the Kultschitzky type of cells[1]. Carcinoid tumours arise from such cells so their serotonin content is very important[2]; apparently, the majority, or maybe all, the symptoms of the so-called carcinoid syndrome must be attributed to the presence of serotonin at the level of the tumour[3].

Histochemically, the serotonin contained in Kultschitzky cells and in carcinoid tumours may be demonstrated as intracytoplasmic granules intensely argentaffin and faintly positive or negative to chromaffin reaction[4].

The parenchymal cells of pinealoma (Fig. 1) behave as Kultschitzky cells and carcinoid cells; thus, they reduce silver ammoniacal solutions and do not show affinity for chromium salts. For that reason it is possible that they also contain serotonin. This substance is very abundant in the nervous tissue of the brain, especially at the level of the basal nuclei, close to the pineal body. Here, serotonin could be secreted, stored or eliminated by the brain[5].

We do not know of any reference relating serotonin to either pineal body or pinealomata, nor any which refers to a clinical syndrome comparable with the carcinoid tumour in pinealoma. Since this syndrome was not discussed until 1956, it is possible that it has not been noticed in patients with pinealoma either; on the other hand, only in malignant carcinoids having hepatic metastases has this syndrome been demonstrated, and no cases of metastatic pinealoma are so far known.

Pinealoma is a rare tumour. From Frazier-Grant's collection we were only able to utilize one, and from Vincent's collection we obtained five more. Since all of them had been fixed in 10 per cent formalin for a long time we could not undertake biological determinations of either serotonin or catecholamines. On the other hand, the content of serotonin and of norepinephrin is relatively low in the normal pineal body, but such a consideration

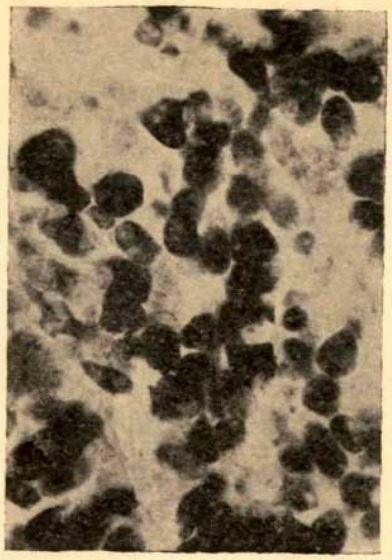


Fig. 1. Pinealoma. The parenchymatous cells of the tumour have numerous intracytoplasmic argentaffin granulations (Rio Hortega method)

does not clarify the histochemical significance of argentaffin cells in pinealomata, since in the normal organ the number of argentaffin cells is scarce, and its morphology is notably different from that of neoplastic argentaffin cells.

Similar differences and resemblances are found in other tumours formed by argentaffin cells. Special mention should be made of carotid body chemodectoma. In this tumour, parenchymal cells emit a long process ending in a juxtavascular club-shaped thickening[6], and they are quite similar to the normal pineal body parenchymal cells[7], as demonstrated by tissue culture[8]. Such unipolar cells are associated in chemodectoma with argentaffin cells, in such a way that in the latter they closely embrace the former[9]; in the narrow space between the embracing argentaffin cell and the unipolar chief cell, nerve endings running towards the Hering nerve are located. In the case of pinealoma, synaptic complexes similar to those in chemodectoma are demonstrated utilizing Rio Hortega's method. Moreover, both the carotid body and normal epiphyses receive abundant efferent nerve supply. Notice the similarity between the normal carotid body and chemodectoma on one hand, and between pineal body and pinealoma on the other hand. If we add to such similarities the observation by Hughes and Smith[10], who have described a chemodectoma in the pineal region, it seems justified to insist on the hypothesis of the aforementioned authors according to which the pineal body could be a chemo-receptor organ.

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IMMUNOLOGY

Effect of 8-Azaguanine on the Specificity of Antigens synthesized by *E. coli* B/r_2

Roblin *et al.* synthesized azapurine analogues of naturally occurring purines and showed their ability to inhibit the growth of *E. coli* and *S. aureus*[1]. Since then many investigators have utilized analogues of purines and pyrimidines in studies of cell metabolism and protein biosynthesis[2–7]. The purpose for our initiating an investigation of the effects of purine analogues on growing cells was to explore the possibility of using such analogues to induce metabolic errors in the biosynthesis of protein antigens that would result in immunologically altered proteins. It was anticipated that this work would also contribute to our knowledge of protein biosynthesis because immunochemical methods are particularly well suited for differentiating even closely related substances on the basis of their immunological specificities.

E. coli B/r_2 and Ehrlich–Lettré mouse tumour ascitic cells were selected as examples of bacterial and mammalian cell types. The following is a preliminary report on the effects of 8-azaguanine on antigens synthesized by *E. coli* B/r_2.

Cells were cultured in 2-l. Erlenmeyer flasks with continuous shaking. Fifteen ml. of a 12-h. old culture were used to inoculate 500 ml. of a synthetic medium, and growth was continued for 48 h at 36° C.

E. coli cells were similarly grown in a medium containing 600 μg/ml. of 8-azaguanine, a concentration resulting in a 50 per cent decrease in the turbidity of the cell suspension after 10 h of growth. The cells were gathered by centrifuging the cultures, washed twice with citrate–saline buffer and resuspended in buffer containing sodium cholate in a final concentration of 1 per cent to minimize the degradation of RNA by nucleases. The suspension was sonicated for 2 h and then centrifuged to give an extract.

The extract of cells grown in the absence of 8-azaguanine was used to immunize rabbits. Each rabbit was injected intravenously three times weekly for four weeks, receiving a total of 18 mg nitrogen. Blood was collected from the rabbits one week following the last injection.

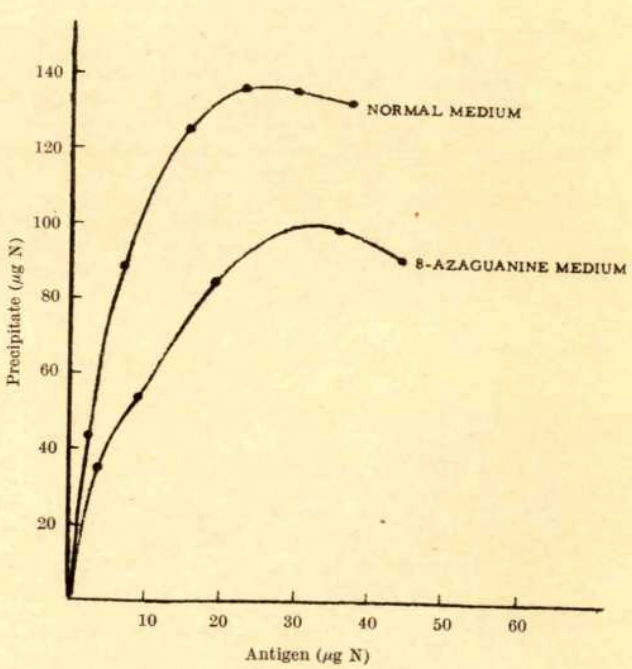


Fig. 1. Quantitative precipitin analysis of antigens in extracts of *E. coli* collected from a normal growth medium or one containing 600 μg/ml. of 8-azaguanine. The ordinate is the quantity of precipitate formed per reaction mixture, each containing 0·2 ml. rabbit antiserum produced by immunization with an extract of normal *E. coli* cells

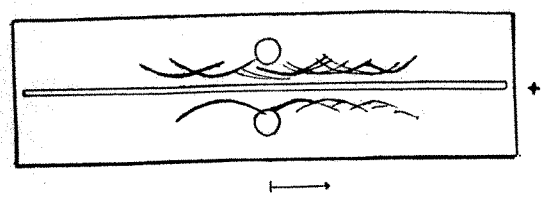


Fig. 2. Immunoelectrophoretic analysis of antigens in the same extracts of *E. coli* used for the quantitative precipitin analysis shown in Fig. 1. The extract of cells grown in a normal medium was placed in the upper circular well and the extract of cells grown in a medium containing 8-azaguanine was placed in the lower well. The rabbit anti-normal *E. coli* extract was added to the central trough parallel to the direction of migration toward the anode (+)

The resulting antisera were analysed for precipitating antibodies, reactive with antigens in extracts of cells grown in the absence or presence of 8-azaguanine, by the quantitative precipitin method of Heidelberger and Kendall[8] or by the gel-diffusion methods of Grabar and Williams[9] and Ouchterlony[10].

The results of the quantitative precipitin analysis are shown in Fig. 1. The maximum amount of precipitate formed during the reaction of antiserum with the extract of cells grown in a normal medium was significantly greater than that obtained in the reaction with the extract from cells grown in the presence of 8-azaguanine. This difference was due either to the deletion of certain antigens as the result of the presence of 8-azaguanine or to a marked alteration in their immunological specificity. A comparable analysis using antiserum against an extract of cells grown in the presence of 8-azaguanine is needed before one can decide between these two possibilities.

In order to determine the number of antigens affected by the presence of 8-azaguanine in the culture medium, the reactions between antiserum and extracts of the two types of cells were followed by immunoelectrophoresis. The results of this analysis (Fig. 2) showed quantitative differences in the concentrations of some of the antigens, particularly one in a slow-migrating fraction. The antiserum was absorbed with an excess of the 8-azaguanine cell extract and analysed by the double diffusion method of Ouchterlony. No antibody was detected in the absorbed antiserum against any antigen in the 8-azaguanine cell extract but it contained at least three antibodies against antigens in the normal cell extract. Thus, the 8-azaguanine was shown to affect markedly only a small number of the antigens synthesized by *E. coli* under the growth conditions used. Chantrenne[6] also reported a differential effect of 8-azaguanine in his examination of the enzymatic specificity of penicillinase and catalase synthesized by *B. cereus*. Because of the great sensitivity of the immunological specificity of an antigen to changes in its structure and because so many cell constituents, regardless of their other biological properties, are potentially immunogenic, the examination of the effects of antimetabolites on cellular constituents as antigens makes it feasible to study their possible effects on the biosynthesis of a greater number of substances.

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RADIOBIOLOGY

Effect of Ultra-violet Light on Skin Collagen

It is now widely accepted that the uncovered sun-exposed skin of man 'ages' more rapidly than covered skin. The most marked effect of such ageing is atrophy of dermal collagen. The mechanism of this atrophy is not known, and we therefore examined the effect of irradiation with ultra-violet light on the collagen fractions of isolated mouse skin.

Thirteen male and nine female heterozygous pigmented hairless mice (obtained from the Institute of Animal Genetics, Edinburgh) were killed by breaking their necks. The skin was incised ventrally and dorsally along the mid-line and stripped off to the ears, snout, paws and tail. Fat was pared off, and the two approximately equal half-pelts from each mouse were blotted, weighed, and pinned out on to cork boards, epidermis uppermost. One half-pelt from each mouse was covered with black paper and the other was left exposed. The skins were put in a deep freeze (temperature $-20°$ C), the top of which was removed. After 30 min for temperature equilibration, ultra-violet light from a medium-pressure Hanovia mercury arc lamp (504/4 type arc tube) was directed vertically down from a distance of 32 cm on to the covered and exposed skins for a period of 15 h at a rate of $1{\cdot}66 \times 10^5$ ergs cm^{-2} sec^{-1}. After thawing the half-pelts were finely cut with a scissors, and the saline and citrate soluble collagen fractions were extracted by the method of Jackson[1]. The residue was heated with 5 per cent trichloroacetic acid and the collagen was measured as hydroxyproline in the residue and in the saline and citrate fractions by the method of Neuman and Logan[2]. A 100 μg/ml. solution of hydroxyproline in 0·46 *N* hydrochloric acid was exposed to the same degree of ultra-violet irradiation for 14 h at laboratory temperature in a soda glass tube. There was no change in the concentration of hydroxyproline.

After thawing, it was noticed that the skins which had been exposed to ultra-violet light looked and felt leathery, and were difficult to cut, while the unexposed control skins appeared to be unchanged.

Table 1. Skin Collagen Fractions in μg Hydroxyproline/g of Original Half-pelt

Mouse	Sex	Control half-pelt			Ultra-violet exposed half-pelt		
		Saline	Citrate	Residue	Saline	Citrate	Residue
1	M	53	120	13,300	37	<10	14,500
2	M	61·5	172·5	11,600	10	<10	8,540
3	M	46	175	12,430	32·5	38	12,900
4	M	54·5	149	13,020	—	<10	12,600
5	M	—	82	7,500	—	<10	6,910
6	M	—	55·6	6,300	—	<10	3,880
7	M	—	49·5	4,350	—	<10	6,350
8	M	—	62	6,675	—	<10	—
9	M	—	50	3,550	—	<10	4,060
10	M	—	187	5,920	—	<10	5,730
11	M	25·7	148	11,300	17·2	27·2	10,766
12	M	19·8	106	9,250	13·1	15·0	9,425
13	M	31·2	84	8,400	14·4	18·7	8,880
1	F	26	53	5,050	<10	<10	5,230
2	F	40	115	4,800	<10	<10	7,650
3	F	29	50	7,150	<10	<10	6,720
4	F	38	103	7,270	<10	<10	5,570
5	F	—	67	6,150	—	<10	10,650
6	F	—	44	8,810	—	<10	8,700
7	F	17·9	37	3,240	<10	<10	3,580
8	F	24·6	49	4,070	15·9	<10	5,650
9	F	25·4	56	3,290	13·1	13·1	4,650

The chemical findings (Table 1) are that the saline and citrate collagen fractions were decreased considerably after exposure to ultra-violet light. Presumably, therefore, ultra-violet light precipitates an insoluble collagen from the saline and citrate soluble collagens. It remains to be seen whether this is due to an increased number of cross-linkages within the collagen fibrils or to denaturation of the collagen. Although the decrease in the soluble collagen fractions was considerable, in absolute terms, the amounts concerned were too small to give a detectable increase in the insoluble collagen residue (Table 1). Although there

was no clear-cut sex difference, the collagen fractions tended to be less in the females than in the males both before and after exposure to ultra-violet light. This is in keeping with our observations on human skin (Shuster and Bottoms, in preparation). Whether or not the 'ageing' of human skin exposed to the Sun is related to the effect which we have observed, it is clear that ultra-violet light has a profound effect on the physico-chemical state of the dermal collagen.

We thank the Medical Research Council for a grant to one of us (S. S.).

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Influence of Temperature and Medium on the X-ray Sensitivities of Leukæmia Cells *in vitro*

Hollaender and Stapleton[1] demonstrated with various strains of *E. coli* that the fraction of cells which survived (that is, retained ability to form colonies) from a given dose of X-rays could be significantly increased if the cells were maintained for some hours before being plated out at either a sub-optimal temperature or in medium enriched with yeast extract (see also Alper and Gillies[2], for restorative action of different media). We have now observed what would appear to be comparable phenomena with murine leukæmia cells *in vitro*. An effect of serum on the radio-sensitivity of mammalian cells has also been found by de Oca *et al.*[3].

Fischer[4] developed a medium in which *L*5178 *Y* cells originating from a radiation-induced leukæmia in a *DBA*/2 mouse can be cloned and grown *in vitro* in suspension culture. These cells are still strain-specific to *DBA*/2 mice in which a tumour can be produced from one cell. Alexander and Mikulski[5] found that in the original Fischer medium these cells showed a semi-logarithmic dose-survival curve with a small initial shoulder typical[6] for mammalian cells in culture. Two mutants, both diploid, and differing by a factor of 2·1 in radiosensitivity, were studied[5]. These are referred to as the sensitive and resistant strains. Following Fischer, it was found that peptone could be eliminated from the medium. This produced a small change in the survival curve and mainly affected the extrapolation number (Fig. 1). Extensive dialysis and ultracentrifugation of the horse serum did not alter the radiosensitivities further, but replacement of the horse serum by the following bovine plasma fractions: V; 0·36 per cent (serum albumins); II; 0·36 per cent (γ-globulins); IV–4; 0·005 per cent (α_2-globulins, etc.) obtained from Armour Laboratories did produce pronounced changes in the X-ray sensitivities (Fig. 1). In this 'simplified' medium the mean doubling time of a sensitive cell suspension was unaltered (11–12 h) but that of the resistant strain was lengthened from 13–14 to 18–20 h at 37° C. Cells grown and maintained after irradiation in the simplified medium were more sensitive to X-rays (220 kVp.); the survival curve becomes steeper in both cases whereas the extrapolation number is little affected (see Fig. 1). The sensitive and resistant strains respond equally to the replacement of horse serum by the bovine plasma fractions when this effect is expressed as the change in the slope of the exponential portion of the survival curve, that is, the dose reduction factor due to the change in medium is 1·7 and 1·8 for the sensitive and resistant strains respectively. For technical reasons it is difficult at present to establish whether the full

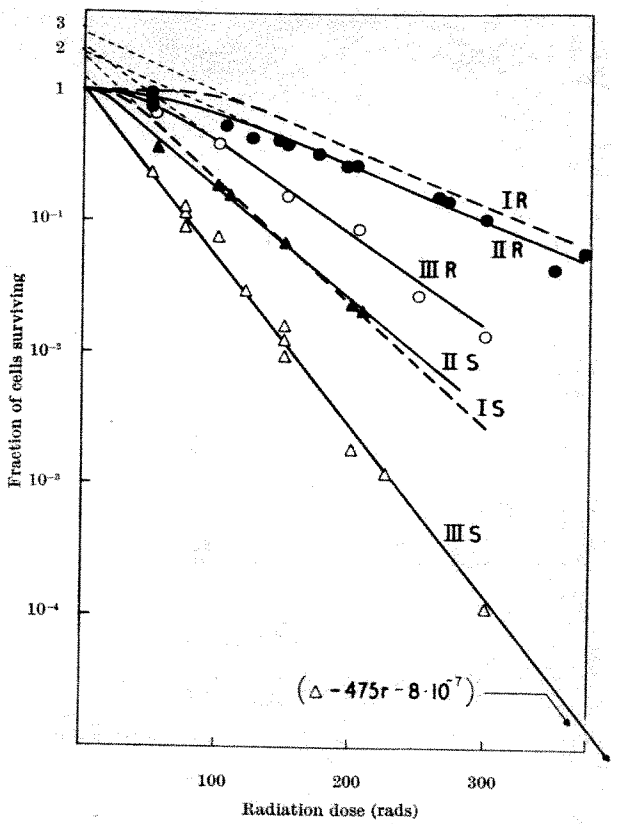


Fig. 1. Influence of culture medium on radiosensitivity. Lymphoma cells grown on Fischer medium with the following additives: I, peptone and horse serum (data from ref. 5); II, dialysed and ultracentrifuged horse serum; III, bovine plasma fractions (see text). Curves labelled *S* and *R* represent the sensitive and resistant mutants respectively

sensitizing effect is obtained by a post-irradiation change of medium, but preliminary experiments indicate that the medium primarily influences post-irradiation processes.

In the simplified medium the survival of the sensitive cells could be varied by changing the temperature at which the cells were maintained in the first hours after irradiation with X-rays. An influence of temperature was first observed when cells were maintained both before and after irradiation at 34° C (mean doubling time 18 h). The cells in these conditions were 1·5 times more resistant (that is, D_0 at 34° C is 51 rads as opposed to 35 rads at 37° C) than those grown normally at 37° C. It became apparent that the major effect of temperature was a post-irradiation phenomenon since cells grown at 37° C showed a higher survival even when placed at the lower temperature only after exposure to X-rays.

Fig. 2 shows that the maximum recovery is attained by keeping cells for 9 h post-irradiation at the lower temperature before returning them to the standard temperature of 37° C. The reverse phenomenon (that is, 'sensitization') can be brought about by placing cells grown at 34° C for various times after irradiation at 37° C before returning them to 34° C (Fig. 2). Again, a period of some 9 h seems to be necessary for the full effect of temperature on survival to manifest itself. Moreover, the cells grown at 34° C, but kept for 9 h at 37° C after irradiation, seem to be somewhat more sensitive than those maintained at 37° C throughout. In other words, this treatment seems to exert a small sensitizing effect while lowering the temperature for some hours after irradiation increases survival. For the resistant cells the influence of post-irradiation temperature in the range studied is very much less than with the sensitive cells and the slopes of the survival curves at the two temperatures are the same within experimental error.

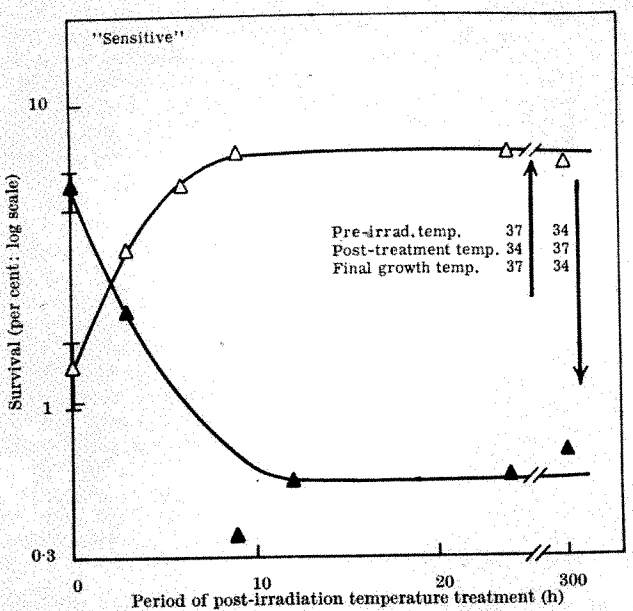


Fig. 2. Influence of post-irradiation temperature on survival of the sensitive lymphoma cell strain after a dose of 150 rads of X-rays. (Cells grown in 'simplified' medium.) △, Cells grown at 37° C and kept after irradiation for various times at 34° C; ▲, cells grown at 34° C and kept after irradiation for various times at 37° C

Indeed, if the two strains of cells are compared in the simplified medium at 34° C, then the difference in their radiation sensitivities is 1·3 (D_0 = 65 and 51 resp.) as opposed to 1·8 (D_0 = 62 and 35 resp.) at 37° C.

Ten years ago Hollaender and Stapleton[1] had found a parallel situation with *E. coli B*, where lowering the post-irradiation temperature aided the recovery of the sensitive strains much more than that of the resistant strain, so that under optimum recovery conditions the strains showed almost identical radiation sensitivities. Their conclusion that the difference in radiation sensitivities of the *E. coli B* mutants is related to post-irradiation recovery potential would appear to apply also to the different lymphoma cell strains in the simplified medium.

That post-irradiation conditions determine the magnitude of the radiation lesion has been known for forty years (cf. ref. 7). The first accurate measurements of such processes in mammalian cells was provided by Elkind and Sutton[8], who found that the sub-lethal damage sustained by cells and manifested as the shoulder in the survival curve was restored under normal culture conditions in some twelve hours after irradiation. We also found this phenomenon with the resistant strain of lymphoma cells in the full medium where the survival curve has a pronounced shoulder. Thus two exposures of 250 r. separated by 6 h gave a survival of 9 per cent as opposed to 1·2 per cent by a single dose of 500 r.

The experiments described here indicate the existence of two post-irradiation recovery mechanisms which are distinct from the Elkind and Sutton effect, since not the extrapolation number but the slope of the survival curve is altered, by medium composition and temperature.

Our experiments suggest that the mechanism of increasing survival of irradiated cells grown on the simplified medium by placing them in the full medium is different from that obtained by lowering the post-irradiation temperature since both mutants respond to medium changes while only the sensitive mutant is affected by temperature in the range studied.

The term 'recovery' has been used rather widely in this connexion since changes in survival can be brought about in many ways and the possibility that the metabolic processes responsible for developing the primary radiation injury are altered cannot be excluded.

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Freeze-drying and Radiation Protection

Gamma-irradiation of *Bacillus subtilis* (N.C.T.C. 8236) spores revealed a marked protection if spores are first freeze-dried from certain carbohydrates. Freeze-drying from water did not produce a statistically significant effect. Protection was demonstrated after freeze-drying

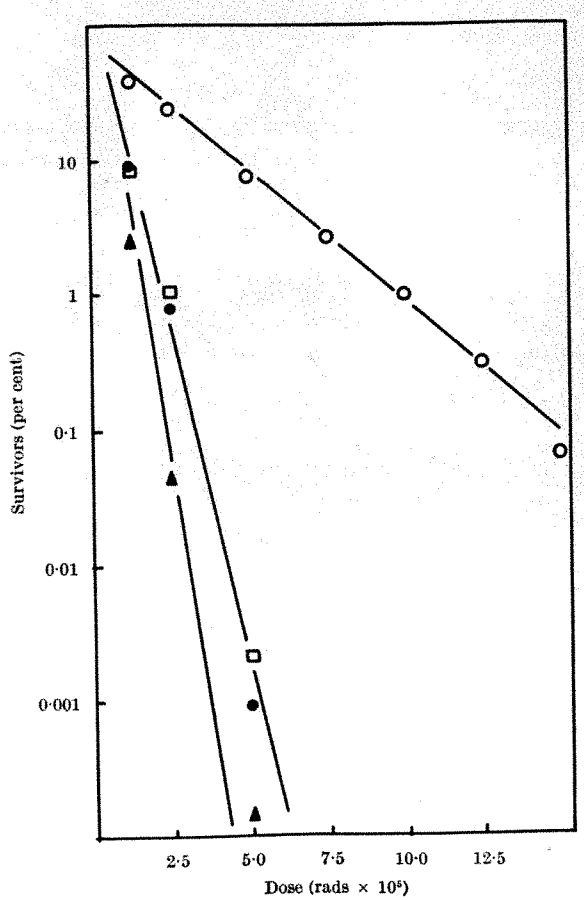


Fig. 1. Gamma-irradiation of *Bacillus subtilis* spores. Regressions of log per cent survivors against radiation dose for: □, aqueous suspension of spores; ●, suspension of spores in 5 per cent aqueous glucose; ▲, spores freeze-dried from aqueous suspension; ○, spores freeze-dried from 5 per cent aqueous solution of glucose

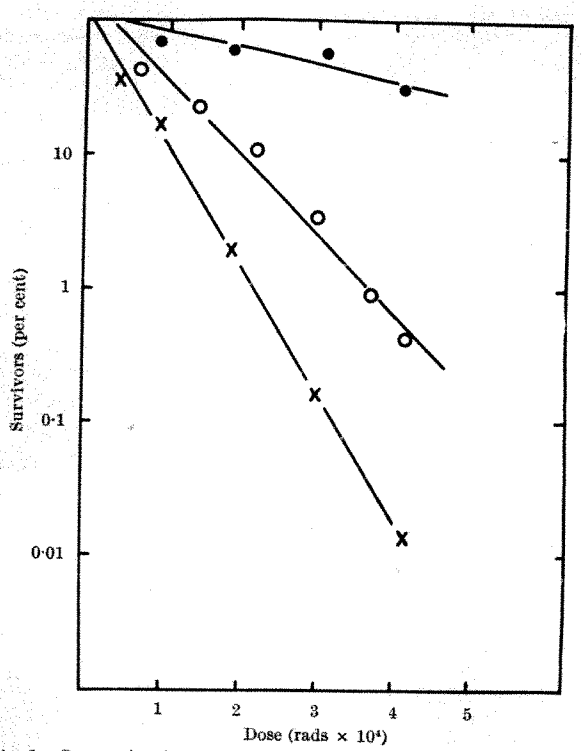


Fig. 2. Gamma irradiation of *Escherichia coli*. Regressions of log per cent survivors against radiation dose for: ○, aqueous suspension; ×, suspension in 5 per cent aqueous solution of glucose; ●, cells freeze-dried from 5 per cent aqueous solution of glucose

0·1 ml. samples at 0·01 mm mercury over phosphorus pentoxide for 16 h from 5 per cent aqueous solutions of glucose, lactose, or fructose, but not from maltose.

Viable counts were performed by a surface spread method (on 'Oxoid' peptone agar) and a tube dilution method ('Oxoid' peptone water). The former gave a higher recovery in every case, although conversion to per cent survivors resulted in there being no significant difference in the slopes of the regressions (log per cent survivors against dose) for the two counting methods under identical conditions.

The protection is apparently associated with the production of a 'glass' during the freeze-drying process. Maltose did not form a glass.

Subsequent work revealed a similar protective effect on *Escherichia coli CN* 1539 after freeze-drying from glucose.

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BIOLOGY

Behavioural Differences between the Larval Stages of *Cirriformia tentaculata* (Montagu) from Drake's Island (Plymouth Sound) and from Southampton Water

A three-year investigation into the ecology of the cirratulid worm, *Cirriformia tentaculata* (Montagu), occurring on an inter-tidal mud-flat in Southampton Water, failed to reveal any trace of pelagic larval stages. These free-swimming stages have been shown by Wilson[1] to exist in a population of worms from Drake's Island.

The development of 'Southampton animals' from fertilized eggs was closely followed in the laboratory. The eggs and young worms were found to be morphologically identical with those from the Drake's Island population (illustrated by Wilson). However, the behaviour of the lecithotrophic trochophores and other larval stages was somewhat different. About 24 h after fertilization, eggs from the Plymouth population became strongly swimming trochophores. They remained pelagic as the trunk elongated, and did not settle on the bottom until they had lost most of their cilia, approximately 7 days later. On the other hand, trochophores which developed from eggs of the Southampton population remained on the bottom of their containers. At this stage, agitation of the water in the rearing vessels could sweep the trochophores into suspension. However, the larvæ were unable to maintain themselves by swimming and would sink to the bottom again within a few seconds. The post-trochophore stages possessed well-developed neurotrochs on which they glided slowly over the bottom of the culture dishes. Only strong agitation of the water could sweep these young worms into suspension.

It may be argued that the young animals did not leave the bottom of the containers because they were less vigorous than those in Wilson's cultures. However, the 'Southampton larvæ' (which were the product of naturally spawned males and females), if anything, grew at a slightly faster rate than the Plymouth forms. The larvæ remained on the bottom of their containers regardless of light conditions, temperature, or the oxygen content of the water.

It is known that certain species (for example, *Spio martinensis*, *Pygospio elegans*) produce pelagic and non-pelagic forms according to the time of the year[2,3]. Such a situation does not exist in the Southampton population of *Cirriformia* where non-pelagic larvæ are produced throughout the breeding season. No observations for a continuous yearly period are available for Plymouth *Cirriformia*.

Clearly, the larval stages of the Southampton population are demersal, the behavioural dissimilarity between the two populations being due perhaps to the existence of two distinct 'physiological races' within the species. Indeed, adult *C. tentaculata* from Plymouth which I have seen are much larger than the biggest adults from Southampton.

I thank Dr. D. P. Wilson and Prof. J. E. G. Raymont for their interest.

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Nemasperm and Pituitary Inhibition

It has been shown that bovine semen—as well as that of man, dog, rat and cock—displays, among its manifold non-gametic biological activities[1], a constant and fairly strong pituitary-inhibiting action[1–3]. This effect does not seem to be proportional to the very low androgenic activity of the semen. Its relation to the well-established œstrogenic activities appears also always to be questionable[2].

It seemed to be of some interest to gain more information on this peculiar activity.

The inhibiting activity of the total semen, or centrifuged, washed and resuspended nemasperm in saline was tested on 120 parabiont pairs (female spayed Wistar albino rat 120–140 g + female immature 60–80 g), forming 9 groups, each of 7–15 pairs, as well as on the gonadotrophin-level in the urine of female spayed Wistar albino rats of 150–180 g: extractions and determinations were according to Loraine and Brown[4,5]. Investigations were also carried out on the spayed female

Table 1. Effect of Some Fractions of Bovine Semen on the Pituitary Inhibition by Parabiotic Albino Rats

No. of pairs	Depr.	Delip.	C-fr.	Homo-gen.	Dial.	Temp. 100°	Temp. 120°	Per cent of falling inhibi-tion (controls = 100)
12	– –	– –	– – –	– – –	– – –	– –	+	100
10	– –	– –	– – –	– – –	– – –	+	– –	60
8	– –	– –	– – –	+	+ (*)	– –	– –	40
8	– –	– –	– – –	+	+ (†)	– –	– –	10
20	+	+	+	+	– –	– –	– –	40
8	+	– –	– – –	+	– –	– –	– –	55

(+ meaning the type of handling.)
* Summer experiments.
† Winter–spring experiments.
Depr., salt-deproteinization; or trypsin digestion and salt deproteinization. Delip., washed 3 times with benzene, 5 vol. C-fr.: conjugate-œstrogens free: incubated 48 h at 37° C with β-glucuronidase + phenosulphatase.; precipitated with sodium sulphite; washed with benzene. Homogen.: Homogenized: centrif. low speed; frozen −40°; ground with carborundum powder; resuspended in the supernatant seminal plasma. Dial.: ultrafiltrated under pressure, φ < 10 μm.

adult Wistar albino rats, using the ascorbic acid depletion method, according to Roe and Kuether[6,7]; and also on the weight of the testis in the immature cockerels, totalling 100 animals, in 2 groups. Finally, we recorded the usual histological modifications in the pituitary of the spayed adult female rat. As a result of this first investigation, it was possible to see that 0·3–0·6 ml. of bull total semen (or reconstituted; also as centrifuged, grounded and homogenized nemasperm) when given intramuscularly, or intraperitoneally, in a single injection, can induce, for example, the following modifications: group of 8 pairs of animals killed after 5–7 days: mean ovarian weight (controls), $48{\cdot}57 \pm 3{\cdot}38$ mg (*S.E.*); mean ovarian weight (treated), $22{\cdot}46 \pm 4{\cdot}23$ mg, $t = 4{\cdot}90$ ($P \leqslant 0{\cdot}01$).

For the gonadotrophin-level, work was carried out on 110 animals: the results, expressed as μg of acetone powder of rat pituitary (see quotations[6]), were as follows: Basal: 26·40 ± 3·8 μg; treated: 12·0 ± 2·2 μg. With the ascorbic acid depletion, we investigated 15 animals.

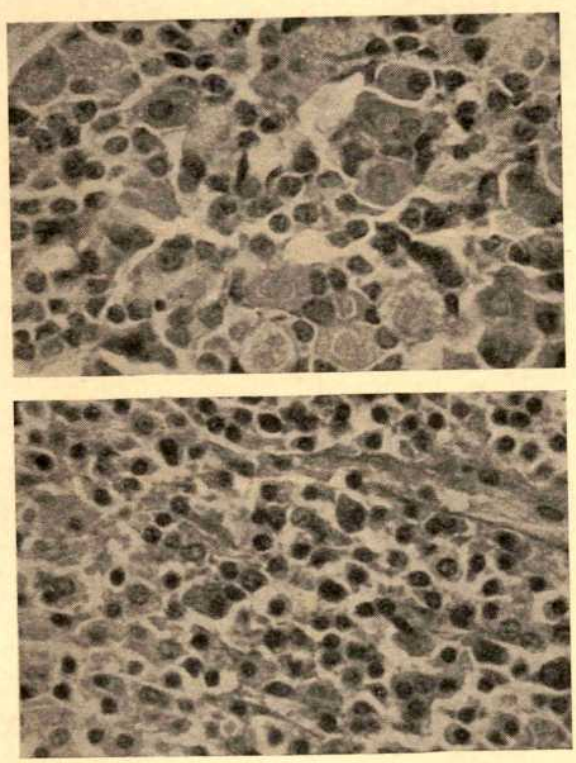


Fig. 1. *A*, control; *B*, treated animals. Spayed rats, 120 g, injected at the 1st, 2nd, 3rd and 4th day with 0·5 ml. of the deproteinized, lipid and œstrogen conjugated-free extract from nemasperm homogenate. Animals killed after 21 days. Hypophysis fixed with Mueller phenolic; col. with periodic acid, Pearse technique (Dr. Spazzoli)

Control: $47{\cdot}5 \pm 5{\cdot}1$/100 mg ovarian weight. Treated: $37{\cdot}20 \pm 4{\cdot}6$/100 mg ovarian weight. Finally, we used 100 Kimber cocks, 45 days old: each animal was given a single dose of 1·5 ml. bovine total semen, intramuscularly. From 50 animals, killed after 50 days, a relative weight of testis (controls) of $0{\cdot}406 \pm 0{\cdot}243$ g was recorded. From 50 animals killed after 50 days, the relative weight of testis (treated) was $0{\cdot}280 \pm 0{\cdot}063$ g (difference 0·406–0·280: significant at $P = 0{\cdot}05$) (data already published in ref. 3). In the animals killed after 50 days, the relative weight of the crest (controls) was 10·252 g; that of the treated 9·144 g. When killed after 90 days (50 animals) the weights of the testis and crest are less than the controls; although statistically not significant.

In the pituitary of the spayed albino rat (21 days after castration) it is easy to see inhibition (Fig. 1).

The activity of some fractions was also tested on 66 parabiont spayed females + immature females, recording the ovarian weights. Table 1 gives the results of these experiments.

From the foregoing data it may be tentatively concluded that nemasperm seems apt to elaborate something capable of inhibiting the gonadotrophic secretions, namely, F.S.H. This action does not seem referable to the androgenic content. The biological activity seems to be referable to a possible non-lipidic, non-proteic, hydrosoluble, dialysable ($\varphi < 10$ mμ), thermolabile ($t > 100°$ C) molecule or groups of molecules. The action of the possible œstrogenic residual content may still be open to question.

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Linear Relationship between Temperature and Uptake of Oxygen in *Euglena gracilis*

Changes in incubation temperature result in changes in the growth rate, the dry weight and the length of the lag phase of the growth cycle of streptomycin-bleached *Euglena gracilis*[1]. It appears that, within limits, low temperatures favour protoplasmic growth whereas high temperatures favour cell division. It was considered of interest to extend these observations to an investigation of the effects of various incubation temperatures on the rate of respiration of *Euglena*.

Experimental and stock cultures of a streptomycin-bleached strain, *SM-L*1, of *Euglena gracilis* var. *bacillaris* were grown axenically in the dark on 250 ml. of the defined Cramer–Myers medium[2], as modified[3], in 1 litre culture flasks. Sodium acetate (0·061 M) was the sole carbon source for growth. Cultures were inoculated from stocks growing exponentially at 25° and began growth at different temperatures (see Table 1) at a cell density of $14–20 \times 10^3$ cells/ml. Oxygen uptake of *Euglena* was determined manometrically by the direct method of

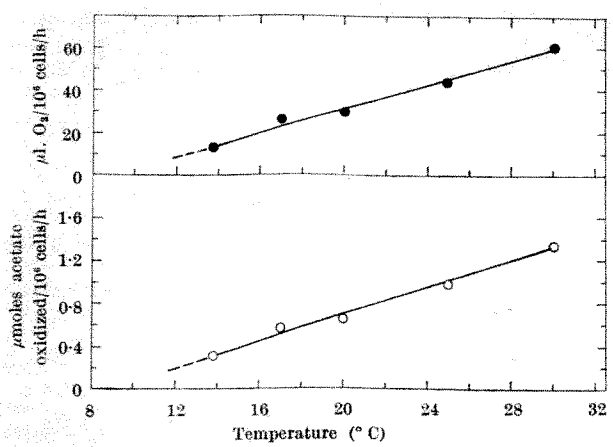


Fig. 1. Uptake of oxygen by *Euglena* at different temperatures (upper graph) and the amount of acetate oxidized by *Euglena* at different temperatures (lower graph)

Warburg[4] at the same temperatures at which the cells were grown. *Euglena* were concentrated by centrifugation at 2,000 r.p.m. for 2 min only, since prolonged or repeated centrifugation inhibits the oxygen uptake of these protozoa[5]. The cells were resuspended to a density of $5{\cdot}1–8{\cdot}5 \times 10^5$ cells/ml. in fresh medium and 2-ml. quantities were pipetted into Warburg vessels. Readings were taken at 5-min intervals for 45–60 min. Quadruplicate dry weight samples were taken at the same time as samples were taken for respiration. Dry weights of *Euglena* at various temperatures have been presented previously[1]. However, in the light of the further finding that the dry weight of these *Euglena* remains constant only during the mid-portion of the logarithmic growth phase (at least at 25°) (ref. 6), the measurements recorded here were taken at each temperature on cells grown to the mid-log phase. Temperatures below 13·8° and above 30° were not investigated because of the extremely slow growth rate of *Euglena* at low temperatures and the inhibition of the growth of the present *Euglena* at 32° (ref. 1).

Dry weight is constant from 17° to 25° but is increased at 13·8° and decreased at 30° (Table 1). The Q_{O_2} (μl. O_2/mg weight/h) of *Euglena* increases as temperature increases and is 7·5 times higher at 30° than it is at 13·8°. When the Q_{O_2} of *Euglena* is plotted against temperature a linear increase in oxygen consumption results between 13·8° and 25° followed by a sharp increase between 25° and 30°. However, when the oxygen uptake of *Euglena* is plotted as μl. $O_2/10^6$ cells/h versus temperature, a linear relationship is observed (Fig. 1) throughout the range of 13·8°–30°. For each degree increase in temperature, the oxygen uptake per 10^6 cells increases 2·85 μl. O_2/h. Therefore, a consideration of the data on a dry weight basis alone would obscure the result that the average cell linearly increases its oxygen consumption over the entire temperature range investigated (Fig. 1). To illustrate further these latter data, the μmoles of acetate oxidized per 10^6 cells per h versus temperature were calculated from the data given in the upper portion of Fig. 1 and are shown in the lower portion of Fig. 1. For each degree increase in temperature, the acetate oxidized per 10^6 cells increases $6{\cdot}37 \times 10^{-2}$ μmoles/h.

It is clear that temperature differentially affects the growth rate, dry weight and the oxygen consumption of the present *Euglena*. The growth rate increases linearly up to 25° and then levels out between 25° and 30° (ref. 1), whereas the dry weight decreases with rising temperature (Table 1). In contrast, the respiration rate increases linearly throughout the temperature range, 13·8°–30° (Fig. 1). Thus, it appears that the dry weight loss at high temperature does not involve the respiratory machinery of these *Euglena*. As a result, temperature becomes a tool for separating, at least partially, variable metabolic processes related to growth in *Euglena*, especially at the high temperatures favouring cell division.

Table 1. Effects of Temperature on the Dry Weight and Q_{O_2} of *Euglena*

Temperature (° C)	Dry weight (mg/10^6 cells)	Q_{O_2} (μl. O_2/mg dry weight/h)
13·8	1·63 ± 0·04*	8·5 + 0·1*
17·0	1·24 ± 0·13	20·6 ± 1·1
20·0	1·22 ± 0·05	23·6 ± 1·4
25·0	1·24 ± 0·03	33·5 ± 1·1
30·0	0·94 ± 0·05	63·7 ± 3·3

*Mean ± *S.D.*

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Seed Dispersal in *Salicornia pusilla*

The method of seed dispersal in the annual glasswort *Salicornia pusilla* (Chenopodiaceae), a local plant of coastal salt-marshes, is of interest. In this species the seeds are deeply immersed in the fleshy tissues of the stem cortex, and are covered (except at the minute orifice) by the long-persistent perianth. In all other European species of this genus, the inflorescence consists of decussately paired 3-flowered cymules. In *S. pusilla*, however, these cymules are single-flowered. A normal fruiting head (Fig. 1) contains 4–10 seeds and is shed from the parent plant, as the seeds ripen, by the development of an abscission layer. The seeds are retained within the cortical tissues even when the fruiting head has become separated.

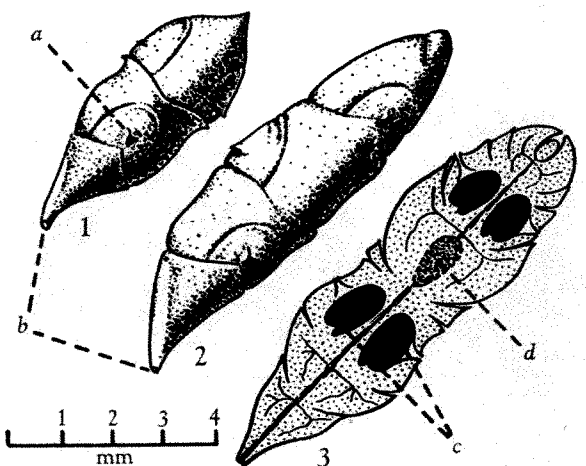


Fig. 1. 1 and 2, Detached heads with, respectively, four and six perianths; 3, longitudinal section through a head, showing immersed seeds. *a*, Orifice to perianth; *b*, points of abscission from parental plant; *c*, pair of seeds in plane of section; *d*, position of pair of seeds in plane at right angles to section

I have shown experimentally that these fallen heads will float in sea-water for up to three months, and that towards the end of this time the seeds may germinate *in situ*. In October and November detached heads may be seen stranded at or near high-water mark, where they have been carried by tidal action. This is presumably the reason for the occurrence of this species in its characteristic habitat just below the often conspicuous zone of *Halimione portulacoides*. *S. pusilla* is found in almost all suitable habitats in south and south-west England, and also in certain more isolated localities on the southern coasts of Wales and Ireland. It occurs also on a few salt-marshes in Brittany.

These outlying localities suggest the possibility of long-range dispersal by means of the floating heads, aided by the longevity of the seeds in sea-water.

Fruits of other salt-marsh plants (for example, *Halimione portulacoides* and *Suaeda maritima*) will also float in sea-water, being buoyed up by the air contained in their tissues, and the naturally isolated seeds of other *Salicornia* spp. will float (if dry) through air being held between the short curved hairs on the testa. I believe, however, that *S. pusilla* is the only example known in the British flora where whole intact fruiting heads may be dispersed by floating in sea-water.

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MICROBIOLOGY

A Photographic Method for the Detection of Rare Bacterial Mutants in the Replica Plating Technique

The replica plating technique, devised by J. and E. M. Lederberg[1], has found wide application to the isolation of bacterial mutants that cannot be automatically selected, for example, of mutants which lack the ability to synthesize a given growth factor or to ferment a given sugar, and of revertants from drug resistance to sensitivity. A great number of colonies should be replicated, if the mutation frequency is low. When several hundreds of colonies per plate are used, however, it becomes difficult, if not impossible, to compare the replicas visually. This communication describes a photographic method which permits the evaluation of plates containing many more colonies than would be feasible by visual comparison.

The method emanated from studies on the resistance of *Escherichia coli C* to bacteriophage φX–174 and its host range mutants[2]. If a wild-type lysate of φX-174 is plated on phage-resistant mutants of *E. coli C* (designated by C/φ), normally a very small number of host-range mutant plaques are observed (one per 10^9 viable particles or less). After a fortuitous finding in similar work with *Shigella*[3] we suspected that a small class of C/φ-mutants (to be referred to as $C/\varphi S$) could be procured, which would permit growth of a much higher number of host-range mutants if wild-type phage was plated (about one per 10^7 particles). Colonies of these mutants could possibly be found among the abundance of C/φ-colonies if they were replicated on to a plate without phage and on to a plate containing a large amount of wild-type phage. A colony of $C/\varphi S$ will not develop on the latter plate when its cells are infected by a host range mutant present in the wild-type lysate. Since in the first experiments no $C/\varphi S$-colonies were found, it became desirable to examine many more colonies per plate.

The method used for handling these dense platings was applied as follows. Of the phage-resistant colonies on the master plate grown after seeding with wild-type bacteria and wild-type phage, two replicas were made, as described here. Of both replicas contact prints (negatives) were made by placing the plates on light-sensitive paper (Dalcopy paper, kindly supplied by Dalco, Soestduinen, The Netherlands) and exposing them to light the rays of which had been made nearly parallel. Pictures were obtained as shown in Figs. 1*a* and *b*. From the print representing the second replica a positive was made on the Dalcopy reproduction apparatus (Fig. 1*c*). The print of the first replica was then placed upside down on this positive in such a way that the white colonies seen on Fig. 1*a* conform to the black ones of Fig. 1*c*. This combination was reproduced in the Dalcopier on another sheet of paper. As, in general, the colonies on the replicas

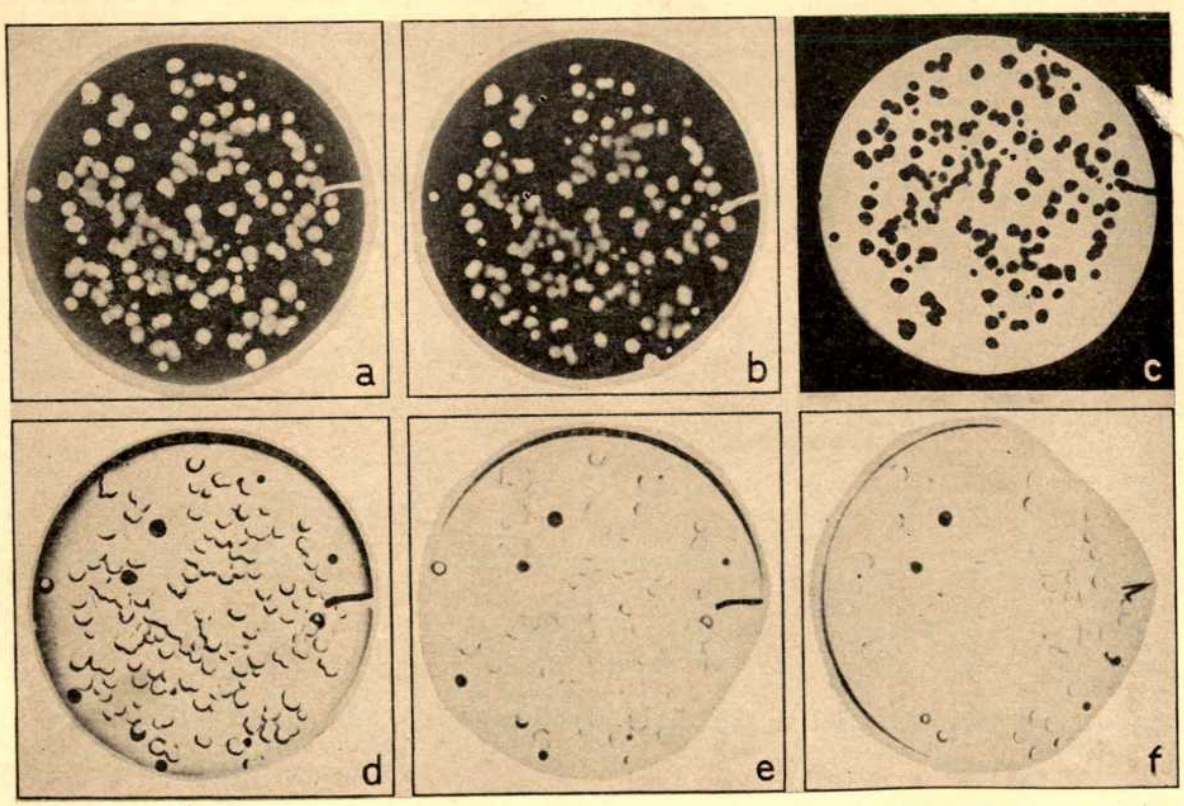


Fig. 1. Prints obtained using the photographic detection method. The master plate was prepared by spreading *E. coli C* and φX-174; after incubation it was replicated fourfold on plates containing: no phage (1), φh-phage (10^5 particles per plate) (2), *idem*. (3), and no phage (4), respectively. *a*, Print of plate (1-); *b*, print of plate (2); *c*, positive of *b*; *d*, relief-print of *a* upside down on *c*, a little shifted and under-exposed; *e*, *idem*, but not shifted and heavily under-exposed; *f*, *idem*. for plates (4) and (3). The two dots occurring at the same site on *e* and *f* represent colonies that proved to be $C/\varphi S$-mutants. They need not necessarily be of independent origin

differ slightly in shape, so that fitting on prints 1*a* and 1*c* is not perfect, the result is a kind of relief-print. This impression is strengthened when the two prints are a little shifted with respect to each other, as is shown in Fig. 1*d*. The print is under-exposed in order to obtain a white background. The relief pattern is helpful in locating the black dots on the first replica, which appear as a consequence of the lacking of some colonies on the replica containing phage. These dots show most clearly on a print like Fig. 1*e*, which is made without shifting and which is heavily under-exposed. The dots may represent the mutant colonies looked for; however, they can also be due either to irregularities in the agar layer of the first plate, for example, air bubbles, or to colonies that have not been replicated on to the second replica. This incompleteness of the replication was especially observed along the edge of the replica.

We therefore consider the following refinement of the method to be of great value. Four instead of two replicas are made on to plates containing no phage (1), phage (2), phage (3), and no phage (4), respectively. By comparison of the relief-print of the plates (1) and (2) with that of (4) and (3), mutant colonies, which should appear on both prints, are easily detected (compare Figs. 1*e* and 1*f*). This photographic method enables testing up to 1,000 colonies per plate.

Master plates were prepared by spreading about 5×10^8 cells of *E. coli C* and 10^4 to 10^6 φX-174 particles on the agar surface of a Petri dish. The number of phage particles should be at least 10^4 per plate in order to obtain confluent lysis of the bacterial layer after incubation (18 h at 32° C). High concentrations of phage should be avoided, however, since otherwise there is a greater probability that colonies of the mutants looked for will be lysed by host range mutants, either brought on the plate or originated in an early stage of phage growth. About 600 colonies could be seen; the diameter was about 2 mm.

These colonies were replicated by means of short-poled velveteen on to four plates (see above) containing broth agar, described by Zahler[4]. Two plates had been seeded with about 5×10^{10} wild-type phage particles, about 20 min before use. In order to obtain small colonies we incubated the replicas for 18 h at 28° C. Phage growth is not greatly depressed at this lower temperature, since neither efficiency of plating nor plaque size varies appreciably on incubation between 26° and 37° C.

For demonstrative purposes we present in Fig. 1 a series of prints of an experiment, in which a host-range mutant (φh) of the phage was used instead of the wild-type. This affords a much better selection for the mutants, as it might be expected that all $C/\varphi S$-colonies would be lysed by the host range particles. The latter do not inhibit the growth of the common C/φ-mutants (detailed information will be published elsewhere[2]). In this way a smaller number of C/φ-colonies needed to be replicated in order to obtain positive results.

This photographic method may be regarded as a useful and economical amplification of the replica plating technique, especially if low mutation frequencies are to be expected. It was successfully applied to the isolation of rare phage-resistant mutants of *E. coli C* that permit growth of a relatively high number of host range mutants if wild-type bacteriophage φX-174 is plated on these strains.

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VIROLOGY

Electron Microscopy of Myxoma Virus

Ruska and Kausche[1] and Farrant and Fenner[2] demonstrated a close similarity, both in size and superficial morphology, between myxoma and vaccinia viruses. This, together with the demonstration of similarity in the pathogenesis of myxomatosis and other pox virus diseases, was considered by the latter authors to justify the inclusion of myxoma virus within the pox group. Further justification for this view has now been obtained by a comparison of the morphology and fine structure of myxoma and vaccinia viruses using the negative contrast technique of Brenner and Horne[3].

Monolayers of a rabbit kidney cell line (*Gl. RK* 13, kindly supplied by Dr. Christofinis of Messrs. Glaxo, Ltd.) were infected with an input multiplicity of three infective units per cell of the Cornwall strain of myxoma virus. After incubation for 48 h at 35° C the infected cells were

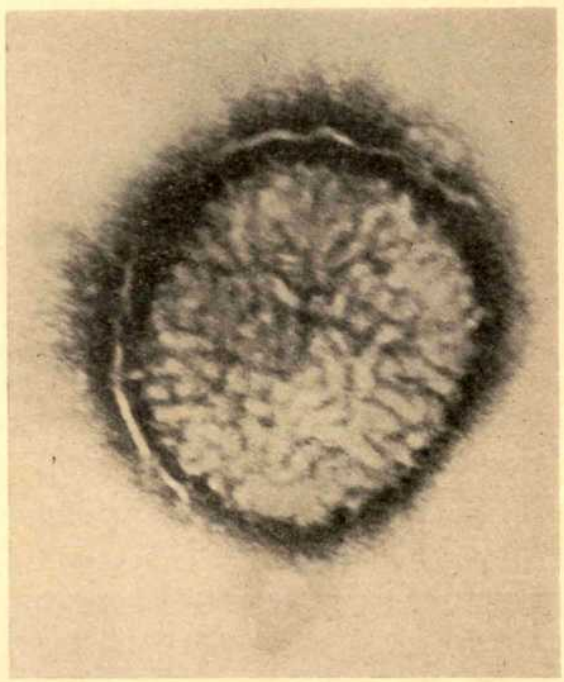


Fig. 1. Myxoma; no PTA penetration (× 160,000)

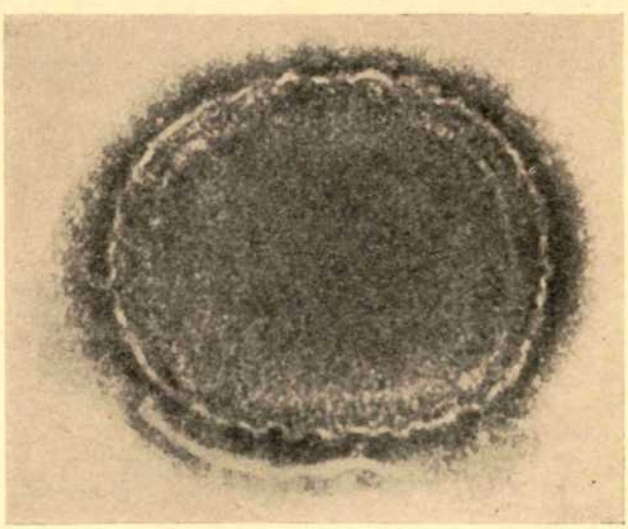


Fig. 2. Myxoma; PTA penetration (× 160,000)

washed free of medium, scraped off the glass and ultrasonically disrupted in a small volume of tissue culture medium. Cell debris was removed by centrifugation at 3,000*g* for 10 min and the virus then deposited at 40,000*g* for 30 min. The pellet containing the virus was dispersed by ultrasonic treatment in a small volume of distilled water and mixed with an equal volume of 2 per cent sodium phosphotungstate, *p*H 6·0. After standing at room temperature for 10 min, drops of the mixture were placed on carbon-coated 'Formvar' grids and examined in the Siemen's 'Elmiskop 1' electron microscope using double condenser illumination at 80 kV accelerating voltage and magnification, 80,000 times.

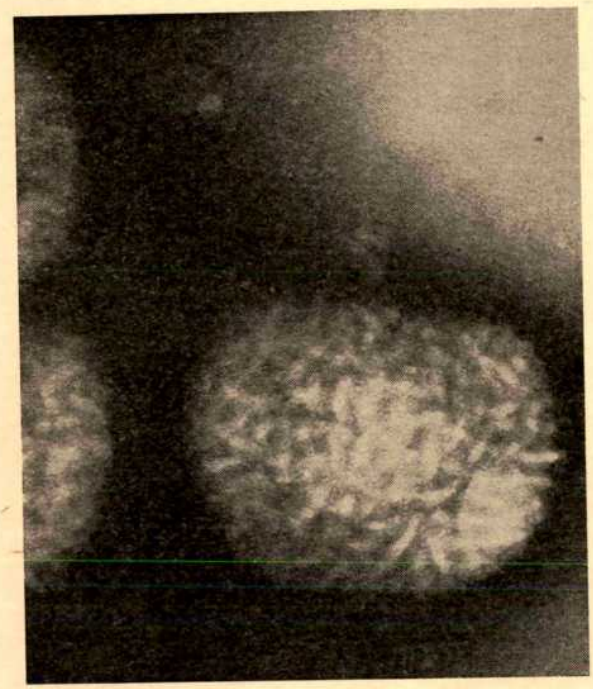


Fig. 3. Vaccinia; no PTA penetration (× 160,000)

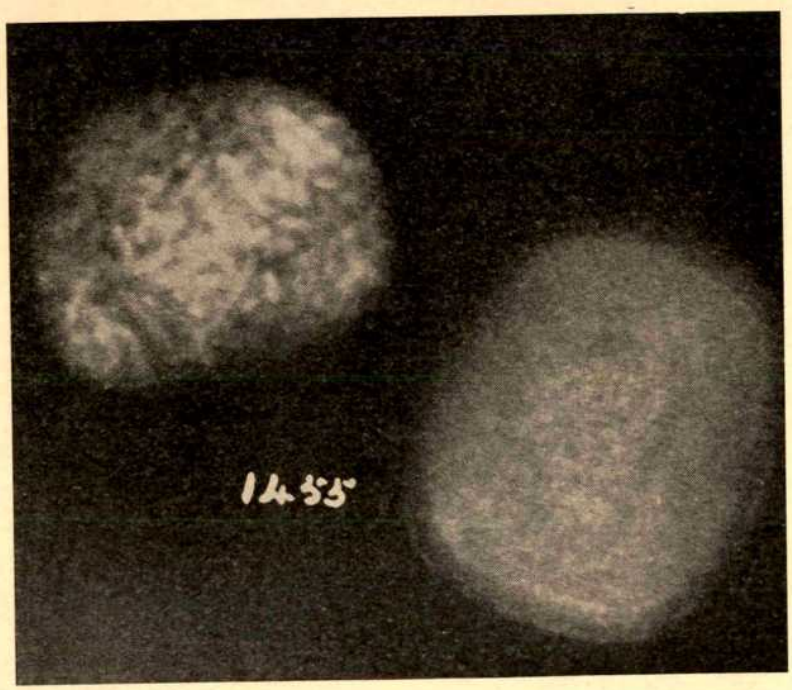


Fig. 4. Vaccinia; PTA has penetrated the particle on the right (× 160,000)

Figs. 1 and 2, showing the two types of particle observed, may be compared with Figs. 3 and 4, showing the corresponding morphological forms observed in similarly treated suspensions of vaccinia virus. Figs. 1 and 3 represent particles of virus into which the phosphotungstic acid (PTA) has failed to penetrate, the superficial beaded structure of the virus surface being clearly revealed. Enveloping membranes similar to the fragment seen in Fig. 1 are also frequently seen in vaccinia preparations. Figs. 2 and 4 show particles into which the stain has penetrated, revealing in each case a superficial 'capsule' of complex structure, surrounding a smooth inner body. Both types of particle have been reported in vaccinia virus preparations[4–6]. The details of their relationship and the significance of the fine structure observed in them will be the subject of a later communication.

In addition to the similarities in size, shape and fine structure revealed by negative staining, recent work (Fenner, F., personal communication) has demonstrated a fundamental serological relationship between the myxoma–fibroma group of viruses on one hand and the vaccinia–ectromelia group on the other. On the present evidence, therefore, the myxoma–fibroma viruses may be included with confidence as a sub-group of the pox viruses.

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Synthesis of Viral Ribonucleic Acid by a Chemically Inactivated Influenza Virus

It has been shown that the fowl plague virus, which belongs to the influenza *A* group, can be inactivated by an ethylene-iminoquinone (Bayer *A* 139, a gift of the Bayer-Werke, Leverkusen, Germany)[1] or by hydroxylamine[2,3] without losing its antigenic, neuraminidase, or hæmagglutinating activities. Bayer *A* 139 mainly reacts with the phosphate groups of nucleic acids, and in this way the sugar-phosphate 'backbone' is broken[4,5]. Hydroxylamine attacks the pyrimidines of the nucleic acids without breaking the RNA-strand[6]. Since during inactivation of the fowl plague virus with both compounds a phenomenon interpreted as multiplicity reactivation was observed[1,3], one should still expect viral RNA-synthesis in cells which were infected with partially or totally inactivated virus. Experiments designed to clarify this point are described here.

The methods applied for the determination of the biological activities are described in refs. 1 and 3. The amount of viral RNA mixed with normal newly synthesized cellular RNA was calculated from the shift of the oligonucleotide pattern of the RNA labelled with phosphorus-32 after RNase digestion of infected cells towards that of viral RNA[7,8]. The synthesis of normal cellular RNA is not inhibited by infection. Viral RNA-synthesis is an additional event[7,8].

Fig. 1 shows the inactivation kinetics of fowl plague virus with Bayer *A* 139 and

hydroxylamine, which is in accordance with former results[1,3]. If the partially or totally inactivated virus samples were added to chick fibroblast tissue cultures, the results presented in Fig. 2 were obtained. While the infectivity (plaque-forming units = PFU) and, to a lesser extent, the hæmagglutination titre (HA) drop quickly with increasing time of inactivation, the yield of viral RNA stays almost constant over a relatively long time. The oligonucleotide patterns of the 18-h points are identical with those of the controls, while the total radioactivity in the RNA (18 h) is, on the average, 10 per cent lower than in the controls. The complement fixation titre (CF), which measures the amount of the *s*-antigen, shows nearly the same dependence on the inactivation time as the viral RNA.

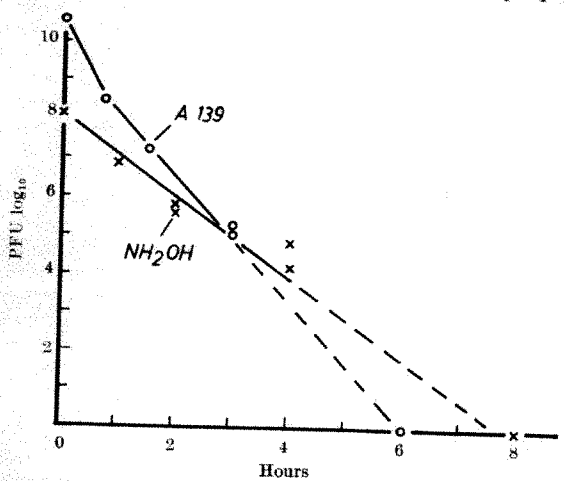


Fig. 1. Inactivation of fowl plague virus by Bayer *A* 139 and NH_2OH, respectively. Purified virus was inactivated by: (*a*) 1 per cent Bayer *A* 139 in 0·1 M *tris*-maleinate buffer *p*H 6·65; (*b*) 0·2 M NH_2OH in sodium chloride–phosphate buffer *p*H 7·0. Temperature, 21° C. For experimental details, see refs. 1 and 3. With higher inactivation it was not possible to determine the PFU exactly because of multiplicity reactivation (refs. 1 and 3). In those cases two points can be seen in the curves, which were calculated from two different dilutions

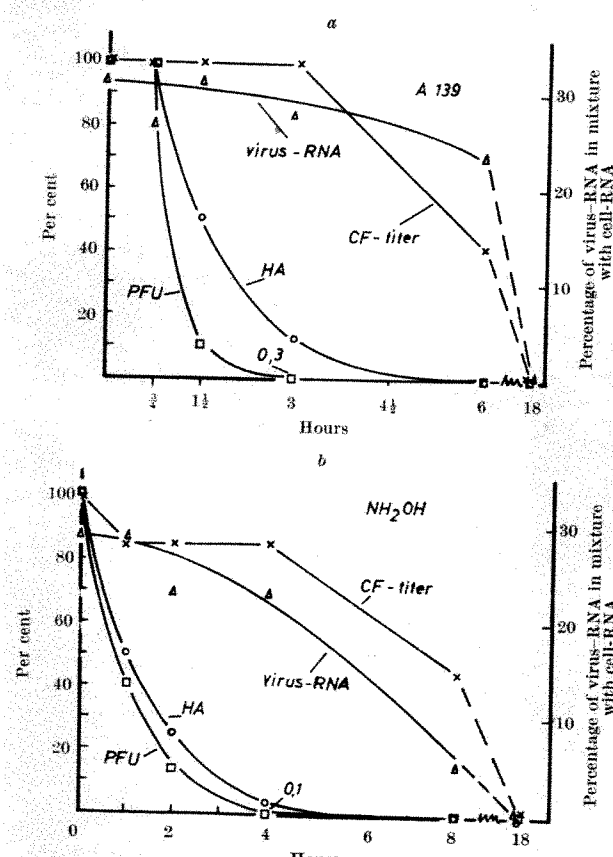


Fig. 2. Synthesis of virus-specific material in tissue cultures infected with partially or totally inactivated fowl plague virus. Chick fibroblasts were infected with virus samples inactivated by Bayer *A* 139 (*a*) or NH_2OH (*b*) for times as indicated at the abscissa of Fig. 1. On the left ordinate the percentage of these activities compared with controls infected with non-inactivated virus (= 100 per cent) is shown. The right ordinate represents the percentage of virus specific RNA in mixture with normal cellular RNA. For experimental details, see refs. 1, 3, 7 and 8

Although no material specific for this virus can be demonstrated in the infected tissue culture, if the virus is inactivated for a longer time (18 h or more), a high degree of interference has been found in these cells. Cells which were superinfected about 20 h after treatment with a virus preparation inactivated either with Bayer *A* 139 or hydroxylamine showed a virus yield 5–11 per cent of that of non-treated control cultures. This means that viral RNA-synthesis is not necessary for the interference phenomenon.

There is no significant difference between the activities of the virus samples treated for corresponding times with either Bayer *A* 139 or hydroxylamine. Therefore, it seems to make no difference whether the backbone of the nucleic acid is broken or only the pyrimidines are attacked. In both cases one has to assume that the viral RNA of the inactivated virus invades the cell and exerts there some activity even if the size of the RNA is lowered to some degree.

Prior to fowl plague viral RNA-synthesis the formation of 'early protein(s)' is necessary[8,9]. It may be that so long as that part of the virus RNA which codes for the 'early protein(s)' is not touched, the total amount of viral RNA is synthesized independently of some breaks or changes of the pyrimidines. If the total amount of the viral RNA is necessary for the formation of an infectious virus progeny, only a fraction of the virus RNA should code for the 'early protein(s)'. Because of the insensitiveness of the tests and some other uncertainties, like the relatively high multiplicity of virus particles per cell, even a rough estimate on the size of that fraction cannot be made. The hæmagglutinin has a rather complex structure of protein and carbohydrate. Therefore, it is conceivable that its formation is more sensitive to inactivation than the other activities (except PFU), which are assumed to be less complex.

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VETERINARY SCIENCE

Concentrating Ejaculated Sperm for Electron Microscopy

In previous work on the ultrastructure of bull sperm[1] semen samples cooled on ice during transportation to the laboratory were used. These samples were centrifuged immediately after arrival, but 24–48 h usually had elapsed from time of ejaculation to time of fixation of the specimens. Since various details of several of the membrane systems of the sperm were generally rather poorly pre-

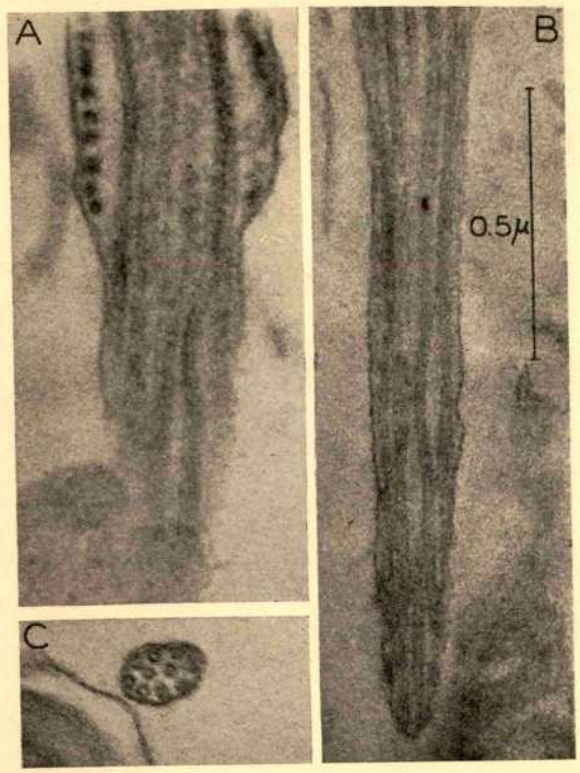


Fig. 1. *A*, Longitudinal section of the distal end of the tail of a bull sperm. The transitory region between the principal piece and the end piece of the tail is seen. Note the termination of the fibres of the fibrillar sheath, and the continuation of the intact cell membrane (lead hydroxide counter-stained, × 72,000). *B*, Longitudinal section of the very tip of the end-piece of a tail of a bull sperm. Here the central fibres (the tube fibres) are covered only by the intact cell membrane (lead hydroxide counter-stained, × 72,000). *C*, Cross-section of the end piece of the tail of a bull sperm. Only ten fibres distributed at random are present (lead hydroxide counter-stained, × 72,000)

served, subsequent centrifugations and fixations were carried out at the centre where the bulls were kept. Thus, fixation could be initiated within approximately 30 min after ejaculation. On such material, some new observations were made[2], but for optimal preservation of many delicate structures a more gentle preparative technique was obviously called for. The two factors considered to be mainly responsible for the less satisfactory results were: (1) The rather long time that had elapsed from the moment of ejaculation to the time of fixation; (2) The centrifugation for 15–20 min at 2,500–3,000 r.p.m. necessary for concentrating the sperm to a suitable number for further processing. A method which removes these two obstacles will be described.

Previously, Bloom and Nicander[3] have described a method for fixation of sperm material from the cauda epididymis. They placed drops of the concentrated sperm suspensions obtained from this source on small paper strips which were then floated on to the surface of the fixative solution. After fixation the specimen was stripped off from the surface of the paper, and cut in pieces of suitable size for dehydration and embedding.

If filter paper is used instead of ordinary paper a convenient method of concentrating ejaculated sperm for sectioning and electron microscopy is available, as a sufficient number of cells for further processing are able to penetrate into the pores of this paper. The method is simple and rapid, and at the same time a very gentle way of concentrating sperm cells prior to fixation and dehydration. The procedure is as follows:

A track is made on a piece of filter paper by drawing two parallel pencil lines 3–5 mm apart. Of a freshly ejaculated semen sample a 'smear' is obtained by distributing a few drops of semen along this track by means of a Pasteur pipette. Apparently the sperm cells are restricted to the area of the track whereas the sperm plasma is sucked into the pores of the paper beyond this track. After 2–3 min the paper has absorbed all the plasma, and a yellow-white, glossy deposit of concentrated sperm cells is left within the borders of the track. This region is now cut into strips 1 mm wide and 5–10 mm long which are quickly immersed in the fixative (2 per cent osmium with sucrose in veronal–acetate buffer $p\mathrm{H} = 7{\cdot}2$, ref. 4).

The entire procedure from receipt of ejaculate to immersion of strips in the fixative need not take more than 10 min. After fixation for the appropriate period of time dehydration and embedding of pieces of a suitable size are performed according to standard procedures. Until now embedding in 'Vestopal-*W*'[5] has been used. Sectioning of the accompanying cellulose fibres can present some difficulty, and the glass knives do wear out more rapidly than when sectioning blocks which do not contain filter paper. However, high-quality sections can be obtained, and the preservation of structural details in the sperm thus prepared is definitely superior to any we previously prepared from samples of ejaculated sperm. Perfect sections may also be obtained more easily if high-quality ash-free filter paper (for example, J. H. Munktell, Sweden, type *OOH*) is used instead of ordinary filter paper.

So far very promising results have been obtained with this method. Particularly the cell membrane and other membranous structures of the sperm seem to be better preserved than hitherto. This is illustrated in the accompanying Fig. 1 in which an intact cell membrane at the very tip of the end piece of the tail (the end piece of Retzius) as well as in the transitory region between principal piece and end piece is shown. Fig. 2 shows well-

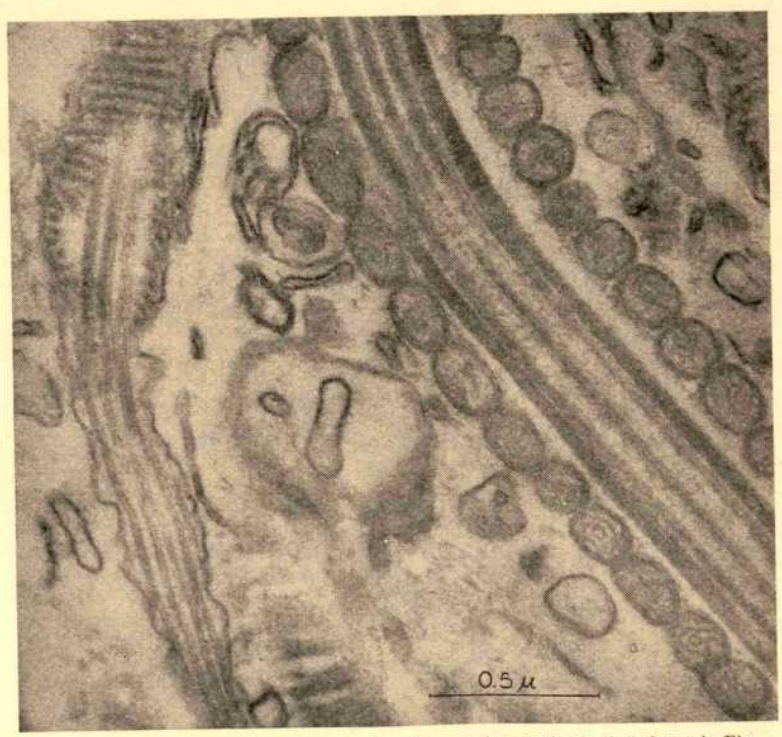


Fig. 2. Left, a longitudinal section of a transitory region similar to that shown in Fig. 1*A*; right, longitudinal section through the middle piece of a bull sperm. Note the mitochondrial helix with well-preserved inner membranes (cristæ). Between the cells several circular profiles of laminated structures and some membrane fragments are pictured. These structures are the contents of the cytoplasmic droplets normally disposed of by the sperm during ejaculation (uranyl acetate counter-stained, × 46,000)

preserved inner membranes or cristæ in the mitochondrial helix of the middle piece of the tail. These structures are generally very difficult to obtain in a well-preserved state in ejaculated sperm. The same applies to various membranous structures in the head of the sperm as well as in the so-called implantation region between head and tail. In these latter cases also, the filter-paper method has proved superior to other previously used methods. Finally, it should be mentioned that this method of concentration is less selective than centrifugation. Sections of material prepared by the new method contain a great number of circular, laminated profiles between the sperm cells. These structures can easily be identified as the characteristic contents of the cytoplasmic droplets (distal droplets) which the sperm cells normally dispose of during the ejaculation process. These profiles are always left in the supernate, and consequently lost when the sperm are concentrated by means of centrifugation.

We thank Mr. Hans Philipsen, chief veterinary officer, Ringsted, Denmark, for his assistance in supplying the samples of bull semen.

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PSYCHIATRY

3,4-Dimethoxyphenylethylamine and Other Amines in the Urine of Schizophrenic Patients

Friedhoff and Van Winkle[1] have reported the presence of 3,4-dimethoxyphenylethylamine in urine from 15 of 19 schizophrenic patients while there was none in urine from 14 normal subjects. Because of the clear biochemical difference between both groups and the structural resemblance of the amine with mescaline, it was considered to be important to confirm their results in a different laboratory. The urine amines were examined first according to their method; but resolution of compounds was not satisfactory on the paper chromatograms and several compounds having similar R_F values interfered with the identification of 3,4-dimethoxyphenylethylamine. Therefore, an improved method was devised in order to obtain a better chromatogram of the amines.

An amount of urine containing 50 mg creatinine was acidified to *p*H 2 by the addition of concentrated hydrochloric acid and heated in boiling water for 30 min. Initial purification of the amines was carried out with a modification of the method used by one of us[2] for the purification of urinary phenolic amines: A column (1 × 4 cm) of 'Dowex 50 (H^+)' was used for the collection of amines and the column was washed with 10 ml. of 0·1 N sodium acetate and 10 ml. of water successively. Amines were eluted with 8 ml. of the ammoniacal ethanol. The eluate was evaporated to dryness in a vacuum desiccator over sulphuric acid, and the residue was dissolved in 1 ml. of 0·1 N sodium borate buffer *p*H 9. The solution was shaken with 5 ml. of chloroform and the organic layer was evaporated in a stream of air at room temperature. The dried residue was dissolved in a few drops of ethanol and subjected to two-dimensional paper chromatography with the solvent systems previously used for phenolic amines[2]. The compounds were visualized with ninhydrin reagent[1]. The recovery of authentic 3,4-dimethoxyphenylethylamine added to urine which was then subjected to the procedures was about 90 per cent. Resolution of the amines on the chromatogram was satisfactory and many interfering substances were eliminated by the purification procedures. The spots observed frequently on the paper chromatograms are shown in Fig. 1. The compounds were identified by co-chromatography of authentic compounds with those from urine, and by several qualitative reactions.

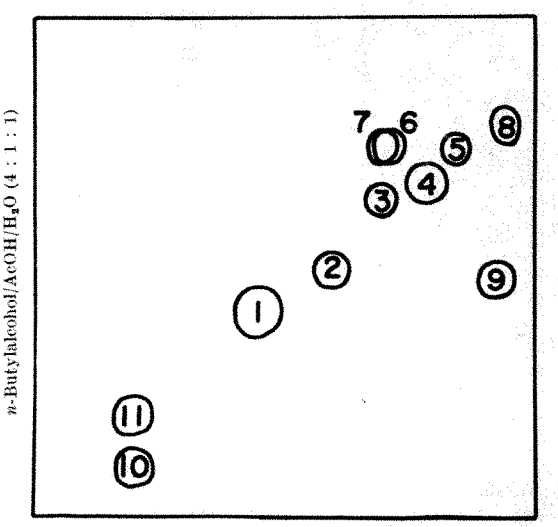


Fig. 1. Paper chromatogram of amines prepared from human urine with the methods described in the text. Tentatively identified compounds are threamine (2), 3-methoxytyramine (3), *p*-tyramine (4), 3,4-dimethoxyphenylethylamine (5) and 10-(3-aminopropyl)-2-chloro-5-oxyphenothiazine (6). The numbers in parentheses correspond to the numbers in the figure

Urine specimens were obtained from 78 schizophrenic patients, 21 psychoneurotic patients and 46 normal subjects. Of the 78 schizophrenics, 16 patients were under treatment with psychotropic drugs, usually chlorpromazine, and the other 62 patients had not received any medications for a minimum period of three weeks before they were studied. About half the schizophrenics had been sent to hospital. Normal controls were selected from the hospital staff and their families so that the sex and age distributions matched those of the patients. The frequency of the occurrence of 3,4-dimethoxyphenylethylamine in urine is listed for each group in Table 1.

Table 1. APPEARANCE OF 3,4-DIMETHOXYPHENYLETHYLAMINE IN URINE

	No. examined	Frequency of appearance of 3,4-dimethoxyphenylethylamine
Normal subjects	46	21/46
Psychoneurotics	21	14/21
Schizophrenics under no medication	62	57/62
Schizophrenics under drug therapy	16	13/16

Although the frequency of the appearance is higher in urine of schizophrenics than in normal subjects, the occurrence of the amine in urine is not so specific to schizophrenia as shown by Friedhoff and Van Winkle. Moreover, the concentrations of the other amines were observed to be generally higher in the urine of schizophrenics, which might explain the higher frequency of the appearance in urine of schizophrenics. It may be recalled that, in general, larger amounts of aromatic acids, particularly the *m*-hydroxyphenyl acids, are observed in urine from schizophrenics as compared with normal controls[3,4].

As suggested by Friedhoff and Van Winkle, 3,4-dimethoxyphenylethylamine might be a metabolite of dopamine. This possibility was examined with negative results as follows. The amount of 3,4-dimethoxyphenyl-

ethylamine in the urine of two normal subjects did not increase after oral administration of 0·5 g of 3,4-dihydroxy-L-phenylalanine and the amine could not be detected in the urine of two patients with pheochromocytoma. In urine of three groups of five rats each given intramuscular injections of 3,4-hydroxy-L-phenylalanine (100 mg/kg body-wt.), dopamine (10 mg/kg) and norepinephrine (1 mg/kg) respectively, 3,4-dimethoxyphenylethylamine could not be detected. Considering the chemical structure of the amine, an endogenous precursor would probably be either dopamine or 3-methoxytyramine. A large amount of the latter compound was consistently detected in urine from both human subjects and rats after the administration of either 3,4-dihydroxy-L-phenylalanine or dopamine. These results imply that 3,4-dimethoxyphenylethylamine originates from dietary sources or is a product formed by intestinal bacteria.

An amine indicated as No. 6 in Fig. 1 was frequently observed on the chromatograms of urine from schizophrenic patients who had received chlorpromazine. It was purified by a combination of ion exchange chromatography and counter current distribution techniques, and was tentatively identified as 10-(3-aminopropyl)-2-chloro-5-oxyphenothiazine from the results of elemental analysis, infra-red and ultra-violet absorption spectra. The occurrence of this compound in human urine as a metabolite of chlorpromazine was reported by Fishman and Goldenberg[5].

It is of interest that another unidentified compound (Fig. 1, No. 7) was observed more frequently in the urine of schizophrenics (25/62) than in the urine of normal subjects (2/21) and psychoneurotics (2/21). This substance was detected as a purple spot with ninhydrin reaction and did not react with any of several other reagents. This compound might also originate from dietary sources because the amount excreted daily varied widely. It should be mentioned that the R_F value of this amine is similar to that of 3,4-dimethoxyphenylethylamine in the first solvent system, which is the system used by Friedhoff and Van Winkle. Therefore, the metabolite of chlorpromazine and the other unidentified substance might be confused with 3,4-dimethoxyphenylethylamine. The other amines numbered 1–4 in Fig. 1 were observed in all specimens of urine and the amines numbered 8–11 were detected frequently.

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The preceding communication confirming the presence of 3,4-dimethoxyphenylethylamine in the urine of schizophrenic patients[1,2] is of considerable interest. The fact that their procedure differed in some respects from ours adds further support to our identification of this compound.

It should be pointed out that interfering compounds have been described by us. We have sometimes found it necessary to resolve 3,4-dimethoxyphenylethylamine by the use of 2 successive solvents. Several satisfactory solvent systems are described in our publications. In general, however, 3,4-dimethoxyphenylethylamine can be distinguished from other chromatographically similar compounds by means of the specific colour reaction for β-phenylethylamines that we have developed[3].

The results of Takesada *et al.* are particularly impressive because of the unusually high percentage of schizophrenic patients who excrete identifiable 3,4-dimethoxyphenylethylamine. Their incidence is higher than that found in our group of patients. This suggests either that their methods are more sensitive than ours or that their diagnostic classification or patient population is somewhat different from ours.

Their detection of 3,4-dimethoxyphenylethylamine in the urine obtained from a normal control group may have resulted from the increased sensitivity of their analytical methods, but it is pertinent that our normal controls were selected with deliberate intention to exclude any subjects with even mild schizoid tendencies. If schizophrenia is a genetically determined disorder, it would be expected that a significant percentage of the 'normal' population would have incomplete penetrance of the disease. It would seem, therefore, to be crucial not only to do qualitative studies but also quantitative determinations of 3,4-dimethoxyphenylethylamine in normal subjects and patients. Also, a specific comparison of patients according to diagnostic criteria should be carried out inasmuch as some cases of schizophrenia in one country would be classified as psychoneuroses in another.

The attempt to synthesize 3,4-dimethoxyphenylethylamine from dihydroxyphenylalanine and dopamine carried out by Takesada *et al.* did not result in the formation of the dimethoxy amine. It should be pointed out that the conversion rate of infused precursors of this compound may be very low. In any event, it seems most fruitful to examine this conversion in schizophrenic patients where the conversion is most likely to occur. Inasmuch as rats do not appear to excrete 3,4-dimethoxyphenylethylamine, it is unlikely that they would form it from precursors. We are now carrying out investigations in schizophrenic patients using tritiated dopamine as a precursor.

The data of Takesada *et al.*, when subjected to simple χ^2 analysis, reveal that their combined schizophrenic group differs significantly from their normal control group ($P < 0{\cdot}001$) in the incidence of 3,4-dimethoxyphenylethylamine in urine. This finding alone plus the pharmacological nature of 3,4-dimethoxyphenylethylamine suggest that many additional investigations of this compound should be carried out. It is of uncommon interest that 3,4-dimethoxyphenylethylamine has profound central effects without appreciable peripheral effect[4]. This is compatible with the symptoms characterizing schizophrenia. In addition, 3,4-dimethoxyphenylethylamine has been shown to produce experimental catatonia in cats[5,6].

Takesada *et al.* have confirmed the presence of a potentially psychotoxic compound in urine. They suggest that this compound is of dietary or microbial origin, but they provide no data to support this.

We feel that considerable caution should be exercised in the interpretation of the relationship of 3,4-dimethoxyphenylethylamine to schizophrenia. At the present time, therefore, it must be concluded that the origin and significance of this compound has not been ascertained. The unusually high incidence of 3,4-dimethoxyphenylethylamine in the urine of the patients studied by Takesada *et al.* should stimulate further interest in investigating the possible significance of this compound in mental illness.

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[3] Friedhoff, A. J., and Van Winkle, E., *J. Chromatography* (in the press).
[4] Epstein, D., Gunn, J. A., and Virden, C. J., *J. Physiol.*, **76**, 224 (1932).
[5] Noteboom, L., *Proc. Acad. Sci. Amsterdam*, **37**, 562 (1934).
[6] Ernst, A. M., *Nature*, **193**, 178 (1962).

FORTHCOMING EVENTS

(Meeting marked with an asterisk is open to the public)

Wednesday, July 17—Tuesday, July 23

FIFTH INTERNATIONAL PESTICIDES CONGRESS (at Friends House, Euston Road, London, N.W.1).

Thursday, July 18

COUNCIL FOR THE PRESERVATION OF RURAL ENGLAND (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 2.30 p.m. —Annual General Meeting. Address by the Rt. Hon. Viscount Colville of Culross.

Friday, July 19

IMPERIAL COLLEGE OF TROPICAL AGRICULTURE, U.K. ASSOCIATION (at Uganda House, Trafalgar Square, London, W.C.2), at 2.45 p.m.—Dr. Harry Evans, O.B.E.: "The Practical Application of Scientific Procedures for the Improvement of Agricultural Productivity in British Guiana".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (with a degree in agriculture or equivalent qualification, sound experience in practical farming, and preferably previous teaching experience) IN AGRICULTURE at the Cumberland and Westmorland Farm School, Newton Rigg, near Penrith, and for lecturing, demonstration and advisory work in the two Counties—The Director of Education, 5 Portland Square, Carlisle (July 18).

ASSISTANT LECTURERS or COLLEGE LECTURERS (2) (preferably with special qualifications in pure mathematics, and experience as a lecturer or teacher)—The Secretary and Bursar, University College, Dublin, Republic of Ireland (July 20).

LECTURER IN PSYCHIATRY—The Registrar, The University, Sheffield (July 20).

LECTURER or SENIOR LECTURER IN SOCIAL GEOGRAPHY—The Registrar, The University, Keele, Staffs (July 20).

RESEARCH ASSISTANT IN ELECTROCHEMISTRY, to work with a group developing the study of the kinetics of organic electrochemical reactions and of new techniques for electrochemical synthesis—Head of the School of Chemistry, King's College (University of Durham), Newcastle upon Tyne 1 (July 20).

RESEARCH ASSISTANT (preferably with experience in the interpretation of air-photos, and a degree in geography) IN THE GEOGRAPHY DEPARTMENT, to work on the organization and classification of a vast library of air-photos, mostly of parts of Europe—The Registrar, The University, Keele, Staffs (July 20).

RESEARCH ASSISTANT (trained research worker) IN THE DEPARTMENT OF PHYSICAL CHEMISTRY, to work with a group developing the study of the kinetics of organic electrochemical reactions and of new techniques for electrochemical synthesis—Head of the School of Chemistry, King's College (University of Durham), Newcastle upon Tyne 1 (July 20).

SECOND CHAIR OF CHEMICAL ENGINEERING—Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne (July 20).

LECTURER (preferably with a medical qualification) IN THE DEPARTMENT OF PHARMACOLOGY—The Secretary, The Welsh National School of Medicine, (University of Wales), 34 Newport Road, Cardiff (July 22).

RESEARCH WORKER (with a good honours degree, not necessarily in psychology) IN THE DEPARTMENT OF PSYCHOLOGY, for work in the field of programmed instruction—The Registrar, The University, Sheffield (July 22).

ASSISTANT LECTURER (preferably cellular biochemist or physiologist) IN ZOOLOGY—The Secretary, The University, Aberdeen (July 24).

ASSISTANT LECTURER IN PHYSIOLOGICAL CHEMISTRY—The Registrar (Room 22, O.R.B.), The University, Reading (July 27).

COMPUTER OFFICER IN THE SUB-DEPARTMENT OF NUMERICAL ANALYSIS in the Department of Applied Mathematics—The Registrar, The University, Liverpool, quoting Ref. No. CV/354 (July 27).

LECTURER (graduate interested in the quantitative husbandry aspects of farm management, and with qualifications or experience in statistics or mathematics) IN AGRICULTURE—The Registrar (Room 22, O.R.B), The University, Reading (July 27).

PROFESSOR OF ANIMAL HUSBANDRY AND HEAD OF THE DEPARTMENT OF ANIMAL PRODUCTION; PROFESSOR OF TROPICAL CROPS AND HEAD OF THE DEPARTMENT OF CROP PRODUCTION, and LECTURER or SENIOR LECTURER IN GENETICS AND PLANT BREEDING IN THE FACULTY OF AGRICULTURE, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana—The Assistant Registrar, Kwame Nkrumah University of Science and Technology, 15 Gordon Square, London, W.C.1 (July 29).

HISTOCHEMIST and a TUMOUR IMMUNOLOGIST (medical or non-medical graduates), for work concerning certain aspects of induced mammary tumours (encouragement will also be given to develop other lines of research) —The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2 (July 31).

JUNIOR RESEARCH FELLOW IN BOTANY—The Registrar, The University, Sheffield (July 31).

RESEARCH SCIENTIST (with a background in any of the geological, biological, chemical or physical sciences), to initiate and carry out an electron microscopic study of ultra-microscopic organisms in sedimentary rocks in the Sedimentary Research Laboratory, Department of Geology—Prof. P. Allen, Sedimentary Research Laboratory, The University, Whitenights Park, Reading (July 31).

RESEARCH STUDENT (with a good honours degree or equivalent in chemistry, physics or geology) IN THE DEPARTMENT OF CHEMISTRY, to work on the crystal structure of zeolites, using electron microscopy and diffraction techniques—Prof. G. M. Burnett, Department of Chemistry, The University, Old Aberdeen (July 31).

SCIENTIFIC OFFICER (with a first- or second-class honours degree) IN THE BIOCHEMISTRY DEPARTMENT, to assist in research on the chemistry and biochemistry of casein—The Secretary, The Hannah Dairy Research Institute, Ayr, Scotland (July 31).

SENIOR LECTURER IN CLINICAL GENETICS tenable conjointly at University College Hospital Medical School and University College, for research in human genetics, under the direction of Profs. M. L. Rosenheim and L. S. Penrose—The Secretary, University College, Gower Street, London, W.C.1 (July 31).

UNIVERSITY DEMONSTRATOR IN INORGANIC CHEMISTRY—Dr. F. B. Kipping, University Chemical Laboratory, Lensfield, Cambridge (July 31).

UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF PHYSICS—The Secretary of the Appointments Committee of the Faculty of Physics and Chemistry, The University, Lensfield Road, Cambridge (July 31).

STATISTICIAN (Senior Scientific Officer or Principal Scientific Officer grade) as HEAD OF THE DEPARTMENT OF BIOMETRICS—The Secretary, The Grassland Research Institute, Hurley, Maidenhead, Berks (August 1).

PLANT PHYSIOLOGIST (Senior Research Officer or Principal Research Officer) (with a Ph.D. degree in plant physiology or biochemistry, or postgraduate research experience of an equivalent standard and duration supported by satisfactory evidence of research ability, and research experience in post-harvest physiology) IN THE DIVISION OF FOOD PRESERVATION, Commonwealth Scientific and Industrial Research Organization, Ryde, New South Wales, Australia, to take a leading part in the development of research on the physiology of fruits after harvesting, especially in relation to disorders occurring during ripening and storage—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 300/368 (August 3).

LECTURERS or ASSISTANT LECTURERS (3) IN PHYSICS at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 8).

CHAIR OF AGRICULTURE at the University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 10).

LECTURER or ASSISTANT LECTURER IN ANIMAL HUSBANDRY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 10).

LECTURER or ASSISTANT LECTURER (with an honours degree in botany or agricultural botany with a special interest in plant physiology, and preferably with experience of university teaching and/or research) IN AGRICULTURAL BOTANY at Makerere University, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 10).

VETERINARY PARASITOLOGIST (with a degree in veterinary science or equivalent qualifications, with postgraduate field experience, preferably in parasitology), at the Animal Health Research Laboratory, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, to assist in investigations of helminth infections in sheep in southern Australia in collaboration, when necessary, with the staff of the McMaster Laboratory, Sydney—Chief Scientific Liaison Officer, Australia Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 201/190 (August 10).

ANIMAL HUSBANDRY OFFICER; and an ANIMAL NUTRITION CHEMIST in the Institute for Agricultural Research at Samaru (Ahmadu Bello University, Northern Nigeria)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 14).

SENIOR LECTURER or LECTURER IN GEOGRAPHY at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 14).

CHAIR OF MATHEMATICS at the University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 15).

LECTURER/ASSISTANT LECTURER (with at least a good honours degree, suitable teaching and research experience, special qualifications in angiosperm taxonomy, and an enthusiastic field worker) IN THE DEPARTMENT OF BOTANY in the Faculty of Science, University of Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, August 15).

SENIOR LECTURER, and a LECTURER (preferably with qualifications in one or more of the following fields: educational psychology, educational sociology, educational statistics, or science education) IN EDUCATION at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 16).

LECTURER (preferably with a special interest in electronics) IN APPLIED PHYSICS—The Registrar and Secretary, Durham Colleges in the University of Durham, 38 North Bailey, Durham (August 17).

SENIOR LECTURER or LECTURER IN THERAPEUTICS in the Department of Materia Medica and Therapeutics—The Secretary, The University, Aberdeen (August 17).

READER IN ORAL MEDICINE AND PATHOLOGY at the London Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 22).

SENIOR LECTURER (with a doctorate in social and/or cultural anthropology, and some teaching and field experience) IN THE DEPARTMENT OF ANTHROPOLOGY, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 24).

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF OPHTHALMOLOGY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (August 26).

RESEARCH ASSISTANT (with experience in embryology and/or biophysics) IN THE DEPARTMENT OF ZOOLOGY, for work on the effect of drugs on the uptake and distribution of water in the developing embryo—Dr. P. H. Tuft, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh (September 2).

CHAIR OF GEOLOGY at the University of Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, September 30).

SENIOR RESEARCH FELLOW (with a Ph.D. degree or equivalent qualification, or a distinguished record of scientific research), for research at the Forest Research Institute of the New Zealand Forest Service—The Director of Research, Forest Research Institute, Private Bag, Whakarewarewa, Rotorua, New Zealand (November 30).

ANALYTICAL CHEMIST (Soils) (under 45, with a good honours degree in an appropriate subject, and experienced in soil survey and land use planning) in Eastern Nigeria, to carry out, with Soil Surveyors, a soil survey of the sites for development as settlements and institutes—The Director of Recruitment, Department of Technical Co-operation, Great Smith Street, London, S.W.1, quoting RC.213/103/01.

ASSISTANT CONSERVATOR OF FORESTS (aged between 20–45, with a degree in forestry, and preferably previous experience in South-East Asia and/or a knowledge of the Malay language) IN THE DEPARTMENT OF FORESTS, Sarawak, for general forest administration—The Director of Recruitment,

Department of Technical Co-operation, Great Smith Street, London, S.W.1, quoting Ref. TRC324/128/01.

Assistant Lecturer (with an honours degree and industrial experience, and preferably a qualification in telecommunications) in the Department of Electrical and Control Engineering—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Head (with good academic qualifications with teaching and/or industrial experience, and able to organize research in the department) of the Electrical Engineering Department—Clerk to the Governors, Woolwich Polytechnic, London, S.E.18.

Nematologist (male, with an honours degree in zoology or entomology and at least one year's postgraduate training or experience, preferably in the study of nematodes) in the Ministry of Agriculture, Uganda, to undertake a survey of the occurrence of harmful nematodes on crops; to develop techniques for testing host reactions and for controlling nematodes of economic importance—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Rev. CI/RC.213/153/07.

Oil Palm Agronomist (between 26 and 45 years of age, with a degree in agriculture or natural science, followed by two years postgraduate training, and experience on an Oil Palm Research Station as an Agronomist) in North Borneo, to deal with the general agronomics and detailed field experimentation of oil palms in North Borneo and in particular at the Oil Palm Research Station—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. T/RC.213/107/04.

Publications Officer (preferably male or female graduate with some knowledge of languages), to be responsible for the publication of research papers, reports and the quarterly journal—The Director, The British Ceramic Research Association, Queens Road, Penkhull, Stoke-on-Trent.

Radioisotope Officer (with an honours degree in chemistry or physics and experience of the use of radioisotopes as tracers in biological research), to supervise all work with radioisotopes and collaborate with other officers in research on diseases of farm animals—The Secretary, Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh 9.

Research Assistant (competent in quantitative chemical analysis and an interest in animal physiology), for various types of biological research, mainly biochemical in character, together with some preparation of material for advanced classes—The Administrator, University Laboratory of Physiology, Oxford.

Research Assistant, for work on the biochemistry of migraine—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1.

Research Biochemist in the Department of Physiology, to work upon the isolation of radioactively labelled carbohydrates and derivatives in tissues (animal)—Prof. A. St. G. Huggett, St. Mary's Hospital Medical School, Paddington, London, W.2.

Scientific Officer for Microbiological Assay Unit (Vitamin B12 and Folic Acid)—The Hospital Secretary, City General Hospital, Sheffield 5.

Senior Technicians and Technicians, for general duties in the physics laboratories to assist with research, lecture preparation and photography—Professor of Experimental Physics, Physics Laboratory, University of Sussex, Falmer, Sussex.

Smith and Nephew Research Fellow (preferably with research experience in the field of protein biosynthesis) in the Empire Rheumatism Council Research Unit at King's College Hospital Medical School—Dr. M. J. H. Smith, Chemical Pathology Department, King's College Hospital Medical School, Denmark Hill, London, S.E.5.

Superintendent (with a degree in biological science or equivalent qualification, for example, F.A.T.A., and experience in some branch of animal work) to take charge of the Physiology Department's goat and small animal houses—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, Berks, quoting Ref. 63/2.

Technician (Male) in the Department of Anatomy—Prof. D. V. Davies, Anatomy Department, St. Thomas's Hospital Medical School, London, S.E.1.

Technicians (2) (preferably with experience in a hospital laboratory) in the Biochemistry Department, for work largely of a clinical nature, but including participation in research projects—J. S. Leahy, Huntingdon Research Centre, Huntingdon.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Teaching Machines and Programmed Learning: a Guide to the Literature and other sources of Information. By R. D. Gee. (Published in connexion with a Symposium on Teaching Machines and Further Education held at Hatfield College of Technology on 3rd May, 1963.) Pp. 68. (Roe Green, Hatfield: Hertfordshire County Council Technical Library and Information Service, Hatfield College of Technology, 1963.) 4*s*. [215

The Effects of High Altitude Nuclear Explosions on Scientific Experiments. Pp. 8. (Cmnd. 2029). (London: H.M. Stationery Office, 1963.) 8*d*. net. [215

World List of Forensic Science Laboratories. Pp. iii+30. (London: The Forensic Science Society, 1963.) 7*s*. 6*d*.; 1.50 dollars. [215

Department of Scientific and Industrial Research. Forest Products Research 1962: Report of the Forest Products Research Steering Committee with the Report of the Director of Forest Products Research. Pp. vi+57+12 plates. (London: H.M. Stationery Office, 1963.) 6*s*. 6*d*. net. [215

Armour Hess Chemicals Limited. Armeens and Duomeens. Pp. 25. (Leeds: Armour Hess Chemicals Limited, 1963.) [215

Relativity To-day: a Symposium Broadcast in the BBC Third Programme, with contributions by Prof. Hermann Bondi, Dr. D. W. Sciama, Dr. T. E. Cranshaw and Prof. Stanley Mandelstam. Pp. 32. (London: British Broadcasting Corporation, 1963.) 2*s*. 6*d*. [215

Psychiatry and Mental Health in Britain: a Historical Exhibition 4th March–9th July, 1963. Exhibition Guide No. 1. Pp. 39. (London: The Wellcome Historical Medical Library, 1963.) 2*s*. [215

The British Hydromechanics Research Association. Fifteenth Annual Report, October 1961 to September 1962. Pp. 40. (Harlow: The British Hydromechanics Research Association, 1963.) [215

Annual Report for 1962 of the Timber Research and Development Association. Pp. 36. (High Wycombe: Timber Research and Development Association, 1963.) [215

The International Nickel Company (Mond), Ltd. Designing for Life: The Role of Nickel in Commercial Vehicles. Pp. 12. (London: The International Nickel Company (Mond), Ltd., 1963.) [215

Murphy Chemical Co., Ltd. Pest Control in Farm Stored Grain. Pp. 5. Agricultural Leaflet No. 5: Brussels Sytam and Rogor 40 for Control of Cabbage Aphid. Pp. 4. Agricultural Leaflet No. 6: Potatoes—Pest and Disease Control—Haulm Destruction. Pp. 4. (Wheathampstead: The Murphy Chemical Co., Ltd., 1963.) [215

Department of Scientific and Industrial Research: Geological Survey and Museum. A Guide to the Geological Column exhibited in the Museum of Practical Geology. By the late Dr. R. L. Sherlock. Third edition, with some additions by Dr. C. J. Stubblefield. Pp. ii+6. (London: H.M. Stationery Office, 1963.) 1*s*. net. [215

Bulletin of the British Museum (Natural History). Entomology. Vol. 14, No. 1: The Coccinellidae of the Third Mount Everest Expedition, 1924, (Coleoptera). By A. P. Kapur. Pp. 1–48. 13*s*. Zoology. Vol. 9, No. 9: Mites of the Genus *Macrocheles* Latr. (Mesostigmata) Associated with Coprid Beetles in the Collections of the British Museum (Natural History). By G. Owen Evens and K. H. Hyatt. Pp. 325–401. 27*s*. (London: British Museum (National History), 1963. [215

The Newcomen Society for the Study of the History of Engineering and Technology. General Index to Transactions, Volumes I-XXXII, 1920–1960. Pp. 111. (London: The Newcomen Society for the Study of the History of Engineering and Technology, 1963. Obtainable from W. Heffer and Sons, Ltd., Cambridge.) 30*s*. [215

An Expansion and Teachers' Guide to Physics for Grammar Schools. (A Section of Part II of the Science and Education Report issued by The Association for Science Education. To be read in conjunction with "The Policy Statement" and "Physics for Grammar Schools"). Pp. 72. (London: John Murray (Publishers), Ltd., 1963.) 2*s*. net. [215

Ciba (A.R.L.), Ltd. Technical Notes, No. 244, (April 1963): Hydromechanics Models. Pp. 8. Technical Notes, No. 245, (May 1963): The Domenichino Frescoes. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1963). [215

The Royal Society. Taxonomy: Report of a Committee appointed by the Council of the Royal Society. Pp. 10. (London: The Royal Society, 1963.) [215

Other Countries

Universidade de Sao Paulo. Contribuicoes Avulsas do Unstituto Oceanografico. Oceanografia Biológica—1962, No. 2: Length-Weight Tables of some Fish Species from Southern Brazil. By H. Nomura. Pp. 4. Oceanografia Biológica—1962, No. 3: Preliminary Results on the Study of the Zooplankton Standing Stock off the South Brazilian Coast at 25° Lat. S. By M. Vannucci. Pp. 28. (Sao Paulo: Universidade de Sao Paulo, Instituto Oceanografico, 1962.) [215

United States Department of the Interior: Geological Survey. Bulletin 1118: Geology of Phosphate Deposits of Northern Peninsula, Florida. By Gilbert H. Espenshade and Charles W. Spencer. Pp. v+115+plates 1–12. Bulletin 1121–E: Geology of the Nefsy Divide Quadrangle, Crook County, Wyoming. By C. L. Pillmore and W. J. Mapel. Pp. iv+52+plates 1 and 2. Bulletin 1141–E: Cenozoic Geology in the Mammoth Area, Pinal County, Arizona. By L. A. Heindl. Pp. iv+41+plates 1–3. Bulletin 1144–C: Strontium Sorption Studies on Crandallite. By Irving May, Marian M. Schnepfe, and Charles R. Naeser. Pp. iii+17. (Washington, D.C.: Government Printing Office, 1963.) [215

Osmania University. Contributions from the Nizamiah Observatory, No. 1: Photometric Observations of YY CMi. By K. D. Abhyankar. Pp. 9. (Bagempet, Hyderabad-Deccan: Nizamiah Observatory, 1962.) [215

National Science Foundation. NSF 63–11: Federal Funds for Science XI-Fiscal Years 1961, 1962, and 1963. (Surveys of Science Resources Series). Pp. ix+161. (Washington, D.C.: Government Printing Office, 1963.) 1 dollar. [215

Publications de l'Institut National pour l'Étude Agronomique du Congo. Carte des Sols et de la Vegetation du Congo, du Rwanda et du Burundi. Notice Explicative de la Carte des Sols du Rwanda et du Burundi. Par A Van Wambeke. Pp. 76. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1963.) [215

Indian Journal of Chemistry, Vol. 1, No. 1, (January 1963). Pp. 1–56. Annual Subscription Rs. 15.00 Single Copy Rs. 2.00. (New Delhi: Council of Scientific and Industrial Research; London and New York: Pergamon Press, 1963.) [215

Indian Journal of Experimental Biology, Vol. 1, No. 1, (January 1963). Pp. 1–62. Annual Subscription Rs. 15.00. Single Copy Rs. 2.00. (New Delhi: Council of Scientific and Industrial Research; London and New York: Pergamon Press, 1963.) [215

Indian Journal of Pure and Applied Physics, Vol. 1, No. 1, (January 1963). Pp. 1–52. Annual Subscription Rs. 15.00. Single Copy Rs. 2.00. (New Delhi: Council of Scientific and Industrial Research; London and New York: Pergamon Press, 1963.) [215

Indian Journal of Technology, Vol. 1, No. 1, (January 1963). Pp. 1–60. Annual Subscription Rs. 15.00. Single Copy Rs. 2.00. (New Delhi: Council of Scientific and Industrial Research, London and New York: Pergamon Press, 1963.) [215

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais. Dundo—Lunda—Angola. Museu do Dundo. Subsidios para o Estudo da Biologia na Lunda. Publicacoes Culturais No. 61: Check-List of Angolan Birds. By Melvin A. Traylor. Pp. 250. (Lisbca: Companhia de Diamantes de Angola, 1963.) [215

Kungl. Sjöfartsstyrelsen, Stockholm. Lovö Geomagnetic Observatory—Year Book 1959. Pp. 37. (Stockholm: Kungl. Sjöfartsstyrelsen, 1962.) [215

National Metallurgical Laboratory, Jamshedpur, India. Integrated Mineral Beneficiation Pilot Plant—Descriptive Brochure. Pp. 28. (Jamshedpur: National Metallurgical Laboratory, 1963.) [245

Some Aspects of the Relative Efficiencies of Indian Languages: a Study from Information Theory Point of View. By B. S. Ramakrishna, K. K. Nair, V. N. Chiplunkar, B. B. Atal, V. Ramachandran, and R. Subramanian. Pp. xii+90. (Bangalore: Department of Electrical Communication Engineering, Indian Institute of Science, 1962.) Rs. 4.00; 6*s*.; 1 dollar. [245

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ORGANIZING SCIENTIFIC AND INDUSTRIAL RESEARCH IN BRITAIN

THE report of the Council for Scientific and Industrial Research for 1962* records a year of continued expansion in which gross expenditure for the year ended March 31, 1962, was almost £2·25 million above the gross expenditure of £14·5 million in 1961–62, while further expansion is contemplated in the near future. This growing effort of the Department in industrial research, like the Government's programme of expansion in technical and higher education, is related to the objectives referred to by the National Economic Development Council in its recent report *Conditions Favourable to Faster Growth* (*Nature*, 198, 1123; 1963), as the Parliamentary Secretary for Science said in the House of Commons on May 28. On that occasion Mr. Freeth also said that the Research Council was to consider further the section of the latter report relating to the extension of the use of civil development contracts to industry and to co-operation between Government and industry. According to the report of the National Economic Development Council, the Council for Scientific and Industrial Research has already suggested that Government and industry should co-operate to identify promising fields for research and development. Recognizing the importance of the scale of Britain's research and development effort being appropriate to the needs of the programme for growth, the National Economic Development Council has undertaken to examine and consider these various matters together with other related questions. Included would be the supply of technicians and scientific manpower generally, the contribution which the social sciences could make and the effective integration in civil industry of any patented benefits from defence research.

It could not have been expected that in its present report the Council for Industrial and Scientific Research could make more than passing reference to its consultation with the National Economic Development Council, but it is curious that although the report discusses two general aspects of expansion which are mentioned in *Conditions Favourable to Faster Growth* the National Economic Development Council is not mentioned. Of the first of these matters, that of establishing the right priorities since there are always new ideas for research competing with one another and with the work in hand for limited resources, the Research Council observes that it must take the initiative in establishing these priorities so that no important research project or programme is neglected. Hence, the research effort on behalf of different industries and in different scientific disciplines is brought into balance. Secondly, the Research Council comments on the extent to which, as the part played by science in national life grows and research and development become more complex, more organizations are concerned with the planning, execution and application of research, it is called on to play a part in the necessary co-ordination.

These twin functions of co-ordination and initiation serve two main purposes: they ensure that the whole research field is covered without overlap, and they present a coherent scientific picture as a basis for executive decisions. Obviously, the Council for Scientific and Industrial Research has a vital part to play in both functions, but it is a limited part. The other Research Councils have their own contribution to make, but the major responsibility for co-cordination surely rests with the Advisory Council for Scientific Policy.

These two functions, as they affect co-operation within the Government, with industry and with universities and colleges, are discussed in the opening part of the Research Council's report, which represents the main contribution of the report to the discussion of industrial research and development generally. Reference is also made to: (1) the completion by the Economics Committee of studies of the marine engineering industry in the United Kingdom and of research and development in foundry engineering and equipment; (2) the approval of arrangements with the Manchester College of Science and Technology for the study of the structure and economy of the building industry; (3) the establishment, in May 1962, of the committee to consider the present standing of mechanical engineering design in relation to the United Kingdom engineering industry and practice overseas, and to recommend any changes likely to result in improved design of British products. Comment on the work of the Department's own research stations is limited to that of the Building Research Station, the programme of space research at the Radio Research Station, the National Physical Laboratory on standards of measurement, the Government Chemist's Laboratory on the fluoridation of water supplies, the Forest Products Research Laboratory on the stress grading of timber, and the Torry Research Station on the quick freezing and low-temperature storage of fish at sea. The position of the research stations in relation to research and development generally is not considered, and the paragraphs dealing with the research associations likewise—apart from a reference to foreign membership of the associations—do not refer to general problems. The work of the Information Services is discussed in a page and a half, and the international activities and responsibilities of the Department are described in another two pages.

Apart from the Information Services, reports are published separately by most, if not all, of the research stations and research associations supported by the Department. With the further reports covering the Department's support of universities and colleges by research grants and training awards and on the research associations as a whole, it is understandable that the Council's own annual report should no longer deal in any detail with these individual activities. That, however, makes it the more desirable that the Council's own report should be careful to fill any lacuna in these other reports.

There is, in fact, a brief comment on the report of the Gibb–Zuckerman Committee on the management and control of research and development, in which the Research Council reaffirms its belief in the value of a steering committee as a normal feature in running a research establishment contrary to the view of the Gibb–Zuckerman Committee. However, two notable omissions are the absence of any discussion of the Department's responsibilities in regard to overseas research or any indication that the suggestions advanced at the British Association for the Advancement of Science meeting last September regarding the reorganization of research along technological lines

* Department of Scientific and Industrial Research. Report of the Research Council for the Year 1962. Pp. 62. (Cmnd. 2027.) (London: H.M.S.O., 1963.) 4*s*. net. (See also p. 233 of this issue of *Nature*.)

rather than that of individual industries are being examined (see *Nature*, 197, 222; 199, 106; 1963). Concerning the latter, despite the obvious inherent practical difficulties of the suggestion made by Mr. L. Holliday, it offers possible advantages that might well be worth the effort. It would have been encouraging to find evidence in this report that the Research Council was aware of such new potentialities and prepared to explore them. With its silence in regard to overseas research and to the National Economic Development Council, the present report is disturbing. If not exactly dragging its feet, the Research Council is not so alert as it might be and may be missing opportunities.

Nevertheless, much of the report is unexceptional. The Research Council aims to take a more positive part in the influence which science exerts on the operations of government, seeking to anticipate, so far as possible, the questions to which practical answers will be needed. The leading part played by the Department of Scientific and Industrial Research in the study of the medical and meteorological aspects, as well as the physical and chemical aspects is instanced as one example. Since 1955 an inter-departmental committee of which the Department provides the chairman, and, including representatives from the appropriate executive department and the Ministry of Housing and Local Government, has co-ordinated the whole subject. Water supplies are a further example, and while for many years the Geological Survey and Museum has been co-operating closely with the Ministry of Housing and Local Government in this field, a Hydrological Research Committee has now been set up to perform a co-ordinating function parallel to that of the inter-departmental committee on air pollution research.

Again, in co-operation with the Ministry of Health, the Research Council has recently established a Committee for Research on Dental Materials and Equipment to investigate the use of materials of greater strength and other improved physical properties which could offer considerable savings in conservative and prosthetic dentistry. Besides the Ministry of Health and the Department, the Medical Research Council is represented on the Committee, which also includes prominent academic and other research workers in this field. Care is also taken, through a Committee established in 1945, for which the Department provides a chairman and secretariat, and of which the Chairman of the University Grants Committee is a member, to ensure that each Department placing research contracts with universities is informed of contracts placed by all other Departments, so that all the contracts are placed on similar terms and there is no overlapping or duplication. This Committee is thus well placed to see that the proposed researches do not distort the pattern of university research.

All this organization must clearly be considered in any review of the structure of civil research, and there can be little doubt that while the Council for Scientific and Industrial Research has not the range of responsibility of the Advisory Council for Scientific Policy, it plays a much larger part than the other Research Councils. In co-operation with industry, the Department is, of course, a natural instrument, and while the pattern of co-operation varies, the Department appears to be functioning effectively. A steering committee has now been established, on which industry is represented, to guide co-operation in industry and Government laboratories in research and development in computers, and the report also cites an instance in research into methods of automation. An experimental project at the Parc Mine, near Llanwrst, North Wales, seeks to provide the basic data for the automatic control of mineral processing, notably the separation of valuable minerals from naturally occurring ores, and it should also indicate whether existing machinery, instruments and control equipment are suitable for use in automatically controlled, continuously operating plants.

As regards co-operation with universities and colleges, the Council of Scientific and Industrial Research observes that it is increasingly required to consider carefully how funds should be allotted between and within the scientific disciplines, and, in particular, what steps are necessary to ensure that neglected subjects are duly encouraged. It is, of course, in this connexion that Mr. Holliday's suggestion regarding the interaction of technologies and the concentration of research associations is important. In its present report, the Council expresses its view that more fundamental and technological research on engineering materials should be encouraged, and it instances the fundamental study of brittle fracture of which the Department is to act as the focal part, and the establishment by its Research Grants Committee of a new Sub-committee in Metallurgy and Materials. A Machine-Tool Research Co-ordinating Committee has also been set up, on which are represented the main laboratories in which the Department is financing research in this field.

The Council's latest report thus amply illustrates the widespread ramifications of the work of the Department of Scientific and Industrial Research and the key position which the Council occupies in the structure of Britain's civil research effort. If the report no longer supplies the conspectus of that work which earlier reports used to do that is almost inevitable, and provided that such reports as *Research for Industry* (*Nature*, 196, 97, 115; 1962) and the reviews of the Department's support for universities and colleges are comprehensive and up to date, that need not be material. It is, however, essential that the Council should ensure that all aspects of the Department's work are adequately carved in some such way and that it should be alert not only to see that its own report makes good any unavoidable gaps in the current year but also that any lack of prospective is corrected. Further, relieved, and justifiably so, from reporting on much detail, it is reasonable to expect that the Council should be concerned in its own report not merely to supply that wider perspective but also the forward look and especially to see that the significance of present-day developments is brought out to the fullest possible extent. It is in these respects that the present report falls short of what might have been expected.

NUCLEAR RESEARCH REACTORS

Programming and Utilization of Research Reactors

Proceedings of a Symposium held in Vienna, October 16–21, 1961. Vol. 1. Pp. xvi + 328. Vol. 2. Pp. xviii + 528. (London and New York: Academic Press. Published for the International Atomic Energy Agency, 1962.) Vol. 1, 63*s*.; Vol. 2, 105*s*.

AT the 1955 Geneva Conference on the Peaceful Uses of Atomic Energy most nations of the world presented statements of their possible needs for nuclear energy in the decades ahead, and many indicated the

great educational effort which would have to be devoted to training in nuclear science and engineering to prepare their countries for the advent of nuclear power. A few countries had built large research reactors for the production of isotopes and to study the behaviour of nuclear fuel, but the small training reactor had not been developed. Since then, research reactors have been constructed all over the world, and now more than 250 are in use. The 1961 Vienna International Reactor Conference was devoted to an overall review of problems of organization of research reactor centres, selection and training of staff, experience gained in operation and on the scientific use of research reactors. More than a hundred papers were presented and are published here in three volumes: the first on the organization of reactor centres of research, the second on experiences gained in the use of research reactors, and the third on more detailed statements about research programmes planned or being carried out in such reactors; but the classification is not very rigid, and there is much in Vol. 2 which concerns the subjects of Vol. 1. The present review deals only with Vols. 1 and 2. All reactor organizations should possess these volumes, for they contain a wealth of experience bought at great cost, and careful study might avoid waste and perhaps accident. Reactors can be dangerous: some accidents indeed have been serious and the utmost care is called for; constant vigilance and rehearsal of safety drills are essential as generations of students take over the controls. Hazard evaluation must be completed before a reactor reaches criticality and the right kind of operators must be chosen: as the Massachusetts Institute of Technology has found, "alertness is the primary requisite for a safe operation and by encouraging this quality problems are caught and rectified before serious consequence can develop".

The first requirement for a research reactor centre is to decide on the appropriate power output; 10–100 kW reactors can be permitted in built-up areas, those in the MW range must be in sparsely populated areas because of the danger from the iodine fission product if released from well-irradiated fuel elements. The papers from the U.S. Atomic Energy Commission, Associated Electrical Industries of Great Britain, the Technical Hochschule of Munich, the Atomic Energy Commission of Austria, and the Atomic Energy Commission of Australia deal with the health and safety aspects of the large research reactors, and it is clear that ensuring safety is an expensive item in the cost of a research centre. These reactors are capable of a large volume of research output; for example, as many as 100 simultaneous investigations are envisaged for the Munich reactor. For the training of students, the 10-kW reactor such as the *Triga* or *Argonaut* is ideal and very many of them are installed in universities with no special precautions about siting: their declared goal is the increase in national scientific manpower versed in nuclear science and engineering and, as Dr. Bradley of the U.S. Atomic Energy Commission pleads, these ought to come as additional tools to existing nuclear research and teaching and not be the main justification for the setting up of a national centre. Somewhere in between lie the group of reactors which can operate at 100-kW: rather large for training, rather small for extensive research into the damage created by fast neutrons. There are, however, many of these and more are planned like those for the universities in Great Britain, Queen Mary College, Imperial College of Science and Technology, and the Liverpool and Manchester colleges; the authors believe these will be mainly devoted to research, and many programmes are listed in the relevant papers.

From a long experience with the setting up of centres for training and research, Dr. Bradley pleads for a more careful assessment of the programme for which the reactor is required and then of the cost, staff and ancillary apparatus: many of the papers will help newcomers to make this assessment more comprehensive than they might have done; the great experiences of Oakridge and the Argonne Laboratories are given in detail. All the questions posed by General Atomics based on the sale of a score of reactors should be answered before deciding on a certain type of reactor.

There are good descriptions of the organization of the large reactor centres in Munich, Denmark, Australia and Canada, where 5- or 10-MW reactors are in operation, some on a basis of three shifts per day. It is a fairly common experience, specially mentioned in the paper from Lucas Heights, that initial assessment of the magnitude of the research programme which can be attempted with a given staff is over-optimistic, and the magnitude of maintenance is stressed by the Chalk River paper.

In Vol. 2 the experiences gained from some years of operation are listed in considerable detail. The Massachusetts Institute of Technology has high- and low-intensity beam tubes and has found that investigators prefer to wait their turn for the high flux from the 5-MW reactor rather than expose for long times at the lower intensity port. As the Massachusetts Institute and the Battelle Institute have tried to balance their budgets, they have given great thought to efficient operation and they emphasize the need for planning the experiment and testing the jigs most carefully before the reactor is ready to accept them. One penalty of arranging an enormous number of experiments to proceed simultaneously is the frequency with which the reactor 'scram' has been found to operate; greater care in design of safety circuits can alone cure this fault. There are many cost figures which appear to be very realistic, not extravagant, but not tinged with the familiar over-optimism associated with university ideas of costs. Another point of great value is the emphasis on space required: it has been found at Lucas Heights that congestion around the reactor is too great. To overcome this, the *Merlin* type reactor was designed to have a core capable of being raised to a second operating position; but experience with *Merlin* and with the modified *Merlin* called *Herald* weighs against this flexibility for sound technical reasons. The *Herald* paper draws valuable conclusions about the use of light water versus heavy water moderator for a pool reactor. Both these British reactors being pioneers of their kind contained features which would have been eliminated if more reactors of the same general pattern had been built. Emergency schemes for the higher powered reactors must be carefully planned and constantly rehearsed. A few papers deal with this, but only one boldly. In fact, in the discussion on operating experiences one author replied, "Normally, one does not bring the existence of emergency problems out into the light of day" (at least not in isolation, but only when mentioning advantages as well as risks !); other speakers and papers took the opposite line and referred to co-operation with police and Civil Defence. This seems to be the accepted policy, especially as pool-type reactors up to 5-MW do not need to operate in a pressure-proof building when built in the country.

This volume lists the many experiments performed with these powerful research reactors, including the medical therapeutic use of neutron beams in the Massachusetts Institute of Technology, the *Triga*-pulsed source at Brookhaven, and the thermoelectric generators at San Diego. These are necessarily brief accounts but they have the advantage of being listed all in one volume. They are too numerous to summarize or even to classify. It may be that from the numerous small reactors overseas little contribution to basic knowledge will come, but Dr. Hoffman, of General Atomics, rightly says, "Many of the human problems . . . arising in societies which are changing from an agricultural to a technological way of life are subject to an attack through an interdisciplinary approach; a handful of men working in this way with a research reactor may bring about a major human advance in those fields which directly affect the welfare of humans".

T. E. Allibone

HAND-BOOK OF CHEMICAL ANALYSIS

Official, Standardised and Recommended Methods of Analysis

Compiled and edited by S. C. Jolly. Pp. xx+577. (Cambridge: W. Heffer and Sons, Ltd., 1963. Published for the Analytical Methods Committee of the Society for Analytical Chemistry, 1963.) 126*s*.

THIS book from the Analytical Methods Committee of the Society for Analytical Chemistry contains in its first section a collection of well-tried analytical procedures which are judged to be the most reliable and unequivocal methods available for each determination described, while the second part of the text is devoted to an extensive classified bibliography of selected literature references for the analysis of diverse technical materials.

The procedures adopted and formulated carefully in Part 1 bear behind them the weight of opinion and expert knowledge of the leading analytical chemists and analytical laboratories in the United Kingdom, and do not represent the chosen or preferred procedures of any one individual or laboratory. Not unnaturally, a very great part of this first section is concerned with the analysis of organic materials; but the text opens with an account of the determination of metallic impurities, principally arsenic, copper, lead and zinc, in organic materials. Chief attention is directed, however, to the methods used for destruction of the organic substance since this is the most obvious point of difficulty in dealing with such analyses. Next the text concerns itself with the determination of vitamins in various matrixes such as green-plants, cereals and meat, making extensive use of column and paper chromatography for separation and identification followed by spectrophotometry for determination. The analysis of essential oils is a less well-defined area, and warning is given at the outset that even slight variations in the adopted procedure are responsible for significant differences in results. Accordingly, very detailed instructions are given for these particular analyses, practically all of which are titrimetric in nature following initial saponification, etc. Methods are also outlined for the determination of the principal physical constants required with these determinations.

The analysis of milk products for solids, fats, sucrose, etc., appears to rest on a more satisfactory basis. Here, the analytical methods range from sedimentation and optical rotation measurements to chloramine *T* titrations. A thorium nitrate procedure is recommended for the determination of fluorine in foodstuffs following a steam distillation separation. Details are also given for the determination of fibre in 'national flour' and for water (oven-drying) ash, chloride, nitrogen, creatine, etc., in meat extracts, and for fatty acids in oils and fats. The methods requisite for the analysis of unsaponified fat, free alkali, etc., in domestic and silicated soaps are also outlined. A large section is next devoted to the analytical problems associated with trade effluents. These involve sampling, *p*H, suspended and dissolved solids, titratable acidity or alkalinity, hardness, organic matter, oxygen-level, etc. This is followed by more specific analyses for seventeen inorganic cations, and ten non-metals, including organic substances such as phenols, formaldehyde, synthetic detergents and organic nitrogen, and others such as cyanide, fluoride, etc. In these analyses the procedures adopted are largely those of micro- or trace analysis, and range from biochemical assay and spectrophotometry (principally) to flame photometry and classical titration.

This most valuable section of the book ends with the determination of a few selected pesticide residues such as DDT, benzene hexachloride, malathion and mercury (extraction, chromatographic clean-up, dechlorination or nitration, etc., followed by colorimetry), and a final chapter on the assay of crude drugs, for example, aconite, ephedra, gelsemium, jaborandi, rauwolfia, etc. These last-mentioned methods are mainly spectrophotometric or titrimetric following extractive separation.

Part 2 (pp. 235) provides a well-set-out tabular summary of the literature with regard to official, standardized and recommended or tentative methods of analysis for a very great range of materials as diffuse as antibiotics, paper and cellulose materials, cement, dairy products, disinfectants, explosives, fertilizers, solid fuels, hormones, ferrous metals, paints, rubber, soils, starches, wood, etc. This is undoubtedly a most valuable compilation. At a glance the reader can find the literature key to innumerable analyses with guidance ranging from the choice of an adequate sampling procedure, without which any analysis is largely meaningless, through to the opening-out treatment and a range of analytical 'finishes'.

At times one may feel that the Committee has been rather conservative in its selection of analytical methods, but this is probably inevitable where co-operative committee work is involved. There are, for example, instances where one feels that the use of radiotracer technique, activation or polarography could profitably have been used. The burden of analysis is by spectrophotometer and burette throughout, and it is, of course, appropriate since many of these analyses require to be done in reasonably equipped small independent laboratories, not backed by the huge resources of major industrial concerns.

Undoubtedly this is a serviceable and invaluable compilation which reflects credit on the individuals who undertook the tremendous amount of work involved, and on the Society for Analytical Chemistry, which devoted so much of its time and energy to the production of this handsome and well-produced volume.

T. S. West

GAS CHROMATOGRAPHY

Gas Chromatography

By Dr. Howard Purnell. Pp. vii+441. (New York and London: John Wiley and Sons, Inc., 1962.) 90*s*.

THE author of this new text on *Gas Chromatography* is already well known as a physical chemist and as an expert on the theory and practice of packed gas-liquid chromatographic columns. The book reflects these interests, but also gives a complete and up-to-date coverage of a technique which is still rapidly expanding.

It begins with a fairly full treatment of the essential physical chemistry relevant to gas-liquid and gas-solid chromatography, the thermodynamics of solution, adsorption, partition and vaporization and the kinetics of diffusion and of gas-flow. This section should be especially valuable to those who use gas chromatography but feel that their understanding could be broadened by greater knowledge or revision of the physico-chemical principles. The second section is an extensive account of the theory of gas chromatography and the manner in which parts of this have been or may be tested. The discussion is lively and great care is taken to relate the theory to practice. Points of present dispute are clearly brought out, and although, as in other parts of the book, the views of the author are emphasized, it is made quite clear where this is done.

The third and largest section is concerned with gas chromatographic practice; equipment and materials, detectors and analytical and non-analytical applications. Constructional details are given of all types of gas chromatographic and ancilliary apparatus. Stress is laid on katharometer (thermal-conductivity) detectors and on simple yet effective equipment such as may be constructed in the laboratory. Again the author draws extensively from his personal experience.

The description of the application of gas chromatography to various problems is deliberately curtailed,

although the main principles are well set out as is the influence which the various experimental parameters will have on the choice of method for a particular type of problem. Each chapter has its own bibliography and there is a helpful subject-index, but the treatment does not lend itself to an index of chemicals. A collected list of symbols would be an improvement in subsequent editions. The style is clear and vigorous, and the text is full of interesting speculations. All workers in gas chromatography will find it stimulating reading, and with its emphasis on principles it should be particularly suited to students.

C. S. G. PHILLIPS

REVIEWS OF PHARMACOLOGY

Annual Review of Pharmacology

Vol. 2. Edited by Windsor C. Cutting, in association with Robert H. Dreisbach and Henry W. Elliott. Pp. vi+477. (Palo Alto, Calif.: Annual Reviews, Inc., 1962.) 7 dollars.

THE second *Annual Review of Pharmacology* adheres to the form which has been so successful in the longer established series of *Annual Reviews* in biochemistry, in physiology and in medicine. There can be no question of the value of *Annual Reviews* to research workers and to teachers; however, there are those who would prefer a slightly different presentation. The lists of references given at the end of each review article are numbered, sometimes in alphabetical order sometimes in the order in which they appear in the text; standardization would ease the reader's task. In the text the references are given sometimes as the author's name followed by the number of the reference and sometimes by the number alone; if the reader wishes to know who was the author in the latter case, it is necessary to turn to the end of the article. The question of dates of publications is important, particularly when the reviewers refer to a number of related investigations. Although these are *Annual Reviews*, by no means every field is covered annually, in fact, the literature cited usually covers periods of 5–20 years: in one article the earliest publication cited is 1821 while in another it is 1959.

There is much to recommend to the publishers of *Annual Reviews* another commonly used system of citing references: to give in the text the names of the author(s) followed by the year of publication, and to arrange the list of references alphabetically. Since library facilities for most readers are far from perfect an additional convenience would follow if the titles of the papers were given, preferably in their original language, or if translated, with the original language indicated.

Of the articles in the present volume, Dr. Gaddum's history, "The Pharmacologists of Edinburgh" is the only one that can be read with enjoyment by the non-specialist. Chancey Leake's "Review of Reviews" is also of general interest. The reviews on the pharmacology of China, India and Central Europe are valuable in diverting attention outwards from the American–Western European pharmacological nexus. It is to be regretted that the international situation is such that Chinese pharmacology was not reviewed by a pharmacologist working in China. Dr. Chen's review was heavily weighted towards practical medicine, presumably reflecting the Chinese literature; perhaps this is as it should be in a rapidly emerging nation.

Each of the remaining thirteen review articles deals competently with various specialized aspects of pharmacology. Most of the contributors admit to some degree of selection in referring to the literature: clearly this is unavoidable if *Annual Reviews* is to be more than another abstracting service. On the other hand, a critical assessment of published literature has been attempted by only some of the contributors. If the objects of *Annual Reviews* are to collect together the most important recently published work in given fields, but to avoid presenting the opinion of the reviewers, they have been successfully achieved.

M. J. RAND

SCIENCE AND DEFENCE POLICY

The Rise of the Boffins

By Ronald W. Clark. Pp. xix+268+16 plates. (London: Phoenix House, Ltd., 1962.) 25*s*. net.

MR. CLARK, who was a war correspondent with the Canadians, gives us in this book a vivid account of the influence of science on defence policy, and more particularly of the relations between scientists themselves in that field and between scientists and the Services. It is far more than a competent piece of journalism; while he is concerned largely with personalities, they are not allowed to become obsessive, and the Tizard–Cherwell clash is handled with fairness and judgment in which the author's own leanings are obvious but not allowed to obscure the issues. It may not be a scholar's book, and it is written in a style which should deservedly commend a popular as well as scientific audience. Nevertheless, although Mr. Clark relies in this book very largely on unpublished material, including the archives of the Royal Society, and the help of many of those concerned with the events with which he deals, his book is better documentated than many more pretentious works. Apart possibly from Tizard's own Haldane Memorial Lecture and Messel Memorial Lecture, his brief bibliography omits scarcely a major reference although, admittedly, as regards the development of relations between scientists and the Services during the First World War, much material is scattered through autobiographies and biographies of the type of Sir Frederick Sykes's *From Many Angles*, on which it is a matter of opinion as to whether it should or should not be included.

As a piece of exposition, the book could not easily be bettered, and scientists themselves would do well to note this demonstration of the practicability of easy communication free from jargon; even the curse of abbreviations is held tightly and reasonably in check. It is also a useful contribution to the study of the interrelations between the scientist and public affairs, correcting, for example, a certain imbalance in Lord Hailsham's recent book, *Science and Politics*, and not the less effectively because Mr. Clark never assays specifically to discuss such general issues. From his brief initial account of interrelations during the First World War his chapters describe successively and fluently the development of relations during the inter-war years to the establishment of radar and the first crude attempts to mobilize Britain's scientific resources at the onset of war. Specific contributions of science, both defensive and offensive, are clearly described as the account continues into the war years, and technical details are not allowed either to daunt the general reader, or to cover weaknesses or failures in system or personality at various levels. It underlines much that is soundest in Sir Charles Snow's lectures on "Science and Government" and in Lord Hailsham's more recent book, illustrating, for example, the crucial importance of the three desiderata which the Minister for Science lays down for Britain's educational system. It is not for a book of this character to describe, much less prescribe, the means, or the mechanism of decision; there could, however, be no more effective demonstration of the imperative necessity for reason to be at the cutting edge, and the suggestion that reason indicates that the best solution to most defence problems often lies in not going to war at all in no way detracts from that demonstration.

It is a pity that the author uses the vulgar expression 'boffins'.

URGE AND MOLECULAR BIOLOGY*

By Dr. PETER T. MORA

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LIVING entities, at all levels and in almost all their manifestations, have something of a directed, relentless, acquiring and selfish nature, a perseverance to maintain their own being and a continuous urge to dominate their surroundings, to take advantage of all possible circumstances, and to adjust to new conditions. The more 'vital' a living thing, the stronger this urge. Often this domineering, this taking advantage, this fighting is directed against other living entities; but the acquisition or absorption of metabolites, the urge of self-continuance goes on also in the absence of other living entities, for example, when a bacterial or mammalian cell is growing and multiplying in a nutrient medium.

Although life is relentless when it can express itself, it is not necessarily active all the time, but may alternate active with dormant periods when rest is more favourable or necessary. Periodic or cyclic activities are quite common in living processes.

Of course, all this urge and activity is temporal so far as any individual living entity is concerned. It declines with age and ceases with death, but its existence, while it lasts, makes living entities recognizable and distinct from non-living.

Biochemistry and molecular biology are unravelling nowadays more and more of the fascinating molecular details of living processes. We know, for example, that in reproduction complex macromolecules participate in a sequence of events: DNA, RNA, proteins, various enzymes using the energy supplied by certain phosphates (ATP) build up and down the recognized units of living material, the molecules of various sizes and complexity. We are beginning to know something about the sequences, the complex interrelations of such processes, and to recognize certain of the signalling and guiding elements, all as expressed by changes in molecules. It is natural for us to think in terms of molecules, since molecules are our tools of investigation and means of expression, or more exactly, symbols of our understanding of the physico-chemical processes of life.

The question raised here is: Are present molecular approaches sufficient to lead us to the understanding of the difference between the living and the non-living, or more explicitly, of what I call the 'biological urge' ?

I shall proceed first by defining this urge, and then by attempting to show it to be distinct from other biological phenomena now under successful investigation by molecular approaches, such as selection and evolution through random mutation, some form of adaptation, etc. I also shall mention briefly what other approaches, such as cybernetics, the principles of control and communication, feedback mechanisms in biochemistry, especially in microbial genetics, etc., may or may not offer toward the elucidation of the phenomenon of biological urge. Then an attempt will be made to show that the traditional approaches of physics or chemistry, be they thermodynamic or kinetic-statistic or, so far as I understand quantum mechanics, do not seem to be adequate. After that the modern scientific approach or method, as developed since the Renaissance, will be examined to see if it is capable of dealing with the problem of life. Finally, further personal and general opinions will be presented about what we should do in order to be more successful; but I am afraid I cannot be very definite, because I cannot see clearly the nature of the new method, or the extension of the physical methods, I feel it is desirable to find in biology.

* This article was prepared while I was assigned to the Virus Research Unit, Agricultural Research Council, Cambridge, England, and was read during a colloquium in the Department of Biochemistry, University of Oxford, on February 4.

Definition of Biological Urge and Delineation of the Problem

Living entities have several characteristics which make them distinct from non-living. They are autonomous; the individuals are similar but not exactly alike; they group in hierarchical systems; their mature state is more or less invariant, but still they are in continuous flux. They are self-maintaining; they build a complex and highly improbable structure necessary for the fulfilment of their function; they restore this structure when worn out, and repair it when damaged. They continuously adjust themselves to their environment, and they may adjust to even completely novel conditions, which they or their predecessors never could have faced before. Most important of all, a certain urge or drive towards self-fulfilment characterizes the living organism in all its activity and at all levels, from the simplest to the most complex.

Several words have been used for describing at least certain aspects of the last-mentioned attribute. Unfortunately, almost all the words available are anthropomorphic and therefore have some unwanted associated meaning; moreover, they often include properties which can be explained by evolutionary selection. Some of the words, such as 'wholeness'[1,2], 'directiveness'[3], 'orientedness'[4], are not very useful for my purpose, and others, such as 'purposiveness'[5], 'consciousness'[6], 'entelechy', or '*vis vitalis*', are clearly unsuitable, because they imply more than the materialistic approach I intend. I choose 'conatus', which, used in this context (Spinoza[4]), means drive, effort or urge. I hope the word urge is unpretentious enough (actually it is almost vulgar) to prevent my being labelled a metaphysician. At the same time it best indicates to me the most important and peculiar attribute of the living, the relentless striving to survive, to absorb, to expand, to dominate.

The assimilation of metabolites, the continuous use of energy for the sake of the living entity, is an example of the urge. A living entity (for example, a cell in tissue culture) does try to expand, organize the elements around it (the random mixture of molecules in the medium) and, so to speak, subjugate or dominate its vicinity, regardless of whether it encounters other living units or not. Now consider the formation of a clone from a single cell. Replication, and generally reproduction and progeny formation, may be thought of as an extension or even a consequence of the urge of the individual living unit towards self-continuance and a spreading over or overwhelming of the available suitable space. So far as I know, no good reason has been advanced on a molecular level for the occurrence and perpetuation of both these processes. The fact that we now are learning about the key molecules (intermediary metabolites, DNA, enzymes, ATP, etc.), their structure, and some of the details of the mechanism of metabolism and of replication on molecular level (the complicated schemes of intermediary metabolism, DNA strand separation, complementariness of nucleotide bases and the pertinence of this to replication, etc.) does not explain why metabolism and multiplication

occur at all, or why living systems build their improbable but functional structure, or what keeps them living.

The problem I raise of how to approach such obvious phenomena by scientific research is not helped by saying that this is just in the nature of living matter, an inherent attribute[7], the way mass or gravitational attraction, etc., is an attribute of matter[8]. But mass, gravitational attraction, and other physical properties are inseparable attributes of all physical matter, and they are not restricted by time to a certain period. The urge is a peculiar attribute, restricted only to the matter of the living while the living is alive. Fundamentally, I am indeed asking what the difference is between the living and the non-living matter, and whether molecular science as we know it is capable of dealing with this question.

This question then asks why is living matter alive, and actually involves really not the 'how' but the 'why'. One may say that the purpose of science is not to answer the 'why' but the 'how', and the question of 'why' should be left to philosophy or to belief. However, I do not question why living matter exists at all, as this would be a part of a philosophical problem of existence in general; but how to approach scientifically the problem of to what extent and why living matter is different while alive from the non-living matter.

We know, of course, that living matter consists of nothing but molecules. But are not these molecules only agents, means of practical expression of the urge, simply units of the complex interrelated functions in living processes ? In contemporary biochemistry, as Paul Weiss said[9]: "there seems to be a predilection for identifying agents and charging them for responsibility of action". I will make a qualified exception to this, when later discussing viral DNA.

It would be pertinent to my question to consider the functional interrelationships of molecules, their mutual influence, the information transfer between the molecules. Cybernetics and other related approaches claim to give such methods to biology. Indeed, theoretical considerations, such as in cybernetics, or experimentally found details of feedback, such as in metabolic cycles, or in the function of genes controlling some enzymes in micro-organisms[10–13], represent important advances in our understanding of the control of living processes and show how information can be co-ordinated on a molecular level for the benefit of the living entity. For example, the expression of a series of structural genes appears to be controlled by the cytoplasmic product of a regulator gene, called the repressor. The repressor can be either inactivated (induction) or activated (repression) in a medium deficient in metabolites, allowing the bacterium to adjust its enzymatic mechanism to the medium[11]. But this does not answer the question of why such processes occur, and what drives the processes. In other words, I cannot see how cybernetics and details of feedback processes, etc., can give the reason for the continuous drive, the urge of the living entity for self-continuance and reproduction. Furthermore, cybernetics does not claim to say anything about the origin of such processes. Still, cybernetics and the recognition of details of information transfer, of feedback, etc., in dynamic biochemical processes seems the most suitable of the available methods to examine the problem I pose: in living processes, even in the simplest living unit, such as a cell, millions of molecules participate, and their interrelation, their mutual influence or control, their co-functioning is essential in space and time.

Let us consider a process, the virus cell interaction, which is in a borderline area. Viruses, of course, in themselves, do not manifest any urge or any special attribute of the living. They are merely stable macromolecular complexes consisting of nucleic acid surrounded by protein. However, when they are brought by random mechanical forces, such as by Brownian motion in a liquid medium, to the vicinity of a host cell, a whole sequence of complicated molecular processes start in a very directed manner, and the sole objective appears to be to produce a large number of viral progenies. In their resting phase, without the host, viruses might be considered analogues of the living entities in their dormant phase; however, viruses have absolutely no metabolism without the host. Their life-cycle, and the factors influencing or favouring the different cyclic phases (temperature, deuterium oxide) are under investigation[14]. When viruses collide with a host cell, and the conditions are right for the manifestation of their potential, then a whole sequence of amazing, interrelated events begin. For example, the tail sheet protein of the *T*2 phage contracts, probably triggered by fresh SH groups freed after oxidation of thiolester bonds by a metal ion present in the receptor *E. coli* cell-wall. This leads to the penetration of the bacterial cell-wall by the tail core. Then a single[15] molecule of DNA is injected, together with a small amount of internal protein[16–18] and certain amines[19]. Then the cellular processes in the bacterium completely change[20,21]. The synthesis of bacterial DNA and RNA almost immediately stops, there is a breakdown of the bacterial chromosomes by an activated DNase, and a new kind of intermediate hybrid DNA–RNA complex is produced[22]. This is followed by further very complicated and little understood changes in the metabolic processes of the *E. coli* leading to the formation of a considerable amount of fresh phage DNA in the double helix structure, which is eventually packaged in a particular folded form together with the internal protein and the amines, and then this is surrounded by newly formed phage head protein and the complex tail is attached[20]. About 200 identical progenies are produced within 20 min. The progenies are then released automatically, as the bacterial cell is lysed by a freshly synthesized lysozyme.

Similar reproductive processes can be initiated by the nucleic acid of a virus, when conditions are such that bare nucleic acid without the presence of the virus protein will penetrate into the cell; for example, when facilitated by changes in salt concentration. Infection[23] of *E. coli* protoplast by a single stranded ring form[24] of ΦX 174 DNA, or by λ DNA (ref. 25), or of cells of a mouse embryo in tissue culture by the DNA of polyoma virus[26] are such examples. Thus, DNA alone, and apparently only one molecule of DNA, either in double helix or in a single stranded form, has inherently all the information necessary to initiate progeny formation. Truly, it appears, this molecule is a full cause of initiation; but it is not responsible alone for all the processes which happen inside the bacterial cell. Host enzymes, the whole metabolic and energy-yielding apparatus of the host cell and the interrelationships inherent in the host bacterial, plant, or mammalian cell are necessary for the production of viral progenies. The viral nucleic acid represents only a 'misinformation', which starts the host cell on a new and often disastrous path[27] ending in cell disruption, or in lysogeny, which is a form of a symbiosis, or in neoplastic transformation[28], which is probably associated with sufficient alteration in the antigenic make-up of the cell[29] to allow it to escape contact inhibition by other cells[30]. Thus, the initiating cause and full information for the particular direction of metabolic activities of the cell are supplied by the DNA.

Recently, systems simpler than a cell were found to exhibit certain aspects of building up of organized structures which resemble or copy the biologic macromolecules. I am referring, for example, to the polynucleotide synthesizing enzyme system of Kornberg *et al.*[31], and to the ribosome extracts containing *s*RNA and fortified with ATP and all essential amino-acids which were found by Nirenberg[32] to support the polymerization of amino-acids as directed by added polynucleotides. Both systems use biologically preformed macromolecules and specific structures (the DNA polymerase[31] or various other enzymes together with soluble RNA and the ribosomes of *E. coli*[32]) which then intereact so that a polymer, a polynucleotide

or a polyamino-acid is synthesized from the existing pool of the monomers. The synthesized polymers resemble in structure the natural polymers or follow the information supplied by the added template. The Kornberg system can produce homopolymers (a simple polynucleotide) or regularly repeating copolymers (cf. *d*AT copolymer[33]) in the absence of a primer polynucleotide, or can produce a polynucleotide or a DNA with the expected sequence and of biological function (cf. transforming activity[64]), determined by the primer. In the Nirenberg system the *E. coli* extract on addition of synthetic polynucleotide (synthetic 'messenger' RNA) can produce polymers of amino-acids with the frequency of the amino-acids (and presumably also the sequence) determined by the information contained in the base frequency (sequence) of the synthetic messenger. In fact, on addition of virus RNA a certain amount of virus-like protein can be produced. For example, on addition of tobacco mosaic virus (TMV) RNA the newly synthesized protein weakly cross-reacts in serological tests with the TMV protein, and also recombines to an extent (about 10 per cent) with TMV RNA[35]. However, these synthetic processes are limited in two respects: (1) they come to an early halt; (2) they represent only isolated small portions of the synthetic processes which occur in living cells. Although they produce a certain amount of absence of randomness, because of the preformed specific directing biological macromolecules (enzymes, templates) and other, still intact, more complex cell components (ribosomes), basically these are polymerization processes which produce polymers and copolymers, some with a specific sequence, using ATP as the energy source, and the initiation and the orientation of which are controlled by the intact biological polymers present.

The purely chemical polymerization or polycondensation processes which give polymers resembling biological macromolecules are more primitive models. Some are random processes, such as the heating of ammonium cyanide in aqueous solution[36], heating of amino-acids[37], sugars[38] or ribonucleotides[39]. Others are polymerization processes in which certain chemical bonds are favoured: Khorana's method of polymerizing thymidylic acid[40], certain forms of amino-acid anhydride polymerization which produce polyamino-acids[41]. In all these cases, however, the restrictions with regards to the particular bonds to be broken or formed, and thus the structure of the resulting polymers, are inherent in the bond energies and structures of the monomers and in the treatment applied (heat, catalyst, initiator, etc.). These polymerizations are only exercises in synthetic organic chemistry. They use similar monomers, but they do not really resemble a self-perpetuating, co-ordinated process, and they do not lead to the synthesis of a living unit with the characteristic urge. They do not even produce functional polymers with specific structure.

But could not similar polymerization processes add up to this, and life be synthesized one day ? Is it not claimed by some, and indeed believed by the majority of scientists that life originated from a suitable mixture of molecules in the distant past in some similar way ?

These are two questions to be argued according to one's disposition; but I do not believe they can be answered at the present time.

The first question will be taken out of the realm of opinion if and when the first living unit with the characteristic urge toward self-maintenance, assimilation and reproduction is synthesized. Until then, it is a matter of one's preference or belief whether this can be achieved or not. Obviously, all the necessary molecules when added together do not function, as in a cell homogenate, where we have destroyed the specific interrelationships and controls. Biochemists will agree that we are very far from knowing how this interrelationship and control comes about in space and time, and we do not, at least not yet, foresee the synthesis of a functioning, living unit from a molecular level. Claim to the contrary comes from insufficient biochemical knowledge or from a belief that the present molecular biology will supply the information imminently. The last tenet is exactly what I am questioning. Characteristically, such claims come more frequently from physicists or chemists who examine biological macromolecules, than from biochemists, and scarcely ever from those biologists who work with a small living unit, such as a cell.

As to the second question, how life originated, I am afraid that, since Pasteur, this question is not within the scientific domain, at least if we consider provability as an essential part of a scientific statement. Some light might be thrown on this problem, however, if we find a certain form of life outside the Earth, and if we analyse that form of life. Scientists nowadays are inclined to expect this, probably because of their pre-occupation with statistical or chance processes and their preference for accounting for facts by existing knowledge, and also from their disinclination to consider the Earth as a singular part of space. They expect that in other parts of space where favourable conditions similar to the Earth's have existed, that is where there was available for a long time a high concentration of complex molecules from polyvalent atoms[61], a self-maintaining, matter-acquiring, energy-producing, reproducing, living unit also appeared.

Even if we find this (and in my opinion we have a better chance to find an answer to this on Venus or Mars, before we synthesize a living unit), we still will not know why this continuous urge exists to maintain and extend life against disrupting, randomizing physical forces, without careful examination of the new type of life. In fact, we might have doubled the problem I pose. However, the statistical, or chance theory for the origin of life certainly would receive very strong support, provided, of course, that a common origin of life (by meteors or by other 'infection' of planets) can be excluded.

In his book on the origin of life, Oparin[5] elaborates on the concept of selection on molecular level as the key process which tips the balance over from the complex aggregates of molecules which continuously build up toward a certain level in the direction of a functioning biologic unit, but break down because of the random physicochemical forces before they cross a theoretical borderline and form the first self-maintaining, absorbing, reproducing, etc., living unit. 'Selection', as Oparin and other biochemists use it in this context, includes a tacit assumption of acquisition, of positive action, of building up the improbable, more complex from the more probable, less complex, and actually increasing stability as complexity increases.

It is more than a passive and statistical phenomenon, common in chemical processes, where molecules are selected or rejected, for example, according to whether they fit or not when they happen to collide. Otherwise, the result would not be a self-maintaining, absorbing, reproducing unit, which will always tend toward more and more complexity as it evolves, but could be only a metastable temporary complex which will tend to disperse more and more as its complexity increases. That the word 'selection' in this context means something different from what it means in a chemical process is not often recognized. One reason might be that each biochemical process, when considered *in vitro*—for example, an enzyme-substrate or antigen-antibody interaction—is controlled by selection, in the passive, traditional physicochemical sense. However, such isolated processes will cease eventually; the molecules will disperse and degrade. There is no justification for expecting selection, on the basis of physicochemical principles, to operate in the positive sense, and there is no justification for involving it to account for the origin and continuance of living processes.

To me it appears that this is an extension of Darwin's use of the word selection in a third sense, which explains evolution of the living, but the Darwinian 'selection' and evolution concept was arrived at empirically, by observing

the reproduction and the balance of dominance of progenies of already-living units, and the spectrum of the living species.

We believe that Darwinian selection and evolution of living entities occurs because of random mutation. Mutation occurs as consequence of a chance phenomenon, and it is explainable statistically on the molecular level: for example, one or more nucleotide base in the DNA of a chromosome may be altered because of radiation, a mistake or other chance effect. The ingenious present work on microbial genetics is indeed very suggestive that selection and evolution occurs because of statistical phenomena on the molecular level.

Then, it appears to many molecular biologists, there is no need to invoke anything further. Living units are those which are selected because they are capable of survival and reproduction under the given conditions and any of their particular functions evolved exactly because of this selection. Any specific property of a living unit such as a certain metabolic activity of a bacterial cell is considered to be a direct consequence of the principles of selection and evolution; unspecific broad statements, such as about urge, etc., are meaningless when considered in this light. There was a high concentration of suitable molecules available and for a very long time before the first living unit appeared; a unique occurrence, probably, as suggested by the similarities in fundamental biochemical processes (ATP as a common energy source, etc.). Then, the aforementioned molecular-statistical-genetic processes led through selection to the evolution of the different forms of life.

The foregoing hypothesis is probably the best that can be proposed at this time, but when one thinks about it, shortcomings are evident. First of all, selection and evolution in the Darwinian sense occur only in the living and because of being alive. To invoke statistical concepts, probability and complexity to account for the origin and the continuance of life is not felicitous or sufficient. As the complexity of a molecular aggregate increases, and indeed very complex arrangements and interrelationships of molecules are necessary for the simplest living unit, the probability of its existence under the disruptive and random influence of physico-chemical forces decreases; the probability that it will continue to function in a certain way, for example, to absorb and to repair, will be even lower; and the probability that it will reproduce, still lower. Statistical considerations, probability, complexity, etc., followed to their logical implications suggest that the origin and continuance of life is not controlled by such principles. An admission of this is the use of a period of practically infinite time to obtain the derived result. Using such logic, however, we can prove anything, including that, after a suitably long time, everything will repeat itself exactly, and I will write again these same lines under exactly the same conditions. When, in statistical processes, the probability is so low that for practical purposes infinite time must elapse for the occurrence of an event, the statistical explanation is not helpful.

There is another escape clause in the statistical hypothesis of origin and continuance of life as coupled with the statistical selection and evolution theory. The simplest, earliest and most labile forms of life, it is explained, are extinct, and this is why we are unable to see exactly how life originated. One may argue that alternative hypothesis that one or a few similar, self-reproducing, living units were brought or appeared on Earth in some relatively advanced stage of evolution. If life appears because of statistical causes, it should continue to appear no matter how rarely, unless conditions have drastically changed, and we should have a chance, no matter how small, of finding surviving units in all stages of development, including the earliest ones. Also, as already mentioned, the similarity of fundamental biochemical processes suggest that life appeared only once; in a statistical theory it is very difficult to explain why it did not happen at least twice, or if it happened more than once, why it happened exactly the same way.

Of course, the alternative hypotheses still do not say how life originated or why it continues. I would like to show that speculations on the origin and continuance of life are in such a vague state that they are not amenable to proof. There is a semantic confusion in the indiscriminate use of the word 'selection' which has at least three different operational uses in these contexts and this then leads to an illusion that it operates in another sense in another field. Our speculations are generally dominated by our present statistical orientation, which is prevalent and successful in physical sciences and in certain aspects of genetics. To invoke statistical and probability concepts to explain the origin and continuance of life does not appeal to me when I consider that the expression and the consequence of life, from which we detect the presence of a living unit, is exactly opposite to what we would expect on the basis of purely statistical and probability considerations. (I refer to the creation of order on a very complex level.) I do not have a scientifically more satisfying theory to suggest in its place, and I beg only ignorance.

However, Darwinian selection and evolution of already living entities is probably under statistical control; it is a consequence of mutability, and so are apparently some aspects of cellular regulatory mechanism[13,65].

In certain cases it is recognized that changes in the cellular regulatory mechanism are the direct consequence of mutation, such as when bacteria mutate so that constitutivity becomes dominant, and the operator becomes insensitive to the repressor. An example of this mutation is when the mutant bacteria will synthesize β-galactosidase and permease in the presence of the repressor. Generally, it was found that the cellular regulatory mechanism reaches back to the gene-level. For example, it is assumed that in the case of β-galactosidase and permease synthesis a regulating gene produces an apo-repressor, and a repressor is synthesized from this only in the presence of a co-repressor, which may be a product of metabolism of the energy source, for example, glucose; while lactose, which is the inducer, competes with the co-repressor and prevents the formation of the repressor. When no repressor is present, the operator gene instructs the structural genes to synthesize the enzymes of β-galactosidase and the permease. However, cellular regulatory mechanism, to some extent, may operate below gene-level. For example, part of the regulation of histidine biosynthesis proceeds through the specific inhibitory activity of the end product, histidine, on the first enzyme of the biosynthetic pathway, and the enzymes in the later stages of the pathway are deprived of their substrates[66]. This is negative feedback mechanism, and the control is corrective.

Another type of regulatory mechanism may depend on the states of aggregation of a certain enzyme protein, for example, glutamic dehydrogenase in the disaggregated state will lose its specific activity, but will acquire a new enzymatic activity, that is of L-alanine dehydrogenase[66]. The ability of a cell to synthesize or not to synthesize this or that enzyme, or to adjust by this or that mechanism to a change in the environment, whatever is the mechanism, is generally looked on by molecular biologists as a consequence of mutability, thus purely of chance. However, chance, while it operates to select the varieties of the mechanism of the existing living organism, is not a sufficient cause.

One may consider evolutionary selection as a passive aspect of the activity of organisms, which are already living and which are capable of reproducing, in the sense that they are under the control, or more properly at the mercy, of the external physical conditions which might lead by chance mutations to progenies more suitable for the changing conditions. To allow selections by mutations, there must be present an ability of the organism to reproduce persistently.

The difficulty in accounting by probability for the occurrence of the first reproducing unit was mentioned earlier. Now we need to account for a persistent ability to reproduce, so that beneficial mutation can occur to select the organism which is more suitable for survival. We know that the frequency of mutation of simple present-day organisms is rather low, and, in any event, mutations may well be deleterious or lethal; as a matter of fact mutability was used to account in a hypothesis for ageing and death of multi-cellular organisms[37]. Mutability should be considered as a consequence, and not the cause, of the persistent ability to reproduce.

A certain amount of selection of macromolecules occur in biology, but again only when living units operate. Anfinsen[42] has presented a plausible story of why we find differences in structures of important proteins, such as in the sequence of insulins in different species, and how complicated enzymes, such as RNase or lysozyme, may have evolved from simpler molecules. This type of evolutionary process might be based on the selection of a macromolecule according to whether it had the best structure for enzyme activity under the given circumstances. But again selection, although macromolecules are selected, occurs within the living unit. Thus selection on a molecular level should not be used as a reason why living units appeared, or explain why a living unit maintains and expands itself in spite of the random disrupting physical forces, or why it reproduces.

It is thought that compartmentalization through membranes, higher concentration of enzymes, different kinetics of enzyme reactions in gel[43], etc., occur in living cells so as to increase efficient enzyme concentration, etc. and thus favour certain processes. Reorientation of linear macromolecules in membranes was proposed as a unifying principle, underlying cellular selectivity[44]. These are, of course, useful concepts, and the latter theory especially might be useful in explaining the details of initiation of many biological phenomena by selective molecular interaction. It is a peculiarity of the living systems, however, that the local concentrations, permeability, etc., are produced and changed continually as benefits the cell. This is not molecular control of cellular activities, but "cellular control of molecular activities"[9]. Even if there was once a favourable selective concentration of molecules, in the primæval coacervate of Oparin, producing the first reproducible unit, why should continuous reproduction occur to allow development of organisms through selection so that they have an ability to control rate processes to take place exactly so as to benefit the living unit at that moment? This is another way of putting the problem, which to me cannot be answered by invoking selection on a molecular level. Keep in mind that Darwin's selection principle is a necessary but not sufficient condition for explanation of development of different forms of living processes.

I am aware that the properties of the building blocks of matter may inherently contain the information necessary to automatic formation of the resulting unit of higher hierarchy with a specific or single structure. For example, amino-acids under certain conditions will polymerize to form an α-helix[45], poly I and poly C will form a DNA-like double helix[46,47], a reduced RNase with sulphydryl groups in the random coil will reassemble into the original tertiary structure with S–S links and recover the full activity of the enzyme when gently oxidized[48], collagen will aggregate[49] *in vitro* with a characteristic spacing of 640 Å, TMV protein and RNA recombines to a normal virus structure with 16 1/3 sub-units of proteins per turn in a helical arrangement with an RNA coil joining them up in their groove[50], etc. These are, however, limited processes, similar to crystallization; an end is always in sight and the organization stops at the next hierarchical level. Most important of all, there is no resulting continuous function, continuous rearrangement or change, or any expression of the urge.

Organization of cells also may occur *in vitro*, apparently controlled by their physico-chemical (surface, etc.) properties. For example, a single cell suspension of a tissue may reassemble in culture to show certain of the characteristic features of the tissue from which the cells came. Again, the information was biologically pre-formed and the reorganization occurs only to a certain level (next hierarchy) and there is no continued function without the control of the whole (in this case the organism). Obviously, this is not the full story in processes such as wound healing, or differentiation, or dedifferentiation. I do not intend to go into these problems, since I do not know enough about them. I simply propose that there are problems in biology on all levels where some kind of urge is evident characteristic of the living; but in detail, so far as we know, it always operates through the well-known physical forces and elements of matter.

Thus, the paradox: well-known molecular forces, exactly the same which operate in the physical realm, appear to operate inside the living units when considering any particular detail, but they are under the control of the total living unit, and in final analysis this is a consequence of an unexpected perseverance of the living unit to maintain of its own being, of an urge of self-continuance, expansion, and reproduction. Living processes persist to a degree improbable in a physical system.

Scientific Approaches

One may approach this problem by several methods useful in dealing with the physical world. One is thermodynamic.

The second law of classical thermodynamics postulates an overall increase of entropy. Of course, certain local areas where the entropy is decreasing are common, such as in crystallization. In crystallization, the full process is determined by the valency, the molecular structure of the components, whether atoms, molecules or polymers. Crystallization occurs because it leads to the lowest energy state, and to the most stable arrangement of atoms or molecules under the given conditions. Crystallization leads to simple, very uniform repeating structures, which are inert. These structures do not function, and are not designed by function. (An exception to this is the case when biologically pre-formed molecules re-combine, such as the aforementioned example of reconstitution of a functioning virus from its protein and nucleic acid component.) In contrast, living structures are organized under the control of other living units, when the molecular conditions alone would not favour their formation; their structure is far from simple or stable. They are designed to function, and indeed they do function; they continuously absorb, expand, reproduce.

Living organisms continuously draw negative entropy from their environment and do this so consistently that this was considered to be their main difference from the non-living[51]. When they die, they lose this ability, and simple molecular forces take over, leading normally to dispersion, but also sometimes to crystallization of certain components. It is possible to crystallize out amino-acids from a cell homogenate, but not from a living cell; actually prevention of crystallization of water and other molecules is necessary to maintain the viability of small organisms or of tissues, when cooled to very low temperatures.

It is fitting to apply the thermodynamics of irreversible processes[52] to biological systems[62]. This thermodynamics considers 'open systems', which continuously exchange both energy and matter with the exterior. In such systems, in the 'stationary state', as Prigogine showed[53], internal irreversible processes always operate in such a way that their effect is to lower the value of the entropy production per unit time. When a system is in a state of minimum entropy production, it cannot leave this state by a spontaneous irreversible change. If some fluctuation

cause deviation, internal changes will take place and bring back the system to its initial state. This would correspond to the tendency of the living organisms, in their fully grown state, to maintain their structure against external perturbation, and it also means that there should be a tendency toward minimum metabolism in this state. The entropy of the matter entering the system has to be smaller than the entropy of the matter given off by the system to the external world. The system 'degrades' the matter it receives, and the stationary state is maintained by this degradation. All the foregoing pertains to the stationary state. Now consider the evolutionary state towards a stationary state, which corresponds to growth. While it was shown by Prigonine that often the entropy of such a system itself may decrease, in other cases, however, it may increase. Actually the latter would always be the case for isolated systems if it were not for the fact that such systems cannot be in stationary non-equilibrium state.

While from the point of view of the thermodynamics of the open and stationary systems the behaviour of organisms is not contradictory with regard to the change in entropy, the problem is far from being fully resolved. There remain the questions why living processes operate as irreversible processes so that the entropy content decreases invariably in the system in the growing state, and why do they immediately revert to increase of entropy on death ? There has been regrettably little research on these important problems, and I have seen no present experimental work pertaining to biological systems.

So far as some aspects of the kinetic approaches are concerned, I expressed myself in a recent article, on "Directiveness on Molecular Level ?"[53]. The gist of the article was a query: whether by the methods of molecular kinetics we can describe the phenomenon of directiveness, or more exactly, prove the full efficiency of an interaction, evident when observing an individual living entity. The term directiveness may encompass much more than directed interactions, and may be used to include phenomena I group under the term 'urge', as E. S. Russell did in a brilliant book[3] in which he surveyed directiveness in the field of animal physiology and behaviour. I must say, however, that many of the phenomena Russell described could very well be described by evolutionary selection.

In my article I considered only the problem of interaction. My approach was to go down the scale from obvious examples in our visible world (an animal goes toward its food or shelter, etc.) to molecular dimensions. The attachment of a bacteriophage on to its bacterial host is a process in which every collision appears to be successful, or, more properly, where infection of the bacterium with DNA occurs every time a bacteriophage happens to collide with the bacterium, and results eventually in progeny formation. This occurs in spite of the fact that only a small percentage of the bacterial cell-wall surface is suitable for penetration and injection of the DNA by a particular phage. Of course, purely physico-chemical factors are responsible for this process when the details are considered: the tail fibres might be complementary to certain areas on the bacterial cell-wall, the rotational diffusion of the head-heavy *T*2 bacteriophage must be greater than the translational diffusion, so that the tail tip or the fibres on the tail tip should have a much better chance of touching the cell surface than, for example, the head protein. Then irreversible attachment follows only when the complementary molecular structures meet. The neutralization of the tail tip of the bacteriophage with its antibody also seems on first sight to be a process where the collisions are fully effective.

In studying interaction on a molecular level, observation is necessarily restricted to the sum total of the effect of a very large number of molecules (as in an enzyme assay, for example), and it becomes clearly impossible to conclude that the process has full efficiency. We can qualitatively demonstrate that, to a certain extent, directed or oriented interactions may occur between molecules: an enzyme such as RNase may attract its substrate, RNA, *in vitro*, so that collisions may be more efficient than they would be when the collisions occurred at random without the attraction caused, for example, by suitably distributed opposite charges on the two molecules[54].

Unfortunately, in neither of the foregoing cases is it possible to prove collision efficiency even within a few orders of magnitude because of inherent theoretical and experimental limitations[63]. Thus, on or near the molecular level, the kinetic approach does not appear to be suitable for dealing with efficiency of collision, while full efficiency of interaction may be obvious on a larger scale, when the fate of each biological entity can be followed unambiguously.

Calculations by classical quantum mechanical methods by Wigner[6] resulted in zero probability of self-reproducing states provided, of course, that those approaches and assumptions which Wigner made are accepted. Wigner noted that his crucial assumption, that is: "a Hamiltonian which governs the behaviour of complicated systems is a random symmetric matrix", is analogous to another assumption, that is "in no system of any complexity is there accidential degeneracy", which was used by von Neumann to arrive at the second law of thermodynamics by quantum mechanical methods. I am impressed that an eminent quantum physicist, like Wigner, concluded that the classical methods of quantum mechanics are not valid so far as living systems are concerned. Thus, Wigner concludes that either quantum mechanics as it stands has to abandon its claim that it comprises a valid explanation of all that exists (and let us remember that the aim of the biochemistry or molecular biology through chemistry and physics is to put understanding of living processes eventually on quantum mechanic basis), or quantum mechanics has to consider aspects not thought of previously, or not necessary when dealing with the purely physical world[6] (see also ref. 34).

Such new aspects, according to Wigner, should include consideration of 'consciousness', which apparently "controls much of the living process, and thus in reverse. living process might be also controlled by it. . . . The description of this phenomenon (consciousness) clearly needs incorporation into our laws of nature of concepts, which are foreign to the present laws of physics". He suggests, that the situation might be analogous to the understanding in the past century of the relationships between light and matter. We had known that matter influences light, but we were so immersed in the investigation of macroscopic phenomena that until the discovery of the Compton effect we had not suspected that light also influences matter. Obviously, Wigner uses the word consciousness differently from its usual anthropomorphic meaning.

Another way out of the deadlock could be the recognition of some kind of law specific to biological systems, a 'biotonic' law, so-called by its proponent, Elsasser[55].

Thus the problem is recognized: the methods of (atomic) science are not sufficient, and they lead to contradiction when we try to explain the peculiar essence of living systems, and when we consider a living system as a whole. Why is this and how did we get into this position ?

The Methods of Science

In a recent book[56] Waddington contrasts the atomistic approach versus a continuum approach in biology, using as an analogy the dualistic approach of dealing with certain manifestations of the elementary particles in modern physics. I believe, however, that there is a different and deeper schism here, one which does not offer an alternative approach, but if we consider the evolution of scientific methods in modern ages, virtually excludes any but simplifying type of approach, which then leads

into a cul-de-sac so far as the description of the total living system is concerned. May I outline here my understanding of this development ?

In the Middle Ages, man's mind was oriented, and to a certain extent restricted, to consider eventual purpose as the principal aspect of each action or phenomenon, or even of any material entity. Scientific investigation and thinking was subjugated to consideration of purpose, and the minds of many eminent natural philosophers struggled with the complexity which is inherent in such a teleological approach: each phenomenon was but a small part of a very complex world, striving towards its final purpose, and had to be considered as such. Any negation of this attitude and seeking of a new approach was difficult and unwelcome.

No wonder that natural philosophy, or physics, was so unsuccessful for centuries during this teleological orientation; everything was so complicated, so much part of its surrounding and of the past, it was so difficult to see clearly the purpose, and there was so much written and thought already that one had to be aware of.

There was no real progress, until slowly, first tentatively and only in part, but later in its full force, the new scientific method emerged: Let us not consider purpose, especially not why things exist. Let us use, as our criterion of validity, the experiment or the observation, fully available to all. Thus the empirical approach, the principle of verification evolved. Let us examine only a limited aspect at one time: let us exclude disturbing influences (cf. Torricelli's experiment of the falling bodies in vacuum) and best, let us examine how only one variable affects a process (the angle of the incline and the roll of the ball; cf. J. S. Mill's *Canons of Inquiry*[57]). This is, in essence, the second and third of the basic principles of method of Descartes[58] which state, that one must divide each of the difficulties under examination into as many parts as possible, and then proceed always from the simplest and easiest to the more complex.

By this three-pronged approach, of: (1) avoiding teleology; (2) verification by observation; (3) simplification, a highly successful natural science was built up in the past three or four centuries. The physical world responded marvellously to our enquiring efforts.

By these methods we have achieved some success also in biology, drugs, antibiotics, the understanding of molecular details of this or that biochemical processes. We may ask, Is it then worth while to abandon this path; would not everything solve itself when all the details are known, and would not the pattern show itself ? Clearly this is the attitude of most scientists nowadays.

I do not advocate abandoning this successful molecular method in the investigation of any particular detail of a biological process. Molecules and the physico-chemical forces between them are the only agents and the only forces, so far as we know, which participate in the living processes. I suggest only that for the understanding of the behaviour of the total biological unit, for the examination of the essence of living, the present scientific method used in physics might not be sufficient. In the simplest living cell the complex interplay between thousands of molecules makes the living cell different from the non-living. A certain kind of exchange of matter and energy within and between the living units and with their media makes the process recognizably biological, and its past history, and the information transfers which exist within the living unit and with their suroundings make it possible to continue to function. This is well recognized, and investigations of enzyme induction, of intermediary metabolism, of cellular regulatory mechanisms[59], etc., pay attention to exactly these aspects, and modern biochemistry is not confined by the principles of Descartes. Indeed, modern biochemistry might lead to a new method of science. Still, the simplifying and analytical approach is generally dominant, and we physical scientists are often afraid, or unable, to deal with too much complexity.

In one respect, in the consideration of purpose, we seem to have a complete mental block. One who brings teleology into science might expose himself to a little ridicule, if not to a gentle inquisitorial castigation by editors of scientific journals or to the expression of exasperation by fellow scientists. But are we not avoiding this consideration of purpose because of the bad memories teleology evokes ? Properly, we try to avoid metaphysics, and teleology has no place in physical sciences. But the 'purposiveness' of living beings (and I quote from Oparin's book[5]) "pervades the whole living world from the top to the bottom, right down to the most elementary forms of life" and it "is an objective and self-evident fact which cannot be ignored by any thoughtful student of nature". I do not propose that in science we think about the purpose or reason for existence. I only wish that we could think freely, and design theories, experiments, and do research keeping in mind a limited[60] and very materialistic purpose each living entity has, which probably is controlled by itself. We should allow teleological considerations to permeate research in molecular biology as it is now permeated with concepts of information transfer. Actually, teleological considerations already appear, although veiled, in molecular biology. For example, when one designs the (tertiary) structure of an enzyme so that it will fit the specific substrate and the specific function, then one sees how physico-chemical characterization data will support or disprove such structure. Research of this type, amenable to verification by observation, should be rich with suggestions for further experiments and knowledge, and thus should be within the scientific domain. But, whether this would bring us to the understanding of the biological urge, of course, I cannot say.

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SYNTHESIS OF COMPLEX ORGANIC COMPOUNDS FROM SIMPLE PRECURSORS: FORMATION OF AMINO-ACIDS, AMINO-ACID POLYMERS, FATTY ACIDS AND PURINES FROM AMMONIUM CYANIDE

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EXAMINATION of simple chemical systems, reacting under conditions comparable with those which may have existed at the time life on Earth began, has indicated that many amino-acids are synthesized from such simple precursors as methane, nitrogen, hydrogen and water. The relation of these experiments to the origin of terrestrial life depends for interpretation on two postulates proposed by Oparin[1]: (*a*) primitive life was heterotrophic, obtaining simple metabolic requirements from the environment rather than by internal synthesis; (*b*) the environment in which organic synthesis obtained was reducing in nature, a condition at variance with the present oxidizing environment. In contrast, hydrogen is still the principal constituent of space, and several distant planets appear to have atmospheres rich in ammonia and methane. A scheme to explain the evolution of an oxidizing atmosphere to replace the primitive terrestrial reducing environment has been proposed and correlative deductions reviewed in detail[2].

Synthesis of amino-acids under possible primitive Earth conditions appears to be successful so long as the medium is reducing[3–9]; but under oxidizing conditions the products are negligible whether a cyclotron generating 40 MeV (ref. 10) or a simple sparking device[4] supplies energy.

Miller[3] examined synthesis of complex organic molecules in a reducing environment under simulated primitive Earth conditions. A continuous 60,000-V spark was produced in a sealed vessel containing methane, ammonia, water and hydrogen, and after one week he found a variety of amino, hydroxy, and aliphatic acids in the reaction mixture. He demonstrated[7] that the first reaction products appeared to be hydrocyanic acid and aldehydes and that the synthesis of amino-acids could be directly related to the formation and subsequent disappearance of these. The significant role of hydrocyanic acid in this reaction was demonstrated experimentally, for added hydrocyanic acid led to a doubling of the yield of glycine.

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Recently, Kliss and Matthews[11] have proposed a unique role for hydrocyanic acid in the synthesis of complex biochemical molecules.

Oro *et al.*[8] examined the products formed by interaction between hydroxylamine hydrochloride and formaldehyde at 80°–100° for 50 h in the absence of sparking. They found that hydrocyanic acid and ammonia were produced and participated in the synthesis of many of the same amino and aliphatic acids identified by Miller. Afterwards, Oro and Kamat[9] investigated the compounds formed from hydrocyanic acid and 3 N aqueous ammonia maintained at 70° for 25 days. By use of paper chromatography they inferred the presence of glycine, alanine and aspartic acid. The identification of adenine in a similar reaction mixture has also been reported[12,13].

The geochemical speculations of Urey and Oparin suggest conditions under which complex organic molecules may have been synthesized and the experiments of Miller and Oro support their hypothesis. Nevertheless, the existence of organic molecules on the primitive Earth would not *per se* lead to organization of these molecules, a prerequisite for the appearance of complex systems of the types found in living organisms. Some evidence for polymer formation is available. Fox reported[14] that many amino-acids undergo spontaneous condensation at temperatures above 150° to yield polypeptides in the presence of an excess of acidic amino-acids. In contrast, the alkaline *p*H of aqueous ammonia is suitable for the polycondensation of both glycine amide[15] and glycine at 100° (ref. 16).

Using a variety of methods, we have examined the products formed when hydrocyanic acid reacts with aqueous ammonia at 90° for 18 h. Our results not only confirm the reports of Oro *et al.*[12–14] concerning the appearance of adenine, glycine, alanine and aspartic acid, but also extend considerably the number of products synthesized. The principal products are complex substances containing amino-acids and unidentified compounds in a bound form which can be released by either acid or alkaline

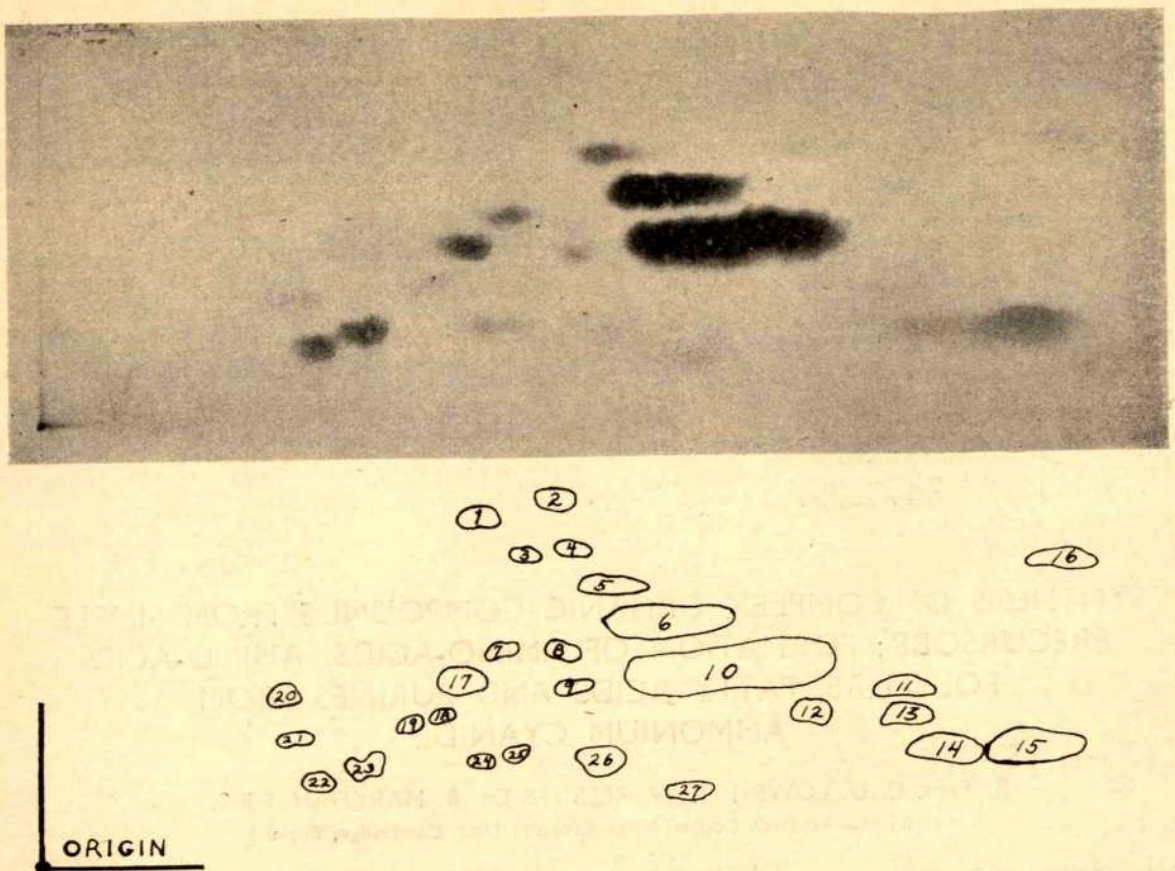


Fig. 1. Resolution of hydrolysed sample, treated by electrolytic desalting, using paper electrophoresis (2 per cent formic and 8 per cent acetic (v/v) for 3 h at 30 V/cm²) and followed by ascending chromatography (*n*-butanol, 4; acetic acid, 1; water, 5; v/v) dried and repeated. Colour developed at room temperature after dipping in 0·2 per cent ninhydrin in acetone
1, Phenylalanine* ?; 2, leucine–isoleucine; 3, methionine†; 4, valine* ?; 5, α-amino-butyric; 6, alanine; 7, glutamic; 8, threonine; 9, serine; 10, glycine; 11, unknown; 12, unknown; 13, unknown; 14, unknown; 15, α-β-diaminopropionic plus unknown; 16, unknown; 17, aspartic; 18, unknown; 19, unknown; 20, unknown; 21, unknown; 22, unknown; 23, unknown; 24, β-alanine; 25, unknown, 26, unknown; 27, unknown

* Not confirmed by column chromatography. † ^{14}C methionine added to reaction mixture.

hydrolysis. After hydrolysis at least 75 ninhydrin reacting compounds appear.

In 9 experiments 1·5 moles of liquid hydrocyanic acid (commercial material containing 0·2 per cent oxalic acid as a stabilizer) were added to 1 l. 1·5 M aqueous ammonia. The oxalic acid produced no obvious effect on the reaction, for the types of products obtained did not differ substantially from those found in a single successful experiment with gaseous hydrocyanic acid.

On addition of liquid hydrocyanic acid to aqueous ammonia, the solution turned amber almost immediately and at the end of the heating period the reaction mixture contained a large amount of amorphous, dense, black material which was removed by centrifugation and amounted to 30–40 g/l. (This complex, and some subsequent complexes, are referred to in the text as polymers. This term is used merely for convenience and is not meant to imply knowledge of the intimate chemistry of these molecules.) The quantity formed appeared in part to be a function of the amount of liquid hydrocyanic acid added. A portion of the supernatant solution was taken to dryness in a rotary evaporator. The residue was extracted with warm water and any insoluble material discarded. Part of the aqueous extract taken to dryness was hydrolysed by heating in constant boiling hydrochloric acid for 24 h at reflux. This represented a 'total hydrolysate'. Another portion of the aqueous extract was placed on an 80 cm × 1 cm column of '*IR* 120' in H^+ form. The large polymers were eluted with water. The free amino-acids and small polymers were retained, and were eluted without fractionation by 2 N aqueous ammonia. After removal of ammonia these were refluxed with hydrochloric acid. Portions of these three fractions (the unhydrolysed aqueous extract, total hydrolysate, and the fraction retained by the resin column) were analysed on an automatic amino-acid analyser (Spinco-Beckman, model 120) by the method of Moore, Stein and Spackman[17]. The results of two experiments are presented in Table 1.

In the unhydrolysed sample the presence of a large number of small complexes interfered with resolution on columns but the positions of glycine, alanine and urea were sufficiently certain for estimation of quantity. In

Table 1. Principal Ninhydrin Reacting Products
Values in μmol/litre of reaction mixture

Product	Exp. 2		Exp. 6	
	Unhydrolysed aqueous extract	Total hydrolysate*	Total hydrolysate*	Hydrosate* of fraction retained by resin column
Aspartic acid		504	576	139
Threonine		2	3	4
Serine†		134	832	255
Glutamic acid			13	
Glycine	58	868	11,420	1,630
Alanine	1·5	84	316	92
Isoleucine		2	8	3
Leucine		2	8	3
β-Alanine			159	216
α-β-Diaminopropionic acid			75	111
α-Amino-butyric acid			4	3
Urea	6,880	15,800	14,520	3,657
Unidentified components‡:				
Amino-acid *a*			19	15
Amino-acid *b*			5	15
Amino-acid *c*		221	306	169
Amino-acid *d*		188	136	26
Amino-acid *e*			73	16

* Refluxed in 6 N HCl for 24 h.
† See footnote 2, Table 2.
‡ Calculation of molar yield based on assumption that colour value with ninhydrin equal to that given by leucine.

Table 2. IDENTIFICATION OF INDIVIDUAL COMPOUNDS

	Automatic amino-acid analyser	Electrophoresis	Special	Volatile solvent column followed by paper chromatography
Arginine*	+	+	+ ‡	
β-Alanine	+			
Glycine	+			+
Alanine	+			+
α-β-Diaminopropionic acid	+	+		
Serine†	+			
Aspartic acid	+			+
Glutamic acid	+			+
Leucine	+		+§	
Isoleucine	+		+§	
α-Amino-butyric	+			
Threonine	+			
Urea	+			
Guanidine*		+	+ ‡	

* Obtained in only one experiment.
† Obtained in each experiment; elution volume identical with that of an authentic specimen. However, yield of formaldehyde after periodate oxidation suggested presence of a substantial amount of second component.
‡ Sakaguchi reaction.
§ Absorbed on charcoal; eluted and analysed by paper chromatography and by amino-acid analyser.

Exp. 1 about 7 per cent and in Exp. 6 about 14 per cent of the glycine formed was present as free glycine, the remainder occurred in complexes. The polymers present in the crude material contained molecules which on hydrolysis produced urea. For example, in Exp. 2, in comparison with the untreated sample, the hydrolysed portion contained more than twice as much urea.

A sample of a total hydrolysate was desalted electrolytically. One portion was chromatographed (Fig. 1) and another treated with urease. The ammonia evolved after 1 h represented about 50 per cent of the urea found to be present by column chromatography.

One total hydrolysate was treated on a 20 × 1 cm column of 'Dowex 2 × 10' in OH^- form; the material retained by the resin was eluted with N hydrochloric acid and was then fractionated on ion-exchange columns by volatile acids as described by Hirs, Stein and Moore[18,19]. Appropriate fractions were pooled, concentrated to dryness, and examined either by paper chromatography or the automatic amino-acid analyser using appropriate markers. Products identified by these means are listed in Table 2.

A total hydrolysate representing 50–400 ml. of original reaction mixture was sequentially treated on columns containing 'Dowex 50' in H^+ form, 'Dowex 2' in OH^- form and finally charcoal. The constituent amino-acids of the eluate could be resolved on the automatic amino-acid analyser. When an amino-acid was tentatively identified by the elution time, a second analysis with a known amount of marker amino-acid added was performed and the shape of the curves were compared for coincidence of the two samples. The amino-acids listed in column one of Table 2 were each identified in this manner. Paper electrophoresis in conjunction with special colour reactions, for example, Sakaguchi reaction for arginine and guanidine[20], were also used. Leucine and isoleucine were adsorbed on charcoal and could be eluted by 20 per cent acetic acid with 5 per cent (w/v) phenol. The amino-acids in the eluent from charcoal were identified both by paper chromatography and the automatic amino-acid analyser.

In several experiments, glycine or alanine labelled with carbon-14 (40 μc., containing 800 μg of carrier) were added to the aqueous ammonia before the addition of hydrocyanic acid. In large part, these were found incorporated into polymers that were not retained on a column of '*IR* 120' in H^+ form. The water eluate was fractionated and the tubes containing these polymers could be found by detecting radioactivity. In general, two radioactive peaks were observed, polymer I and polymer II. The effluent fractions containing these were pooled separately, and after concentration were further treated on a column of 'Sephadex, medium, *G*-25', 40 × 1 cm. The polymers were eluted with water and dried. A portion of this dry material was examined by paper electrophoresis, using a pyridine–acetic acid buffer, *p*H 6·5, at 36 V/cm. From 7 to 10 bands were shown to be present by chlorination[21,22], and those containing radioactive isotopes were located by autoradiography. Three distinct radioactive bands were found in polymer I. These were eluted from the paper with 1 N aqueous ammonia, dried, hydrolysed in 6 N hydrochloric acid and analysed for amino-acid constituents. Table 3 compares the composition of polymer I obtained from two separate experiments with that of the hydrolysed black polymer of the original reaction mixture and the three radioactive polymers separated by electrophoresis.

The black polymer contained several amino-acids, including glycine and a compound yielding urea on hydrolysis. Polymer I contained amino-acids such as serine and amino-acid *d* which were not found in the radioactive polymers resolved from it by electrophoresis. The three radioactive polymers differed from each other and had characteristic ratios of glycine to alanine.

When ^{14}C-methionine was added to the aqueous ammonia prior to the addition of hydrocyanic acid, it was incorporated into a polymer which corresponded in its behaviour on ion exchange and 'Sephadex' columns to polymer I. This suggested that formation of polymers occurred after synthesis of amino-acids and by implication that some dehydrating mechanism was operative in the reaction mixture.

The addition of hydrogen sulphide to the reaction mixture produced major alterations in the patterns of products and we are attempting to isolate sulphur-containing amino-acids.

Besides the ninhydrin reacting substances, other compounds were obtained and several identified. We were surprised at the complexity of the mixture as revealed by ultra-violet light (260 mμ) on a chromatogram run in isopropanol–ammonia; the combined absorbing and fluorescent bands numbered at least 50. Substances with the chromatographic behaviour and ultra-violet absorption spectrum of adenine and hypoxanthine could be found in the total hydrolysate. The position of adenine on a paper chromatogram was found by adding a small amount of ^{14}C-adenine on paper to the edge of a band of hydrolysate, prior to chromatography. The spot was identified by autoradiography and the region cut out,

Table 3. COMPOSITION OF SEVERAL POLYMERS*

Amino-acid	Exp. 6	Exp. 7	Exp. 5			
	Black polymer	Polymer I†	Polymer I†	Polymer I_1	Polymer I_2	Polymer I_3
α-β-Diaminopropionic acid	14 (7)	45 (0·224)	1 (0·056)	? (0·02)	—	—
Aspartic acid	28 (14)	24 (1·22)	13 (0·66)	2·5 (0·128)	1·3 (0·06)	
Threonine	1 (0·05)	1 (0·042)				
Serine ?	22 (11)	14 (0·675)	4 (0·201)	—	—	—
Glycine	1,044 (522)	78 (3·9)	157 (7·88)	67 (3·35)	47 (2·35)	134 (6·71)
Alanine	30 (15)	21 (1·04)	11 (0·54)	13 (0·64)	2 (0·10)	
β-Alanine	1 (1·5)	—	1 (0·065)	—	—	—
NH_3		210 (10·52)	173 (8·65)	243 (12·16)	420 (21·02)	145 (12·26)
Urea	5 (2·5)					
Amino-acid *b*‡	4 (2)	1 (0·06)	1 (0·038)	? (0·02)	—	—
Amino-acid *c*‡	34 (17)	27 (1·14)	7 (0·369)	1 (0·054)	1 (0·05)	
Amino-acid *d*‡	4 (2)	2 (0·12)	1 (0·07)	—	—	—

* Figures are molar ratios; figures in brackets, yield in μmol/sample.
† Analysis on polypeptide I refers to unfractionated polymer. Polymers I_1, I_2 and I_3 were derived from polymer I by paper strip electrophoresis.
‡ See footnote 3, Table 1.

leaving behind the marker. The eluted material was further purified by repeated chromatography and identified by means of absorption spectra and paper electrophoresis. The yield was approximately 1 μmol/l. of reaction mixture. By use of similar techniques several attempts to find guanine were unsuccessful.

An ether extract of a dried portion of the total reaction mixture was examined by paper chromatography using an ethanol–ammonia solvent system. At least three spots behaving as fatty acids were resolved. The presence of formic acid was inferred by mobility and reaction with ammoniacal silver nitrate.

These observations indicate that heating aqueous ammonium cyanide produces a variety of ninhydrin reacting compounds as well as polymers containing these compounds. It is probable that the amino-acids are a racemic mixture, since preliminary observations have failed to demonstrate optical activity. The final verification of these substances will be dependent on isolation and identification using classical chemical techniques. We have no data on the structure of the polymers nor knowledge as to whether they are linear. From a single specimen examined by infra-red spectroscopy the presence of peptide bonds could be inferred.

These experiments provide evidence for a consistent, if not exclusive, hypothesis for primitive Earth synthesis of complex organic substances and macromolecules. A major problem on the primitive Earth concerns persistence of the original organisms. Not only was it necessary to provide the constituent materials but also a medium suitable for survival and multiplication. If at any period in recent cosmic time appreciable quantities of ammonium cyanide existed in the Earth's atmosphere, it is obvious that there would have been ample supplies of organic components which would fulfil these functions.

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SYNTHESIS OF ADENOSINE TRIPHOSPHATE UNDER POSSIBLE PRIMITIVE EARTH CONDITIONS

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IT has been suggested that the pre-biological synthesis of nucleoside phosphates on the primitive Earth was a consequence of the absorption of ultra-violet light by purines and pyrimidines in an appropriate aqueous medium[1,2]. The basis for this suggestion is as follows:

Even the simplest living organisms are statistically unlikely aggregations of organic molecules. The improbability of contemporary organisms is extracted from the field of possibilities through natural selection. But before the advent of self-replicating systems, natural selection as we understand it to-day could have played no such part. The origin and subsequent replication of life must therefore have involved molecules preferentially produced in the primitive environment. Such a view is implicit in the early works of Haldane[3] and Oparin[4]. While it is possible that the fundamental molecular basis of living systems has itself evolved, the simplest working hypothesis holds that the molecules that are fundamental now were fundamental at the time of the origin of life. The production of amino-acids, purines, pyrimidines and pentose sugars under simulated primitive conditions during the past decade lends support to this hypothesis.

There are, however, still several molecular species the involvement of which in the origin of life remains to be demonstrated. Chief among these are the nucleoside phosphates. Adenosine triphosphate (ATP) is the 'universal' energy intermediary of contemporary terrestrial organisms, and one of the major products of plant photosynthesis. The need for its production in primitive times was first emphasized by Blum[5]. Guanosine triphosphate has recently been implicated as the energy source for peptide linkage[6]. The deoxynucleoside triphosphates are the precursors for contemporary DNA biosynthesis[7]. To the extent that the origin of DNA plays a fundamental part in the origin of life, the abiogenic synthesis of deoxynucleoside triphosphates seems indicated[2]. Several fundamental coenzymes of intermediate metabolism and plant photosynthesis (CoA, DPN, TPN, FAD) are nucleoside phosphates. All these molecules contain purines or pyrimidines which have strong ultra-violet absorption maxima near 2600 Å. The possibility then arises that the absorption of ultra-violet photons by purines and pyrimidines provided the bond energy for the synthesis of nucleoside phosphates in primitive times; and it is therefore of some interest to investigate the ultra-violet transparency of the early terrestrial atmosphere.

There is evidence from astronomy[8,9] that the Earth's atmosphere was reducing at the time life first arose. Laboratory experiments have shown that it is far easier to synthesize organic matter under reducing than under oxidizing conditions[10–12]. The molecules O_2 and O_3 are thermodynamically unstable in an excess of hydrogen, and the principal sources of the ultra-violet opacity of the present terrestrial atmosphere cannot have then been present. The ultra-violet absorption which did exist arose from intermediate oxidation state molecules, principally aldehydes and ketones. In experiments in which electrical discharges were passed through simulated primitive atmospheres, the only aldehyde or ketone

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produced in high yield was formaldehyde[13]. Nevertheless, the production of some acetaldehyde[14] and acetone can be expected. Formaldehyde absorption extends longwards of about 2900 Å. Acetaldehyde and acetone absorb throughout the 2400–2900 Å region. Ammonia, acetylene, and other molecules absorb shortwards of 2400 Å.

Therefore, the question of the transparency of the primitive terrestrial atmosphere near 2600 Å turns mainly on the unknown early abundance of CH_3CHO and CH_3COCH_3. Because of the relatively low acetaldehyde and acetone yields in simulation experiments, and because of possible independent biological indications of high ultra-violet fluxes in primitive times[2], it seems likely that the early reducing atmosphere was at least slightly transparent between 2400 and 2900 Å. From models of the evolution of the Sun, and an integration of the Planck function, the ultra-violet flux of wave-length $2900 \text{ Å} \geqslant \lambda \geqslant 2400$ Å incident on the Earth's atmosphere 4×10^9 years ago is computed to be about 7×10^{14} photons cm^{-2} sec^{-1} (ref. 15). Even with substantial atmospheric absorption, ultra-violet radiation in this window will greatly exceed other energy sources for organic synthesis[16].

The synthesis of purines and pyrimidines which absorb in this wave-length region has recently been accomplished in a variety of primitive Earth simulation experiments. Adenine has been produced by thermal polymerization of 1·5 molar hydrocyanic acid in an aqueous ammonia solution[17]; by 5-MeV electron irradiation of methane, ammonia, water and hydrogen[18]; and by ultra-violet irradiation of a 10^{-4} molar solution of hydrocyanic acid[19]. Guanine also appears to be formed in the last experiment. Another guanine synthesis occurs in the thermal copolymerization of amino-acids[20]. Uracil has been produced by heating urea and malic acid[21].

The yields of purines and pyrimidines are sometimes quite high. In the electron beam irradiation of primitive atmospheres by Ponnamperuma, Lemmon, Mariner and Calvin[18], autoradiography indicates that the substance produced in highest yield is adenine. Thus it appears possible that ultra-violet light passing the 2400–2900 Å partial window in the primitive terrestrial atmosphere was strongly absorbed by purines and pyrimidines in the early oceans.

The production rates of organic molecules from reducing atmospheres suggest that the primitive oceans were about a 1 per cent solution of organic matter[2,9]. In addition to purines and pyrimidines the pentose sugars, ribose and 2-deoxyribose can be expected to be present. The laboratory production of 2-deoxyribose has been achieved through the condensation of formaldehyde and acetaldehyde, or of acetaldehyde and glyceraldehyde in aqueous salt solutions[22]. (Indeed, this is an example of a mechanism which keeps the atmospheric aldehyde concentration low.) Both ribose and 2-deoxyribose have been synthesized by either ultra-violet or γ-irradiation of dilute formaldehyde solutions[23]. Phosphates and other phosphorus compounds can be expected in the primitive oceans, even at very early times[24].

It therefore seems of some interest to attempt synthesis of nucleoside phosphates by ultra-violet irradiation of dilute solutions of purine or pyrimidine bases, pentose sugars, and phosphorus compounds, both because of our expectation that such syntheses were easily performed in primitive times, and because ultra-violet irradiation of dilute solutions of adenine and ribose has already produced the nucleoside adenosine[25].

Materials and Experimental Techniques

Adenine-8-^{14}C of specific activity 23·4 μc./mg, adenosine-8-^{14}C of specific activity 7·2 μc./mg, and adenylic acid-8-^{14}C of specific activity 3·1 μc./mg were supplied by Schwarz Bioresearch, Orangeburg, New York. The non-radioactive AMP, ADP and ATP used as carriers were supplied by C. F. Boehringer, Mannheim, Germany. The adenosine tetraphosphate was a gift of Dr. John Moffat of Syntex, Ltd., Palo Alto, California.

The ethyl metaphosphate used in the experiment was prepared by dissolving 150 gm of phosphorus pentoxide in 300 ml. of ethyl ether and refluxing the solution for several hours with chloroform[26]. The excess solvent was removed by evaporation under vacuum, leaving a syrupy residue of ethyl metaphosphate.

The method of irradiation and analysis has already been described[25]. Quantities of the labelled adenine, adenosine and adenylic acid, varying from $1{\cdot}5 \times 10^{-6}$ to $1{\cdot}5 \times 10^{-5}$ moles in various experiments, were sealed in aqueous solution in 'Vycor' tubes with approximately stoichiometric quantities of ribose, phosphoric acid or polyphosphate ester, as shown in Table 1. The final concentration of base nucleoside and nucleotide in each solution did not exceed 10^{-3} moles/l. The solutions were irradiated by four General Electric ultra-violet germicidal lamps, type 782*H*-10, which emit 95 per cent of their light in the mercury resonance line at 2537 Å. The 'Vycor' glass of which the tubes were made transmitted 80 per cent of light of this wave-length. During a 1-h irradiation, the sample absorbed a total of $\sim 10^6$ ergs. During the irradiation the ambient temperature of the samples was $40° \pm 2°$ C.

The reaction products were first analysed by paper chromatography, autoradiography and ultra-violet absorption studies. An aliquot of the reaction products was spotted on a Whatman No. 4 paper and the chromatogram run in two solvents, butanol–propionic acid–water[27] and isobutyric-acid–ammonia[28]. The positions of the carriers adenosine, AMP, ADP, ATP and A4P were detected by shadowgrams[29]. Coincidence both in position and in shape between the carriers on the shadowgrams and the radioactivity on the autoradiograph was the chromatographic basis for the identifications. The formation of adenosine has already been reported[25] A further aliquot was chromatographed in two other solvent systems, trichloroacetic acid–acetone[30] and butanol–formic acid–water[31]. Once again there was coincidence between the carrier as outlined in the shadowgram and the radioactivity on the film.

Separations effected using thin-layer chromatography, and ion-exchange chromatography confirmed the result obtained from paper chromatography.

Results

The results of the investigation are summarized in Table 1 and in Figs. 1–4. Four different categories of experiments were performed. In the first the starting material was adenine, in the second adenosine, in the third adenosine monophosphate, and in the fourth adeno-

Table 1

Exp.	Adenosine	AMP	ADP	ATP	A4P
(1)					
(i) Adenine-^{14}C + ribose					
(ii) Adenine-^{14}C + ribose + phosphoric acid	+ (0·01%)	–	–	–	–
(iii) Adenine-^{14}C + ribose and ethyl metaphosphate	+ (0·01%)	+ (0·08%)	+ (0·06%)	+ (0·05%)	+ (0·04%)
(2)					
(i) Adenosine-^{14}C + phosphoric acid		–	–	–	–
(ii) Adenosine-^{14}C + ethyl metaphosphate		+ (0·5%)	+ (0·2%)	+ (0·1%)	+
(3)					
(i) Adenosine monophosphate-^{14}C + phosphoric acid			–	–	–
(ii) Adenosine monophosphate-^{14}C + ethyl metaphosphate			+ (3%)	+ (0·3%)	+ (0·1%)
(4)					
(i) Adenosine diphosphate + phosphoric acid				–	–
(ii) Adenosine diphosphate + ethyl metaphosphate				+	+

Figures in brackets show conversion as percentage of starting material. With the techniques used in this experiment the lower limit of detectability was 0·001 per cent.

In Exp. 4 no quantitative estimates were performed as unlabelled ADP was used. The ATP in this case was located by shadowgrams.

sine diphosphate. The conversion of adenine to adenosine, adenosine to adenosine monophosphate, adenosine monophosphate to adenosine diphosphate, and adenosine diphosphate to adenosine triphosphate has been established. Experiments using adenine as the starting material have produced adenosine, AMP, ADP and ATP.

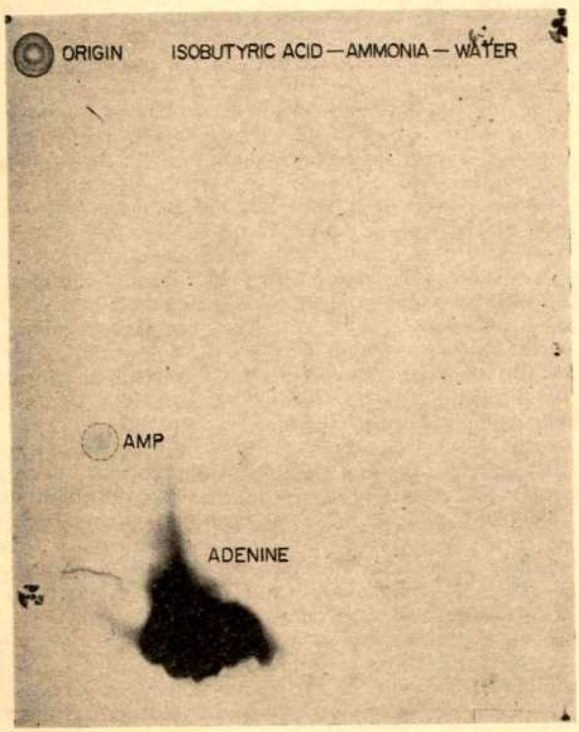


Fig. 1. Autoradiogram illustrating the formation of AMP from adenine, ribose and ethyl metaphosphate by the action of ultra-violet light

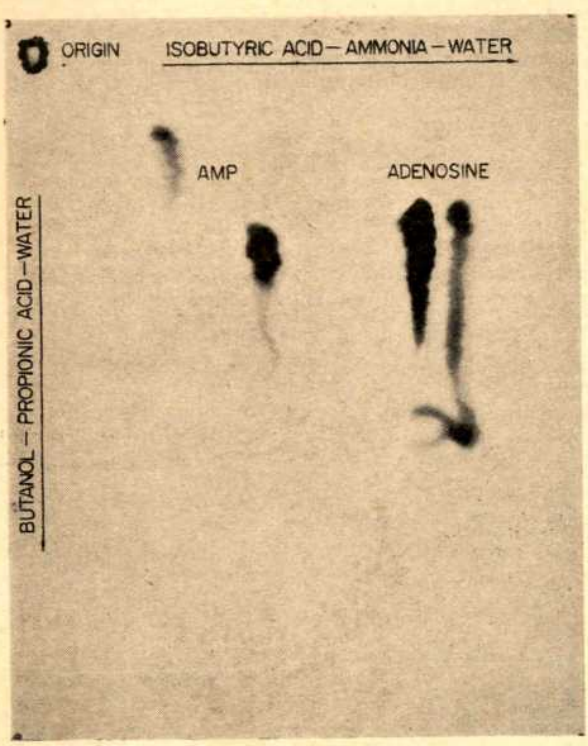


Fig. 2. Autoradiogram illustrating the formation of AMP from adenosine, and ethyl metaphosphate by the action of ultra-violet light. The long feature to the right of the teardrop-shaped adenosine spot is adenine, produced from adenosine photolysis. The dark central feature between AMP and adenosine is at present unidentified

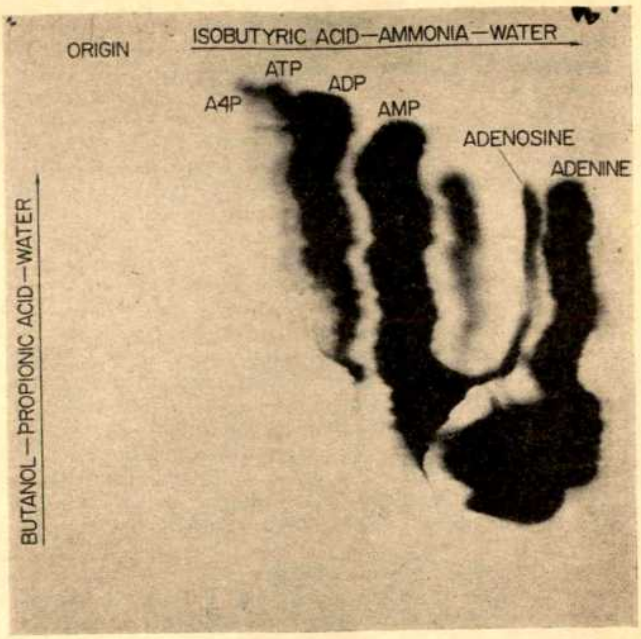


Fig. 3. Autoradiogram illustrating formation of ADP, ATP and A4P from AMP and ethyl metaphosphate by the action of ultra-violet light

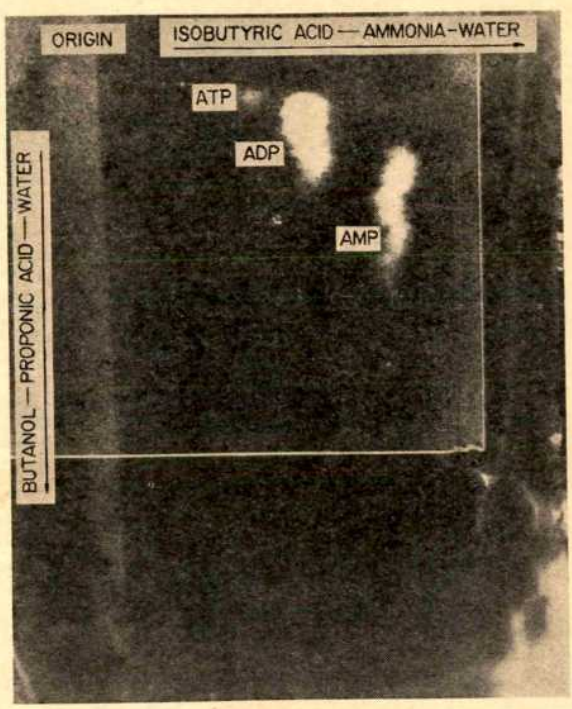


Fig. 4. Shadowgram illustrating the formation of ATP from ADP and ethyl metaphosphate by the action of ultra-violet light. The AMP is a photolytic product

The previously reported experiment showed that adenosine is not produced in detectable amounts in the absence of a phosphorus compound[25]. While adenosine is produced in the presence of both phosphoric acid and ethyl metaphosphate, the nucleoside phosphates were detected only with the use of ethyl metaphosphate. Phosphoric acid was chosen first in the attempt to synthesize the nucleoside phosphates. Ethylmetaphosphate was selected as a possible reagent because of a recent report[26] that it activates carbonyl, hydroxyl and amino groups in organic synthesis. Other phosphorus compounds may also be effective in this synthesis, but they have not yet been investigated.

The yields are given in Table 1 and are further discussed below.

Controls. Two general categories of control experiments were performed to assess two possible modes of biogenic contamination of the reaction products. To test the possibility that the relatively high temperatures (40° ± 2° C) attained by the 'Vycor' tubes during ultra-violet irradiation stimulated the metabolic activities of micro-organisms in the reactants, we placed control tubes at these same temperatures for comparable periods, but without ultra-violet irradiation. In all other respects they were handled and analysed similarly to the irradiated samples. In no case was any yield detected.

An alternative hypothetical source of contamination is the presence in the labelled reactants of micro-organisms which, under ultra-violet irradiation, are photolysed, introducing their metabolic products into the medium. To test this possibility, we introduced into 'Vycor' tubes which had been autoclaved for 45 min at 120° C reactants that had been passed through an autoclaved Seitz filter. These sterile samples were then irradiated with ultra-violet light and analysed. No change in yield was observed. We conclude that the microbiological contribution to the observed yields was negligible.

Discussion

The abiogenic non-enzymatic production of nucleoside phosphates and related molecules under simulated primitive Earth conditions is relevant to the problem of the origin of life. The expected availability of ATP in primitive times suggests that energy was then available in convenient form for endergonic synthetic reactions of large molecules. The question arises why adenosine triphosphate, rather than, for example, the triphosphates of guanosine, cytidine, uridine, or thymidine, were not produced in primitive times and utilized to-day as the primary biological energy currency. There are several possible responses. In primitive Earth simulation experiments under reducing conditions with low hydrogen content, adenine is produced in far greater yield than are other purines and pyrimidines[17–19]. Secondly, no biological purine or pyrimidine has a larger absorption cross-section between 2400 and 2900 Å. Thirdly, adenine is among the most stable of such molecules under ultra-violet irradiation. Finally, the ultra-violet excitation energy is readily transferred, especially by π electrons, along the conjugated double bonds of the molecule; the excited states are very long-lived, and thereby serve to provide bond energies for higher synthetic reaction. All but the first of these properties of adenine derive from the fact that it has the greatest resonance energy of all the biochemical purines and pyrimidines[32,33]. It thus appears that molecules ideally suited for the origin of life were preferentially produced in primitive times.

The yields achieved in these experiments, as shown in Table 1, are relatively quite high. In contrast, quite elaborate methods are ordinarily required for the laboratory synthesis of nucleoside phosphates[34]. For the production of adenosine from adenine, ribose and a phosphorus source, the quantum yield for a 1-h irradiation is $\varphi \sim 10^{-5}$. For production of AMP, ADP and ATP by the use of ethyl metaphosphate, the quantum yields are almost an order of magnitude greater.

It is not now known to what extent the experiments here reported accurately reproduce the environmental conditions in the primitive terrestrial oceans. It can be expected that ethyl metaphosphate was probably not the most abundant phosphorus source; but we do not know how well other, possibly more abundant, phosphate salts may efficiently substitute for ethyl metaphosphate. The irradiation period in these experiments was 1 h. Continued irradiation, with no removal of products, must, by the second law of thermodynamics, ultimately result in lower over-all quantum yields. The influence of inorganic anions on the course and rate of these reactions is largely unknown. Nevertheless, it is of some heuristic interest to compute the production rate of adenosine triphosphate in the primitive terrestrial oceans, where the conditions there were similar to those in the present experiments.

The production rate of ATP in the primitive reducing atmosphere will then be:

$$\frac{d\sigma}{dt} \simeq \frac{Q\varphi\mu}{4N_A} \text{ g cm}^{-2} \text{ sec}^{-1}$$

where Q is the ultra-violet photon flux for 2400 Å $\leqslant \lambda \leqslant$ 2900 Å, is the quantum yield, μ is the molecular weight of ATP, and N_A is Avogadro's number[15]. Taking $Q \sim 7 \times 10^{14}$ photons cm^{-2} sec^{-1}, [15]$\varphi \sim 3 \times 10^{-5}$, and $\mu \sim 550$, we derive:

$$\frac{d\sigma}{dt} \simeq 5 \times 10^{-12} \text{ g cm}^{-2} \text{ sec}^{-1}$$

A feeling for the magnitude of this figure can be obtained by computing the steady-state population of micro-organisms over the entire globe that could be maintained by this quantity of abiologically produced adenosine triphosphate. That is, we assume that the primitive Earth is populated by obligate heterotrophs that obtain all their energy from abiologically synthesized ATP. We will obtain a mimimum population if we assume that the number of ATP molecules required for each replication and the doubling time per cell have values characteristic of typical contemporary organisms. Taking values for *Eschericia coli* of 10^9 ATP molecules per cell for each doubling, and a doubling time of 1 h, we find the required ATP production rate to maintain one cell must be $2{\cdot}5 \times 10^{-16}$ g sec^{-1} cell^{-1}. The steady-state population of micro-organisms that can be maintained over the entire globe by the abiological synthesis of ATP is then 2×10^4 cells/cm^2 column of ocean. This estimate is, of course, extremely approximate. The assumptions that all the ultra-violet light is transmitted by the atmosphere, that it is all absorbed by adenine in the ocean and that the quantum yields used in the ethyl metaphosphate experiments are applicable to the primitive environment probably increase the derived steady-state cell population; while the assumptions that the ATP requirement and doubling time for organisms are the same as for *E. coli* probably decrease the derived steady-state cell population over the true value. Nevertheless, this calculation does suggest that abiogenic ATP production by ultra-violet light in primitive times may have supported quite sizable populations of micro-organisms on the primitive Earth.

Such abiogenic production of ATP is, in effect, photosynthesis without life. One striking conclusion that has emerged from recent work on the mechanism of terrestrial plant photosynthesis is that the production of ATP is the primary, and most primitive, function of the photosynthetic apparatus[35,36]. The experimental results of the present article permit us to understand why this might be so. With rather efficient abiogenic synthesis of so ideal an energy currency as ATP in the primitive environment, the transition from a reducing to an oxidizing atmosphere must have had profound results.

The transition was at least partially initiated by the ultra-violet photodissociation of water vapour in the high atmosphere, and the selective escape of hydrogen to space[8,37]. The ozone concentration of a planetary atmosphere depends approximately logarithmically on the oxygen concentration, down to a certain lower limit of the oxygen concentration[38,39]; thus the steady-state production of even 10^{-4} or 10^{-5} of the present oxygen concentration would have produced enough ozone to diminish the ultra-violet flux in the 2400–2900 Å partial window, and make the rate of ultra-violet synthesis of ATP decline. A premium was then placed on organisms with the ability to utilize visible light for ATP synthesis. One can imagine the metabolism of the primitive organisms to be so keyed to the availability of ATP that the first visible

photosynthetic apparatus evolved would be adopted by all subsequent life forms.

The precise mechanism of synthesis has not yet been investigated. Ultra-violet excitation of adenine accounts for the adenosine synthesis, but the participation of phosphorus compounds in the reaction is obscure. Synthesis of nucleoside phosphates must be more indirect, since it is difficult to imagine the excitation energy being transferred across the ribose molecule, which has no conjugated double bonds. Alternative possibilities, such as the production of activated adenine or ribose phosphates, remain to be investigated.

Further investigation of so far unidentified chromatographic features should both help clarify the mechanisms of synthesis and cast light on other possible prebiological organic reactions. Ultra-violet irradiation of solutions of deoxyribose purines or pyrimidines, and phosphate compounds may have some relevance for the problem of polynucleotide origins.

Bio-assay. To establish whether the ATP synthesized by us was biochemically active, a luminescence assay was performed using dehydrated firefly tails[39]. The method described by Strehler and Trotter was used. (Firefly tails were supplied by Schwarz Bioresearch, Inc., Mount Vernon, New York.) The intensity of luminescence was measured by a Turner fluorometer. The decay curve of the luminescence was identical with that of an authentic sample of ATP. The concentration of ATP in the solution used, as determined by this method, corresponded within the limits of experimental error to the value obtained by spectrophotometric measurements.

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PREDICTION OF FUTURE SUNSPOT CYCLES

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THE dates and intensities of the next few sunspot maxima and minima need to be known in order to predict the lifetimes of present and future satellites (since upper-air density varies greatly with solar activity), to plan future telecommunications, to decide the best dates for international geophysical projects, and to forecast the years when astronauts who venture outside the Earth's atmosphere are least likely to meet lethal streams of charged particles from the Sun. Unfortunately, "no satisfactory method of extrapolating sunspot numbers beyond the present cycle has been found"[1], and predictions of the present cycle[2–5] have not been accurate. The object of this article is to direct attention to a 7-cycle recurrence tendency in rise-time and its possible use in prediction.

Since the regular cycle of solar activity, with period 8–15 years, has persisted for many centuries, the forces controlling the variation are likely to be partially determinate. If so, the time-interval which might be expected to show the greatest regularity is the rise-time t_R—the time for the rise from minimum to maximum activity. Fig. 1 gives values of t_R from ref. 6 for each of the past 15 cycles, from 1788 onwards, plotted against the year in which sunspot maximum occurred. (Records for earlier cycles are less reliable.) Fig. 1 shows that the values of t_R for 1788–1870 (broken line) have an identical up-and-down sequence to those for 1870–1947 (unbroken line), thus indicating a 7-cycle recurrence tendency. If the

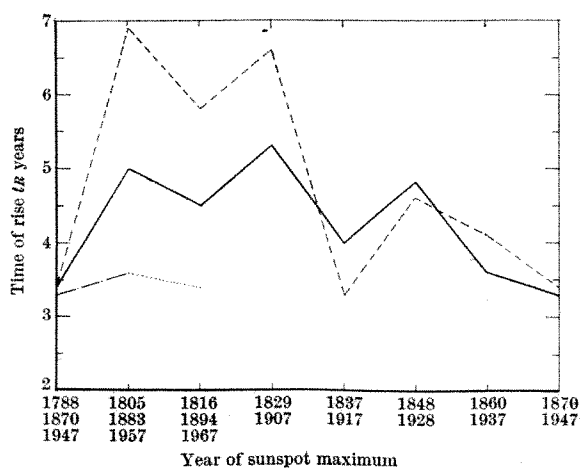


Fig. 1. Time of rise from sunspot minimum to maximum
– – –, 1788–1870; ——, 1870–1947; — · —, 1947–1957; · · · ·, prediction

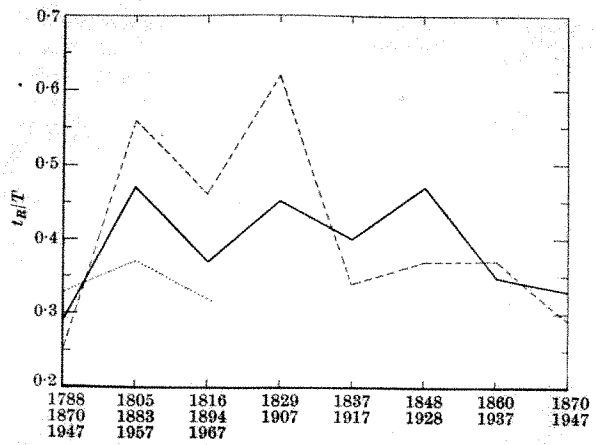


Fig. 2. Time of rise expressed as a fraction of the complete cycle
– – –, 1788–1870; ——, 1870–1947; ····, 1947–1967, predicted

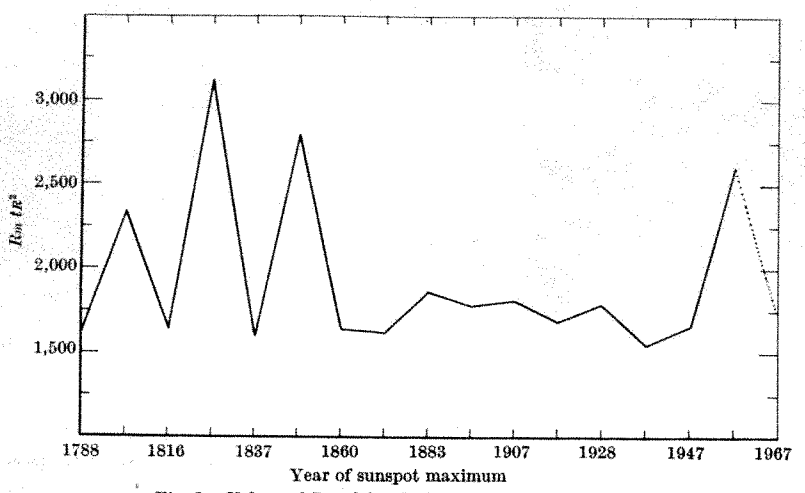


Fig. 3. Values of $R_m t_R^2$ for the last sixteen sunspot cycles
····, prediction

regularity apparent in Fig. 1 persists, the rise-time for the next solar cycle (maximum in 1967) should be about 3·4 years.

Fig. 2 shows t_R expressed as a fraction of the total cycle-length T. Again, the pattern for the years 1788–1870 is the same as for 1870–1947, except that the value of t_R/T for the 1860 cycle is equal to that for the 1848 cycle, instead of being lower. This semi-exception to the rule could, however, be eliminated by taking the 1856 minimum, which was rather indefinite, as a month or two later than the official date of 1856·0 (a change which would not be unreasonable, since sunspot numbers were zero throughout May 1856). If the regularity apparent in Fig. 2 continues, the values of t_R/T for the next two sunspot cycles should be near those indicated by the dotted line: if so, the present cycle would last 9·7 years, declining to its minimum at about 1964·0. This prediction (which makes no use of any information later than 1958) agrees quite well with present estimates, based on short-term extrapolation of current values, that the next minimum is likely to be in 1964. If the values in Fig. 2 are right, the next sunspot cycle should last 10·6 years, having a maximum at 1967·4 and a minimum at 1974·6. (The cycle after that should last 10·0 years, with maximum at 1978·3 and minimum at 1984·6).

The maximum number of sunspots during successive cycles varies widely and shows no such obvious recurrence tendencies as t_R: a 7-cycle recurrence tendency was suggested by Schove[5] on the basis of data given by Gleissberg[7], but failed to show itself in the current sunspot cycle; the 169-year recurrence tendency demonstrated by Anderson[2] also failed in the current cycle. The Zurich sunspot relative numbers[6] show that the highest smoothed monthly number R_m has increased almost linearly in the past four cycles, from 78 in 1928 to 201 in 1957: the 1947 maximum (152) was equal to the previous highest (in 1778) and the 1957 maximum was by far the highest ever recorded. If prediction is to be successful, the uniqueness of these last two cycles must be destroyed and a parameter which exhibits a more uniform trend must be found. Fig. 3 shows values of $R_m t_R^2$, which has the remarkable property of remaining almost constant, at 1700 ± 150, during 9 cycles from 1860 until 1947, though R_m varied by a factor of nearly $2\frac{1}{2}$. In 1957, $R_m t_R^2$ showed a large increase, but since such increases have never before been maintained for two cycles in succession, the value of $R_m t_R^2$ seems likely to return to about 1750 for the 1967 cycle, giving a maximum sunspot number of about $1750/3{\cdot}4^2$ or 150 in 1967—much the same as in 1947. This estimate is in agreement with that of Minnis[1].

These predictions are, of course, disputable. But independent estimates of this kind seem to be needed if the uncertainty which hinders planning in all solar–terrestrial sciences is ever to be dissipated: for example, it would not have been necessary to bring forward the date of the International Years of the Quiet Sun if it had so happened that some credence had been given to the foregoing predictions, which could have been made in 1958.

I thank Mr. W. R. Piggott, of the Radio Research Station, Slough, for suggesting relevant references.

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[3] Herrinck, P., *Nature*, **184**, 51 (1959).
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[7] Gleissberg, W., *Terr. Mag.*, **49**, 243 (1944).

THE ROYAL RADAR ESTABLISHMENT

ON May 24–26, the Royal Radar Establishment, Malvern, was opened to the Press and invited visitors from industry, universities and other Government organizations. The Establishment has not staged such a comprehensive display since, as separate Establishments before the amalgamation in 1953, Telecommunications Research Establishment and Radar Research and Development Establishment held open days in 1948 and 1949.

For administrative purposes the Royal Radar Establishment is organized into five technical groups covering physics, electronics, airborne radar, guided weapons and ground radar. In addition, there are supporting departments in engineering and experimental flying. The basic research activities of the Establishment, which form the main subject of this article, are carried out primarily (but not exclusively) in the physics and electronics groups.

It was possible to discern in the 45 listed exhibits some family resemblances to the Telecommunications Research Establishment of 1948, in spite of the revolutionary developments in electronics over the past 15 years. Thus,



Fig. 1. Map of England by sideways-looking radar. Note the major urban areas. Scale: 60 nautical miles

in 1948, 1 cm was a short wave-length and 3μ (the lead sulphide photo-detection limit) considered far infra-red, but the need to close the gap was uppermost in many minds. The gap is now closed. By the use of various doped semiconductors, notably indium antimonide in a magnetic field at helium temperatures, the Establishment has developed photoconductive detectors spanning the entire infra-red spectrum up to 1 or 2 mm wave-length while essentially conventional microwave receivers have been pushed down to 1 mm primarily by high-precision engineering of which the Establishment can be justly proud. Three decades of the electromagnetic spectrum now lie open for exploitation—for spectroscopy, for plasma physics, for radar. Instrumentation for far infra-red and sub-millimetre spectroscopy was on show, as was the use of high-speed sub-millimetre detectors in plasma investigations. The latter programme is in support of work at the Culham Laboratory of the U.K. Atomic Energy Authority and it was shown how plasma densities and temperatures, as a function of time, can be deduced from emission measurements on magnetically contained plasmas.

With this background, a significant effort at the Royal Radar Establishment on masers and lasers might have been expected. Microwave masers are now primarily a device development rather than a research activity, and formed part of an exhibit showing the impressive improvements made in radar receiver noise performance through the use of improved crystals, parametric amplifiers and masers. With lasers some exploratory applications work is under way—a rudimentary ranging set, using a *Q*-switched ruby laser, was shown—but most of the work is of a more basic nature. The Establishment research on lasers is concentrated on solid-state lasers, for example, ruby, CaF_2, $CaWO_4$, etc., where the problems are at least as much in crystal growth as in optics. The physics group has, over the past ten years, slowly built up a materials section to provide first semiconductor and now laser crystals to meet their various needs. The supply of high-quality crystals, however, throws up new research problems on crystal growth and the results from the investigation of, for example, constitutional supercooling and the 'facet' effect in melt grown crystals were on display, together with related work on vapour phase growth.

Perhaps no single development of the past fifteen years has influenced electronics as much as the miniaturization, and now microminiaturization, permitted by the use of solid-state devices. Two (working) transistors on a pin-head and a four-colour reproduction of the coat of arms of the Royal Radar Establishment by selective etching of an oxide film on a silicon crystal illustrated the power of available techniques. For military electronics at least, all future circuit assembly seems destined to take place under a microscope or in an evaporation chamber, and circuits must be designed with this end in view. An example of this trend —a 60-Mc/s bandwidth amplifier—was on show. Some of the recent developments in computer components—notably cryotrons, magnetic films and tunnel diodes—were exhibited, both as devices and for their basic physical interest. Thus the use of tunnel diode pairs in a time discriminator circuit for non-destructive interrogation of ferrite cores was shown, and an investigation of the effect of magnetic fields up to 150 kilogauss on InSb tunnel diodes. For computers, miniaturization is synonymous with high speed and low cost, at which evaporated devices such as cryotrons are aimed. The demonstrations included equipment for displaying switching transients in magnetic films for switching times of the order of 1 nanosecond, at which speeds the close proximity of components is essential.

The open days represented, in a sense, the baptism of the automatic computer of the Royal Radar Establishment which is a one-address machine with 36-bit words and, at present, an 8,000-word core store. Basic operations (for example, shifting) take about 300 nanoseconds each. The standard input language is *ALGOL*. In common with many modern machines *RREAC* can tackle three programmes simultaneously by time sparing, and this facility formed the basis of the exhibit: while working on a more serious problem, *RREAC* played the game of kails against all challengers.

Another large installation on view to the public for the first time was the High Field House, which contains Bitter solenoids for the production of steady magnetic fields up to 130 kilogauss and the associated equipment to provide power at a rate up to 2·7 MW. The energy is obtained from a large bank of submarine batteries. Present work, which was on show, includes the examination of those superconductors having a β-tungstate structure which show superconductivity even in high magnetic fields. Apart from the 130 kilogauss facility, the Establishment had a number of other large magnets on show. This magnet, which is limited by its core to about 35 kilogauss, is a good example of the work of the engineering department. At present, and for the open days, it is being used for magneto-optical investigations of semiconductors.

Radio-astronomy and satellite tracking proved popular exhibits in spite of the travelling (about 10 miles and 3 miles respectively) involved. The radio telescope at Defford, near Malvern (*Nature*, 190, 1150; 1961) has been used as an interferometer at 610 Mc/s on nearly 100 radio sources and very accurate positions have been derived for about 30 of these. Used as a radar, the Defford installation has been used to examine meteor echoes at 1,300 Mc/s, and more recently measurements have been made on incoherent scatter by electrons in the upper atmosphere. The satellite tracking equipment on show included a recently erected 45-ft. diameter precision radar tracker and its associated equipment. Though not yet complete, visitors were able to watch the aerial lock on to satellites *Allouette* and *Anna*. Included in the exhibit was a 24-in. diameter *F*1 Schmidt camera which can accurately record the position of a satellite or star down to magnitude 9.

An important series of exhibits illustrated the part that the Royal Radar Establishment is playing in the development of a fully integrated air traffic control system. The increasing quantity of air traffic of the present day demands techniques which will match the increasing demands without impairing safety, and it seems that a combination of modern radar with electronic computing techniques may meet the requirements. Experimental systems involving a number of automatic techniques are being investigated at the Establishment and the exhibits showed how simulated situations might be dealt with in a series of exhibits at once elegant and complex.

Many of the exhibits demonstrated the work being carried out to improve the resolution of radar targets against a background of clutter. Pulse compression techniques demonstrated the ability to simulate the effects of short pulses of embarrassingly high-peak power with, in fact, a long pulse of modest peak power. Another showed how it might be possible automatically to compensate for the distortion of a large aerial, for example, in high wind, or under load, and so preserve the accurate profile so essential to accurate angular determination of target direction.

Modern and refined techniques now being made available for airborne use for navigational purposes were on show. These included a new optical line scan technique for aerial photography in which the ground is scanned by a rotating mirror as the aircraft moves forward. The resulting images are transformed into electrical signals which can be used to form a strip photograph. The results obtained with sideways-looking radar techniques were shown, and Fig. 1 shows a map of England and Wales which has been built up from a series of flights which might well be over cloud or by night. High resolution reconnaissance requires high resolution display equipment. A fully resolved television picture measuring $2{\cdot}5 \times 3{\cdot}0$ mm and viewed through a microscope proved an eye-catching exhibit.

To conclude this brief account of the Royal Radar Establishment open day exhibits mention must be made of the Establishment's College of Electronics where students take, among other things, the Higher National Certificate and Diploma Courses in Electrical and Electronic engineering. The College was open for inspection, the demonstrations including a gas laser and a 3-cm marine radar. The College, now some fifteen years old, caters for some 180 student-apprentices, and about 100 craft apprentices and for day-release students.

A. F. Gibson
J. E. N. Hooper

OBITUARY

Prof. J. F. Piccard

The gentle giant, professor emeritus of aeronautical engineering at the University of Minnesota, Jean Felix Piccard, died of a heart attack on January 28, 1963, at the age of seventy-nine.

Prof. Piccard was born January 28, 1884 of French-speaking Swiss parents Hélène (*née* Holtenhoff) of Geneva, and Jules, head of the Department of Chemistry in the University of Basle, Switzerland. He is survived by his wife Jeannette (Ridlon) Piccard, three sons, Donald, John, and Paul, a sister Mme. Paul Lambert of Lausanne and a brother, Dr. Paul Piccard of Lucerne.

Jean and his twin brother, Auguste, attended the Obere Realschule, Basle. After graduation from that school in 1902, Jean Piccard enrolled at the Swiss Institute of Technology in Zurich, took his degree in chemical engineering (1907) and, like his brother, was awarded a doctorate in natural science (1909) from that institution. He received his doctorate in organic chemistry in 1901 as a result of his thesis "The Constitution of Meriquinonique Compounds" which brought him an Institute prize and the silver medal award. He taught at the University of Lausanne during 1914–16, when he was appointed assistant professor at the University of Chicago; but he returned in 1919 to join the staff of the University of Lausanne. Then he returned to the United States and was appointed instructor at the Massachusetts Institute of Technology, until 1929 when he entered private industry as consultant for various organizations. He became a United States citizen in 1931. Afterwards, he worked as head of the Chemical Service Department at the Hercules Powder Co. of Delaware (1929–32). He next assumed the post of research associate at the Bartol Research Foundation, Swarthmore, Pa., during 1933–36. In 1936 Prof. Piccard joined the teaching staff of the University of Minnesota as special lecturer in aeronautics, and attained professorial rank in 1937 from which he retired in 1952 as professor emeritus.

In 1957 Jamestown College, North Dakota, presented him with an honorary doctor of science degree; in 1959 he was honoured by the American Meteorological Society for his pioneer work in scientific stratosphere balloon flight. The University of Strasbourg conferred on him an honorary Ph.D. degree in 1932, and in 1960 he received the United States Distinguished Service Award. He had been named one of Minnesota's 100 "Living Great".

Named the "Mr. Up" of scientific exploration, Prof. Piccard's interest in balloons stemmed from collaboration with Auguste in 1913, when they made the first balloon flight together in Switzerland, and were the first to use a balloon as a physics laboratory. During the First World War, Jean and Auguste served as civilian consultants for the Swiss Army on captive balloons. The 1930's brought his contribution to the forefront when in 1933 Prof. Piccard designed a stratosphere gondola for the Dow Chemical Co., the basis for to-day's gondola design. The following year he ascended in a special balloon piloted by Mrs. Piccard. They took off from the Ford Airport in Dearborn, Michigan, and after attaining a height of 57,579 ft. landed in Cadiz, Ohio. In 1936 he sent up a balloon equipped with a radiosonde set from the University of Minnesota football field. This endeavour proved to be the first known experiment with a plastic non-extensible balloon. One of his helpers on this project was Robert Gilruth, now director of Manned Space Flight Program of the National Aeronautics and Space Administration.

In 1937 Prof. Piccard introduced another 'first' by successfully completing a flight over Rochester, Minnesota, testing the cluster idea of balloons. He made the ascent to about 10,000 ft. in an aerostat, *Pleades I*, using 98

rubber latex sounding balloons fastened to a metal gondola for lift. Of this flight Dr. Gilruth said: "It was a tremendous achievement. For one thing, he used electrically controlled blasting caps to blow off his ballast. Thus he was the first to use pyrotechnics to actuate devices. Now we have 73 such pyrotechnic devices for Mercury capsule".

His interest in high-altitude flight and balloons preceded that of Raven Industries in Sioux Falls, South Dakota; Winzen in South St. Paul; Schjeldahl in Northfield, and General Mills in Minneapolis. He later experimented with 'Cellophane' and other plastic balloons, and helped design the polyethylene *Skylark* series of high-altitude balloons with which the U.S. Air Force sent manned flights to altitudes exceeding 100,000 ft.

Prof. Piccard published many papers on ballooning, and in his earlier specialization, chemistry (organic, inorganic, and physical), and in aeronautical engineering.

His hobbies and interests included sailing, skiing, mountain climbing, riding in 'blimps' and aeroplanes. In regard to this he said: "But to me, riding in a free balloon is the most beautiful sport of all". During his retirement, he and Mrs. Piccard found pleasure in their cabin retreat of a four-acre island in Lake Vermilion. He and Mrs. Piccard were also part-time teachers at St. Paul's Episcopal Day School.

C. C. Chang

NEWS and VIEWS

Royal Society Research Appointments

The Royal Society has made the following appointments in original scientific research: The Tropical Research fellowship has been awarded to Dr. K. Vickerman, of the Department of Zoology, University College, London, to enable him to work on the mechanism of structural and physiological transformation in the life-cycles of trypanosomes. A Stothert Research fellowship has been awarded to Mr. C. N. Hales, of the Department of Biochemistry, University of Cambridge, to enable him to work on the immunochemical investigation of insulin. A further Stothert Research fellowship has been awarded to Mr. P. E. Reynolds, of the Department of Biochemistry, University of Cambridge, to enable him to work on cell-wall synthesis in bacteria, and the effects of antibiotics. The Head Bequest Committee of the Royal Society has awarded the Henry Head Research fellowship to Dr. J. S. Gillespie, of the Institute of Physiology, University of Glasgow, to enable him to continue his work on the automatic innervation of the gut. The appointments take effect from October 1.

Warren Spring Laboratory : Dr. C. C. Hall

Dr. Charles Hall has been appointed director of the Warren Spring Laboratory of the Department of Scientific and Industrial Research at Stevenage. Dr. Hall, at present deputy director of the Laboratory, will succeed the retiring director, Mr. S. H. Clarke, who will take up a post in the Department's Headquarters, with responsibility for supervising the careers of scientific staff in the Department. Both appointments take effect in January 1964. Dr. Hall was born in Croydon and was educated at Beckenham County Grammar School and at Sir John Cass College, University of London, where he graduated (B.Sc.) in 1929 with first-class honours in chemistry; he obtained his M.Sc. in 1930 and Ph.D. in 1934. He joined the Fuel Research Station of the Department of Scientific and Industrial Research in 1930 and was with that Station until it closed down in 1958. During most of the time, Dr. Hall was concerned with the chemistry and technology of the production of liquid fuels from coal. From 1939 until 1958 he was in charge of the Station's largest team working on the Fischer–Tropsch process. After the Second World War he played a leading part in the investigations into the development of the process in Germany. From 1953 until 1958 he was the deputy chief chemist of the Fuel Research Station. In 1959, Dr. Hall was appointed deputy director of the newly-formed Warren Spring Laboratory, which is engaged in research primarily on air pollution, mineral processing and chemical engineering. He was elected a Fellow of the Royal Institute of Chemistry in 1944, was a member of Council from 1957 until 1960 and chairman of the London Section from 1957 until 1959. He was elected a Fellow of the Institute of Fuel in 1954.

International Atomic Energy Agency's Research Laboratories : Prof. Leo Yaffe

Prof. Leo Yaffe (Canada) has been appointed director of the International Atomic Energy Agency's Division of Research and Laboratories. Prof. Yaffe, who took up his post on July 14, was born in 1916 at Devil's Lake, North Dakota. He studied at the University of Manitoba, gaining his B.Sc. in 1940 and M.Sc. in 1941, and received his Ph.D. from McGill University in 1943. He was director of the Radiochemistry Laboratory of McGill University and has been teaching since 1952, and Macdonald professor of chemistry at that University since September 1959. From 1943 until 1952 Prof. Yaffe was project leader in charge of Nuclear Chemistry and Tracer Research with Atomic Energy of Canada, Ltd., Chalk River. Prof. Yaffe was adviser to the Canadian Delegations for both United Nations Conferences on the Peaceful Uses of Atomic Energy at Geneva in 1955 and 1958. His predecessor as director of the Division of Research and Laboratories was Prof. Carlo Salvetti (Italy) until March 1962. In the interim period, Prof. Alexandre Sanielevici (Romania) served as acting director.

Academy of Sciences of the U.S.S.R.

At a general meeting of the Academy of Sciences of the U.S.S.R. a re-organization of the Academy has been approved. Fifteen specialized branches for the guidance of research in the main directions of science have been formed. Each of the previously existing eight branches had covered a very wide range of different fields of learning. Therefore, instead of one biological branch, for example, three new ones have been set up: biochemistry and chemistry of physiologically active compounds; physiology; zoology, botany and evolution. Four new branches, including a branch of nuclear physics and mathematics, have been instituted. A new branch has been set up for the science of the Earth, which unites geologists, geographers, oceanologists, geophysicists, specialists on the physics of the atmosphere, etc. Mstislav Keldysh has been re-elected president of the Academy. Nikolai Semenov, who headed the branch of chemical sciences, has been elected vice-president. Norair Sisakyan, who was the academic secretary of the biological sciences branch, has been elected senior honorary secretary.

Anglo-Russian Exchange of Scientists

Sir Bernard Lovell is visiting Leningrad at the invitation of the Soviet Academy of Sciences. On July 9 he met Vasily Isayev, the Mayor of the City, and other officials, and discussed with them scientific contacts between the Pulkovo Observatory and Jodrell Bank, which, he said, were very useful. Leningrad astronomers supported Sir Bernard's proposal that an exchange of junior scientific personnel should be started. Sir Bernard is planning to familiarize himself with the scientific

achievements of Pulkovo astronomers and to deliver a lecture to them on research carried out at Jodrell Bank. He will discuss with his Leningrad colleagues a programme of scientific exchange and individual observations of interest to both observatories.

Problems of Noise Abatement

THE Minister for Science, Lord Hailsham, said in the House of Lords on July 2 that he had now received the final report of the Committee appointed in April 1960, under the chairmanship of Sir Alan Wilson, to consider the problem of noise, and the Government had already given preliminary study to the recommendations in the report. The Minister of Transport proposed to give effect to nearly all the recommendations of the Committee's interim report on noise from motor vehicles, and new regulations had recently been circulated in draft to the organizations concerned. These regulations would lay down maximum permissible noise-levels for motor vehicles when used on the roads, and would also prescribe the instruments and conditions to be used for roadside tests. The Minister of Aviation would maintain the present strict control over movement of aircraft in and near airports and would continue to support research into ways of reducing aircraft noise at source and to investigate other aspects of the problem. The Ministers of Education and Health would seek to ensure that new schools and hospitals in the close vicinity of airports are designed to minimize noise inside the buildings, and would continue to seek to reduce noise in existing buildings, but the Government was not satisfied that the situation at Heathrow (London Airport) justified the recommendation for grants for sound-proofing private houses there. A number of recommendations relating to the need for additional research, for improved methods of disseminating information about the results of research, and for making industry and the public aware of the need to avoid making unnecessary noise had been accepted and were being implemented. The Medical Research Council and the National Physical Laboratory were co-operating in a joint project, sponsored by the Ministry of Pensions and National Insurance, to survey the effects of industrial noise on the hearing of workers by means of mobile laboratories which will visit factories of various types. Besides recommendations coming within the direct responsibility of the Government, others would be discussed with local authorities and others concerned, and some would require legislation. The necessary consultation would be undertaken as soon as possible.

Research in the Social Sciences

IN a written answer in the House of Commons on June 26, the Home Secretary, Mr. H. Brooke, stated that the other members of the Committee of Enquiry set up under Lord Heyworth's chairmanship (see also *Nature*, **198**, 1041; 1963) would be: Mr. Noel Annam, Prof. C. F. Carter, Sir Austin Bradford Hill, Dame Mary Smieton and Prof. Charles Wilson. The Committee's terms of reference were to review the work at present done in the field of social studies in Government departments, universities, and other institutions, and to advise whether changes were needed in and arrangements for supporting and co-ordinating this research, and it was intended that the Committee's investigation should cover social studies in Scotland as well as in England and Wales.

Output Capacity of Nuclear Reactors in Great Britain

IN a written answer in the House of Commons on July 4, the Minister of Power, Mr. R. Wood, stated that the present output capacity of nuclear reactors in Great Britain was 960,000 kW; by the end of 1964 this was expected to rise to 2,535,000 kW; 1965, 3,100,000 kW; 1966, 3,670,000 kW; 1967, 3,950,000 kW; and 1968, assuming 1 million kW of capacity will be commissioned at Wylfa, 4,950,000 kW.

Smithsonian Institution : Museum of Natural History

A NEW Department of Entomology has been established as the fifth major unit of the Museum of Natural History of the Smithsonian Institution, Washington. The other four Departments are Anthropology, Botany, Geology, and Zoology. Hitherto the entomological activities of the Museum have formed a division of the Department of Zoology. The new Department initially will consist of four divisions: the Division of Neuropteroids (caddisflies, mayflies, dragonflies); the Division of Coleoptera (beetles); the Division of Lepidoptera (butterflies and moths); and the Division of Myriapoda and Arachnida (centipedes, millipedes, and spiders). To-day, the entomological collections of the Museum comprise more than 16,000,000 insects, spiders, centipedes, millipedes, and related creatures. It is the most important and largest such collection in the Western Hemisphere, and, with that of the British Museum (Natural History), the Smithsonian collection is one of the two largest insect collections anywhere. Dr. J. F. Gates Clarke has been appointed head curator of the new Department. He will also serve as acting curator of the Division of Lepidoptera. The Division of Neuropteroids will be headed by Dr. Oliver S. Flint, jun., the Division of Coleoptera by Oscar L. Cartwright, and the Division of Myriapoda and Arachnida by Dr. Ralph E. Crabill, jun.

Abstracts and Indexes in Science and Technology

UNDER the title *Abstracts and Indexes in Science and Technology*, the Borough of Lewisham Library Service has issued an annotated guide to the abstracts, indexes and periodicals containing abstracts in science and technology provided by that Library Service (Metropolitan Borough of Lewisham Library Service. Scientific and Technical Information Service Publication No. 1, *Abstracts and Indexes in Science and Technology—an Annotated Guide to the Abstracts, Indexes and Journals containing Abstracts, provided by the Lewisham Library Service*. Pp. 12. Lewisham, London: Scientific and Technical Library, 1963.) The abstracts and indexes listed cover the bulk of the world's estimated output of 30,000 technical periodicals. A list of *Current Abstracts and Indexes in the Technical and Commercial Libraries of the Manchester Public Libraries*, with a preface by Mr. F. R. Taylor, librarian of the Manchester Technical Library, has also been issued (Manchester Public Libraries. *Current Abstracts and Indexes in the Technical and Commercial Libraries*. Pp. ii+42. Manchester: Technical Librarian, Central Library, 1963. 10*s*. 6*d*.). It contains 287 entries, which are annotated and give the library holdings, coverage, frequency of publication, etc. The entries are arranged alphabetically by title and there is a subject index.

British Cænozoic Fossils

THE first edition of *British Cænozoic Fossils*, published by the British Museum (Natural History) in 1959, was an immediate and deserved success. A second edition of this beautifully illustrated guide to the fossils most commonly found in Britain is now available (*British Cænozoic Fossils (Tertiary and Quaternary)*. Second Edition. Pp. vi+133 (44 plates). London: British Museum (Natural History), 1963. 6*s*.). The plates are unaltered and only minor changes have been made to the text. A useful addition is the provision of references to plate numbers in the fossil distribution lists, and the botanical nomenclature has been brought up to date.

Physics of Electronic and Atomic Collisions

THE third international conference on the "Physics of Electronic and Atomic Collisions" is to be held at University College, London, during July 22–26. Approximately 350 physicists from 21 countries will attend and

contribute 130 papers, relating to elastic and inelastic collision processes involving atoms, simple molecules, electrons and photons. The growth of interest in this field during the past few years, perhaps four-fold, is in part stimulated by power generation, magneto-hydrodynamics and fusion, by the discovery of lasers, and by the need to understand ionizing phenomena in the Earth's atmosphere and in space exploration, as well as problems in astrophysics. However, the central interest remains the development and testing of our understanding of the quantum theory of collisions. In this context it is interesting to note the present spheres of emphasis; the newly discovered narrow resonances in the scattering of electrons by atoms; the anomalous polarization of radiation excited by collision; the exploration of energy-levels in the continuum; the numerous inelastic processes undergone in energetic collisions between two atomic systems; the study of chemical reactions by refined scattering technique; the electron-ion recombination processes in highly ionized gases; and the conditions for the excitation of gases to a state where stimulated emission dominates and the production of coherent radiation by laser action becomes possible. Review papers will be presented by: Sir Harrie Massey, Dr. D. W. O. Heddle and Dr. M. J. Seaton (England); Prof. D. R. Bates and Prof. A. Dalgarno (Northern Ireland); Dr. M. A. Biondi, Prof. R. B. Bernstein, Dr. V. W. Hughes and Dr. C. K. Patel (United States); Prof. C. A. McDowell (Canada). Further information can be obtained from the conference secretaries, Dr. J. B. Hasted and Dr. D. W. O. Heddle, Department of Physics, University College, Gower Street, London, W.C.1.

The Night Sky in August

Full moon occurs on Aug. 5d. 09h. 31m. U.T. and new moon on Aug. 19d. 07h. 35m. The following conjunctions with the Moon occur: Aug. 5d. 23h., Saturn 1° N.; Aug. 10d. 02h., Jupiter 4° N.; Aug. 23d. 05h., Mars 5° S. In addition to these conjunctions with the Moon, Mercury is in conjunction with Regulus on Aug. 2d. 20h., Mercury being 0·6° N. Mercury, Venus and Mars are too close to the Sun for observation. Jupiter rises at 22h. 00m., 21h. 05m. and 20h. 00m. on Aug. 1, 15 and 31, respectively. It is in Pisces, its stellar magnitude is −2·3 and its distance from the Earth on August 15 is 400 million miles. Jupiter is stationary on August 10, after which its motion is retrograde among the stars. Saturn is above the horizon most of the night. It is in Capricornus, reaching opposition on August 13. Its stellar magnitude is + 0·5 and its distance from the Earth on August 15 is 825 million miles. There are no occultations of stars brighter than magnitude 6 visible at Greenwich. The Perseid meteors are active during July 27–August 17, with maximum on August 12, but conditions for observation are unfavourable.

University News: Aberdeen

Two additional chairs, of statistics and education, have been established and will be occupied by the present heads of the departments concerned, namely, Dr. D. J. Finney and Dr. J. D. Nisbet, respectively. The following promotions from lecturer to senior lecturer have been announced: Mr. P. W. Davis (forestry); Dr. W. E. Fraser (geology).

Sheffield

Dr. W. Bartley, at present University demonstrator in biochemistry in the University of Oxford, has been appointed to the chair of biochemistry from a date to be arranged. Dr. A. R. Entwisle has been appointed senior lecturer in metallurgy, and Dr. E. Rothwell senior lecturer in fuel technology and chemical engineering. Dr. I. O. Sutherland has been appointed lecturer in chemistry.

Announcements

Mr. A. Simpson, recently appointed lecturer in aeronautical engineering in the University of Bristol, has gained a Ph.D. degree at the University of Bristol. In so doing he becomes the first holder of the diploma in technology to obtain the Ph.D. of a university.

The second European Anatomical Meeting will be held in Brussels during September 1–5. Further information can be obtained from Prof. P. Dustin, 97 rue aux Laines, Brussels 1.

The conference of the Society for Folk Life Studies will be held in the University of Leeds during September 6–9. Further information can be obtained from J. Geraint Jenkins, c/o Welsh Folk Museum, St. Fagan's Castle, near Cardiff.

The fourth International Congress on Surface Active Substances will be held in Brussels during September 7–12, 1964. Further information can be obtained from the General Secretariat, Fourth International Congress on Surface Active Substances, 49 Square Marie-Louise, Brussels 4, Belgium.

The ninth conference on the European Meat Research Workers, organized this year by the Hungarian Meat Research Institute, will be held in Budapest during September 4–12. Further information can be obtained from the Hungarian Meat Research Institute, Herman Otto ut 15, Budapest 2.

The fourth international conference on "Non-Destructive Testing", sponsored by the British National Committee for Non-Destructive Testing and arranged by the Institution of Mechanical Engineers, will be held in London during September 9–13. Further information can be obtained from the Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1.

The fifth international Biometric Conference will be held in Cambridge during September 9–14. The programme will include sessions on: biometrical genetics; problems of experimentation, mathematical models in biology; multivariate analysis; medicine and bioassay; statistical methods. Further information can be obtained from Dr. R. C. Campbell, School of Agriculture, Cambridge.

The Centennial of Entomology in Canada, sponsored jointly by the Entomological Society of Canada and the Entomological Society of Ontario, will be held at Carleton University, Ottawa, during September 3–6. Further information can be obtained from the Centennial Executive Committee, Centennial of Entomology in Canada, K. W. Neatby Building, Carling Avenue, Ottawa.

A four-week practical course in "Administration and Management of Research and Development", arranged by the Comparative Administration Trust, will be held at Queen's College, Oxford, during September 1–28. The three main topics to be considered are: the nature of the individual and of the team; the teams in their wider context; special problems of science and technology. Further information can be obtained from the Comparative Administration Trust, 30 St. George Street, Hanover Square, London, W.1.

The International Union of Crystallography will be holding its sixth general assembly, international congress and symposia in Rome during September 9–18. The programme will include: a special congress discourse entitled "Crystallography: the Old and the New"; four general lectures; sessions for the presentation and discussion of contributed papers; an exhibition of apparatus and books; symposia on: problems and methods in crystallographic computing; phase transitions in solids; some aspects of lattice dynamics. Further information can be obtained from Dr. W. H. Taylor, Crystallographic Laboratory, Cavendish Laboratory, Cambridge.

GOVERNMENT EXPENDITURE ON SCIENTIFIC RESEARCH, 1962

THE report of the Council for Scientific and Industrial Research for the year 1962 * records gross expenditure in the year ended March 31, 1962, of £16,691,172, compared with £14,467,278 in the previous year, and reduced to £14,378,202 by various receipts from industry and other sources for services rendered, of which £1,120,677 was from other Government departments and £858,170 from industry, £214,143 being received by the National Physical Laboratory. The net increase on 1961 was £1,619,022, and of this, £258,922 is on account of the National Lending Library for Science and Technology, which figures for the first time as a separate item (gross expenditure, £304,249) in the summary of expenditure. The staff again increased, from 6,224 to 6,525. Grants for special researches rose to £2,275,060, compared with £1,495,469 in 1961, and contributions to the European Organization for Nuclear Research remained about £1,359,000, while receipts reduced the contribution of £198,800 to NATO and the Organization for European Co-operation and Development scientific schemes to £63,722.

Postgraduate training awards amounted to £1,289,903 (£1,105,415 in 1961) and the 1,604 new studentships, an increase of 9 per cent, brought the number current to 3,478. Research fellowships increased from 54 in 1961 to 92 in 1962, and there are now 133 current. Of 51 awards for study abroad taken up, 23 were studentships and 28 fellowships, while 8 studentships and 16 fellowships were awarded to assist young British scientists returning from North America; 35 candidates with the diploma in technology obtained awards, compared with 11 in 1961. Of the 3,019 current research studentships, 413 were in biology and biochemistry, 205 in chemical engineering and metallurgy, 899 in chemistry, 128 in electrical and 203 in other fields of engineering, 214 in geology, 57 in the human sciences, 260 in mathematics, and 640 in physics. The advanced course studentships were distributed mainly in mathematics (88), physics (75) and engineering (other than electrical) (70), with 39 in chemical engineering and metallurgy, 29 in human sciences, 28 in biology and biochemistry, 22 in geology, 20 in electrical engineering and 15 in chemistry. All the 73 first-year studentships were again in chemistry, and of the 115 research fellowships, 56 were in chemistry, 29 in physics and 25 in biology and biochemistry, with 10 in geology.

Grants for special researches totalled 724, of which 321 were new, amounting to £9,881,252 in the year ended March 31, 1962; of these, 59 in nuclear physics totalled £3,870,624, 17 in astronomy, £1,045,796, 121 in technology, £1,024,768, and 165 in chemistry, £957,551. The 79 grants in low-temperature and solid-state physics totalled £688,267, 100 grants in biology totalled £535,417, 33 in space research, £520,926, 58 in geology and geophysics, £469,689, 50 in the human sciences, £419,601, and 36 in physics, £328,162. During the academic year 1961–62, the Research Grants Committee considered 500 applications for grants with a total value of £4,204,000, compared with 447 applications valued at £3,156,000 in the previous year, and made 390 grants with a total value of £2,313,000, compared with 362 amounting to £1,961,000 in 1960–61. In addition, five grants were awarded for new major capital projects: £534,200 to the University of Cambridge for construction of a radio-telescope for research under Prof. M. Ryle; £301,000 to the University of Manchester for construction of a 125-ft. steerable radio-telescope for research under Sir Bernard Lovell; and three grants totalling £1,188,000 for nuclear reactors for the University of London as a whole, the Universities of Manchester and Liverpool jointly and the Scottish and Northern Ireland universities. The Human Sciences Committee considered 36 applications with a total value of £232,000 and made 27 awards to the value of £132,000.

With the exception of building research, which decreased slightly from £785,413 gross in 1960–61 to £781,037 gross in 1961–62, and the Geological Survey and Museum, which decreased from £586,640 to £578,969 gross, the increased expenditure was distributed fairly generally over all branches of the Department's work. However, an unexplained jump in receipts by the Tropical Products Institute from £22,300 to £399,472 converted the increased total expenditure on the Institute of £229,751 into a credit of £169,721, an anomaly that seems to call for comment. The major increases are in the expenditure of the National Physical Laboratory (£2,033,109 gross; £1,787,542 in 1960–61), the National Engineering Laboratory (£924,978; £811,164); the National Chemical Laboratory (£409,887; £384,073); road research (£970,403; £872,805); radio research (£320,413; £239,957). For fire research the corresponding figures are £164,438 and £156,130; for forest products research, £194,619 and £185,985; hydraulics research, £232,661 and £203,931; the Torry Research Station, £226,696 and £215,192; the Warren Spring Laboratory, £471,181 and £453,985; water-pollution research, £183,550 and £165,336; and for the Government Chemist's Laboratory, £491,215 and £460,257. Expenditure on headquarters administration decreased from £758,126 in 1960–61 to £615,536, or slightly below the 1959–60 figure.

* Department of Scientific and Industrial Research. Report of the Research Council for the Year 1962. Pp. 62. (Cmnd. 2027.) (London: H.M.S.O., 1963.) 4*s*. net. (See also p. 207 of this issue of *Nature*.)

EDUCATION IN MATERIALS SCIENCE

THE first conference on "Education in Materials Science" and technology in the United Kingdom was held in Banbury during May 18–19 under the general chairmanship of Mr. L. Holliday (Shell Chemical Co., Ltd.). The conference was arranged by the Steering Committee of the Working Party on Materials Science and Technology, set up by the Institution of Chemical Engineers. Some thirty representatives from universities, colleges of advanced technology and industry were invited.

The first session dealt with the situation in materials science in the United States and in Britain and went on to consider possible starting points for materials science courses and research in universities. Sir Harry Melville, secretary of the Department of Scientific and Industrial Research, introduced the subject of the conference and emphasized its importance not only in universities and colleges but also in industry and Government scientific establishments. It was of immediate concern to the Department of Scientific and Industrial Research, which

had to keep new developments in constant review so that finance could be found for worth-while proposals.

Although it was not known what the outcome of the Robbins Committee would be, Sir Harry thought that it was likely that Britain would continue with the three-year undergraduate courses, and because these were now so crowded it would be difficult to introduce in depth the concepts of materials science without risking superficiality in the courses. He thought that this problem would be least serious in metallurgy departments because they have done this kind of work in universities over the years. The limitations of existing courses could be met by the establishment of postgraduate courses, support for which was now provided by his department. Sir Harry considered that one of the serious problems of creating an interdisciplinary subject was to achieve something greater than the arithmetical sum of the parts. This was a problem of achieving real co-operation and co-ordination so as to influence the mental attitudes of the students. He was anxious that Britain should try to meet the developing situation by action now, so as not to be forced to act later under pressure from influences outside the country.

Prof. R. L. Sproull, head of the Materials Science Centre, Cornell University, and chairman of the Advanced Research Projects Agency activity there, reviewed the situation in the United States. He recalled that some six years ago many currents of opinion seemed to be running in the same direction pointing to the absence of adequate materials for the many new technological projects, in particular atomic energy and astronautics. The problems came to a focus within the Atomic Energy Commission and there was a proposal to form a National Laboratory for Materials. It was soon realized that there were not enough men with the right background and attitudes to staff such a laboratory and urgent decisions were taken to provide training to produce this type of man for the future.

It was noticed that in some of the big industrial laboratories in the United States it was possible to bring sophistication from various scientific fields to bear on problems and frequently the outcome from this approach was strikingly successful. It was also noticed that students are more readily attracted to those subjects that contain the word 'science' in their titles. Even so, a very high proportion of graduates who were working on problems of materials had in fact been trained in high-energy physics. This was recognized as wasteful, and it was decided to establish a series of interdisciplinary laboratories in several university institutions devoted to materials science. There are now twelve such interdisciplinary laboratories supported by the Advanced Research Projects Agency, with two more supported by the National Aeronautics and Space Agency and an additional one supported by the Atomic Energy Commission.

University departments had not, in general, been equipped with sophisticated apparatus of the type students would eventually use for research and development in Government and industrial laboratories. Therefore, one objective was that university departments should be equipped to about the same standard as some of the best industrial laboratories. In response to Government support, the chosen university institutions agreed to produce more Ph.D.s, to undertake research which would be broadly in keeping with the trends in materials development and to use an interdisciplinary approach to research. They were in fact trying to produce a new breed of man and not merely to achieve a simple addition of disciplines. Prof. Sproull was in favour of the development of materials science courses without reference to existing disciplines although this had not, in general, been possible in the United States.

Prof. E. W. J. Mitchell (University of Reading) described how he had tackled the problem of achieving depth while covering the essential fundamentals required in materials research work. This could be achieved by a fairly ruthless pruning of the conventional physics course, leaving out treatment in depth of such topics as high-energy physics, electronics and optics, and streamlining the teaching in some other subjects. The objective was to try to impart to the students an understanding of some of the structure-sensitive phenomena and an appreciation that they could be studied in an intellectually stimulating fashion. Prof. Mitchell's undergraduate course forms one of three options within the school of physical sciences at the University of Reading.

Prof. D. A. Wright explained that because there was much activity in high-energy physics at Durham it had been appropriate to introduce applied physics and to concentrate on electrical materials in teaching and research. The University hopes eventually to introduce postgraduate courses in this field.

Profs. A. G. Quarrell and V. N. Douglas emphasized strongly the interdisciplinary nature of all technology. They pointed out the distinctions between solid-state physics and activities such as physical metallurgy and glass technology, which could not avoid the complexities of real industrial problems. Prof. Quarrell warned against thinking that the study of materials science was specifically a new feature. At Sheffield this had been the case for some time where the Faculty of Metallurgy included glass technology and refractories as well as metallurgy. He thought that technological subjects should stand in their own right as university disciplines and that they could not be adequately imparted in short postgraduate courses given to people who had not previously had some understanding of the complex situations involved.

Prof. R. W. Cahn (University College of North Wales, Bangor) presided at the second session when the discussion embraced "the relationship between materials science and other disciplines, the problem of financing materials science teaching and research, and the problem of university recruitment".

Prof. T. K. Ross (Manchester College of Science and Technology), speaking for chemical engineering, said that the relationship between materials science and chemical engineering might at first sight appear slight but was by no means unimportant. The chemical engineer tended to think in terms of constructional materials for process plants and the problem of environment usually represented corrosion. In his opinion, the postgraduate field had most to offer.

Mr. A. Kennaway (B.T.R. Industries, Ltd.) contended that mechanical engineers required to be better educated in materials science. They should know more about the plastic range in metals, the criteria for failure and the technical properties in general in relation to structure. The properties of other materials such as plastics were often investigated by chemists and physicists with the result that the available information was not in a form that the engineer could use. Mr. Kennaway could see no case for the unification of the technologies, as compared with the science of materials.

Prof. A. J. Wildschut (Technical University, Delft), said it was surprising, on reflexion, how little was known about materials. The civil engineer was interested in a wide range of materials—the 'big volume materials' such as clay, soil and stone, which must be cheap, and the 'small volume materials' such as brick, metal and wood. He quoted a number of novel applications and emphasized the importance of durability in structural materials which were difficult to use up to their physical limit. Dimensional stability was important in metals, for example, and stiffness in structure, while a weakness in an expensive joint could impair the success of a major civil engineering project. Improved knowledge of all materials of construction was important to the civil engineer.

Prof. E. H. Rhoderick (Manchester College of Science and Technology) described the courses in materials

science designed for electrical engineers with a bias towards electronics interests, which included subtle and complicated techniques and involved, for example, superconductors and cryogenic techniques. The courses presumed that the materials scientist was not essential for inventions but he was required for the development of inventions. Third-year electrical engineering students at Manchester were given an extensive course in properties of materials, including new materials. A further course was available at postgraduate level. The stimulus came from linking the materials work to electrical engineering.

In the discussion that followed, it was pointed out that a postgraduate course has been designed at the Imperial College of Science and Technology. This represented a co-operative effort between seven disciplines because the interdisciplinary approach was considered to be essential. Another speaker contended that materials science could make its greatest contribution through the development of a common language. We were not disseminating adequately the existing knowledge of materials, and practice in industry tended to lag too much behind research—the growth in knowledge in the field had outstripped the applications. A representative of the Department of Scientific and Industrial Research spoke on the problem of financing materials science teaching and research. Special emphasis was being given by the Department to the needs of materials science, and the long-term financial implications were under investigation. This led to a lively and prolonged discussion on the question of the adequacy of the financial provision, particularly for postgraduate students and courses. Prof. G. Ball (Imperial College of Science and Technology) introduced the subject "the problem of university recruitment" and emphasized what had been said earlier—the tendency of the schoolboy to think in terms of pure science. For this reason alone it might be worth while to retain the word 'science' in the title of courses, with the view of attracting better than average students. In the long term, the social attitude to 'technology' might change. The attitude to materials science as a discipline could be influenced by the provision of suitable text-books for schools. One speaker claimed that pure science was more attractive to the schoolboy by reasons of the so-called 'convertibility' with which it endowed its students, but that it was a mistake, however, to fill higher posts with those trained in narrow specialisms such as rubber or refrigeration technology.

At the third session the chair was taken by Prof. Sproull. Dr. P. E. Evans (Faculty of Technology, University of Manchester), opening a discussion on materials science and the established disciplines, stressed the need for a fresh and unfettered approach to the subject and the importance of engendering thinking in terms of materials from the beginning of the course. A good case could be made out for teaching materials science as a first degree subject. He thought that there might be a danger that the established departments of applied science would regard materials science departments as competitors for limited funds, and, under Britain's present educational system, a limited number of students. The trained materials scientist required is one who not only has specialized knowledge in one field of materials but also has a thorough understanding of materials generally.

Dr. N. F. Astbury (director of Research, British Ceramic Research Association) considered the possible role of a Materials Science Council: whether it could be a viable unit and how it could best operate. He suggested that all rational research is good but only some is timely, and that we are not getting the maximum co-ordination of national effort since much materials research in the United Kingdom is done in a vacuum, thus becoming sterile because it is not stimulated by the materials user. He pointed out that there is no civil agency for co-ordinating materials research in Britain. The relation of a Materials Science Council to existing bodies would be a matter for careful thought, since materials science cuts across several entrenched interests. Opinion would seem to favour a council formed from the relevant existing professional and scientific bodies.

Dr. H. M. Finniston (International Research and Development Co., Ltd.), talking on materials research institutions, suggested that the United Kingdom cannot afford the pursuit of knowledge for its own sake so that 'research' must not always be interpreted as 'pure research'. Any materials research institute would have to be Government supported, interdisciplinary and related to some other continuing function, for example, teaching or industry; it could only be founded at the expense of existing institutions. In general, he was opposed to the foundation of a single national institute. He added that Britain cannot and should not copy the American approach. Mr. Zvegintsov (National Research and Development Corporation), discussing the types of materials scientist needed by industry, said that the paradox in science and technology in this field was the requirement for greater specialization with greater hybridization. He stressed that the essential background knowledge required was the relation of properties to structure and that the need for the imaginative creative step was increasing.

In the discussion that followed, it was suggested that a Materials Science Council could collate and disseminate information on existing and proposed materials science courses in addition to promoting conferences on themes cutting across several disciplines. It was said that Britain cannot afford a proliferation of research institutions.

In conclusion, it was agreed that the following items summarized the tenor of the conference:

(1) There is a real need for an expansion of existing postgraduate courses in materials science.

(2) There should be a limit to the number of institutions offering courses in materials science because such an institution must be of a minimum size before it can be effective.

(3) The best kind of materials science course is organized by cutting across the boundaries of departments of both pure and applied science.

(4) The lack of Department of Scientific and Industrial Research advanced studentships is a grave handicap to the development of materials science courses.

(5) There appears to be no case to support any plan for setting up special research institutions to be devoted to materials science.

(6) Experience in materials science courses so far shows that it will be necessary to interest the schoolmaster and the schoolboy in this subject. There are various ways in which this can be done, but one which calls for attention is the re-writing of parts of school physics text-books.

(7) It has been stated that the reason why schoolmasters recommend that their best students read pure science is because physics and chemistry are more 'convertible' than a subject like materials science or a technological subject. This is questioned. Experience from such universities as Sheffield shows that graduates in technological subjects are at least as adaptable as graduates in pure science.

(8) Sir Harry Melville, secretary of the Department of Scientific and Industrial Research, would welcome the opportunity to meet representatives of the universities and colleges of advanced technology concerned with materials science and technology, to discuss the forward planning of teaching and research.

It was resolved to hold a further conference on the same theme in 1964. Prof. Mitchell, of the University of Reading, has agreed to co-ordinate the arrangements for this.

L. Holliday
J. G. Ball
P. E. Evans
J. B. Brennan

DELTAIC AND SHALLOW WATER MARINE SEDIMENTATION

THE sixth International Sedimentological Congress, held in the Netherlands and Belgium during May 29–June 2, was attended by about 300 members from more than 20 countries. It was preceded by field trips to study the Dutch Holocene deposits, especially the tidal flat sediments of Zeeland and the coastal plain west of Amsterdam, and the lagoonal deposits of the East Flevoland polder.

The earlier sessions of the Congress were held in Amsterdam, the later ones in Antwerp. The main theme of the Congress was "Deltaic and Shallow Marine Deposits" though some papers of a general nature were also presented. After speeches of welcome by the president of the Organizing Committee, Prof. R. Tavernier, the president of the International Association of Sedimentologists, Prof. F. P. Shepard delivered his presidential address on "Criteria in Modern Sediments Useful in Recognizing Ancient Sedimentary Environments". This appropriately dealt in considerable part with the work of the speaker and his colleagues in and around the Mississippi Delta. Prof. Shepard stressed the importance of the extensive Cænozoic tectonic activity on recent sedimentation and commented on the discovery in deep water of an increasing number of sedimentary features once regarded as indicative of shallow water origin.

Only about one-third of the papers accepted by the Congress for publication could be read and discussed at the meeting. Descriptions of recent or relatively recent deltas dealt with those of the Niger (J. R. L. Allen) and of the Rio Grande de Santiago off west central Mexico (D. G. Moore). R. H. Dott discussed the incidence of deltaic deposition in eugeosynclinal belts, with special reference to the United States, and P. Wurster described the deltaic sedimentation of the German Keuper.

Studies of modern shallow water marine sediments included accounts of transportation and deposition along the Dutch coast (A. J. de Groot) and off Brest (A. Guilcher). H. Füchtbauer discussed the varying porosities of sediments from different environments in the southern North Sea. A paper arousing widespread interest was that in which E. J. Degens described the diagenesis of organic substances off the Californian coast and reached the conclusion that the organic matter was almost entirely land derived. Modern carbonate sedimentation in the Persian Gulf was described in papers by D. J. J. Kinsman and by A. J. Wells. The patches of milky water, known as 'whitings', from which large-scale precipitation of calcium carbonate occurs were illustrated by a short film. Two other excellent films were shown in the course of the meeting, one illustrating the formation of various types of ripples, bars and dunes in flume experiments, the other submarine erosion by slow-moving sediments.

Older shallow-water deposits described included, among others, marginal Tertiary sediments of the Paris basin (C. Pomerol) and of the sub-Alpine molasse trough (W. Zimmerle). J. P. Mangin claimed an extremely shallow-water origin for sequences interpreted as 'flysch' in the Cretaceous and Eocene rocks of northern Spain. R. Goldring discussed the significance of the two distinct trace-fossil assemblages commonly met with in shallow-water marine sediments. A number of contributors dealt with British Carboniferous sedimentation in South Wales (G. Keeling and T. R. Owen) and in central and northern England (H. G. Reading, P. M. L. Duff and E. K. Walton). G. V. Chilingar, in a study of more than 600 core samples, showed that for sandstones of comparable grain size a close relation exists between porosity and permeability.

During the Congress a special one-day symposium on "Sedimentology and Ore Genesis" was held in Delft. The opening session dealt with syngenetic sulphide deposition. A. J. C. Bernard discussed the importance of adsorption and of precipitation at surface temperatures in metallogenesis. O. Schulz claimed the lead-zinc deposits of Raibl and Bleiberg as of exhalative sedimentary origin, while H. J. Schneider regarded these and other deposits in Triassic limestones and dolomites of the Eastern Alps as due to syn-sedimentary precipitation in the back reef facies. P. Nicolini drew on Moroccan examples as well as those of Mansfeld and of deposits in the Cévennes in illustration of his theories of sedimentary concentration of copper, lead and zinc. L. G. Love described, with convincing detail, the early diagenetic formation of pyrite in recent muds. G. C. Amstutz illustrated the development of four generations of diagenetic iron sulphide from Ordovician dolomitic limestones in Missouri. In collaboration with P. Ramdohr, he quoted mineralogical evidence interpreted as indicative of pre-deformation and even pre-slumping deposition of much of the sulphide at Mount Isa.

A second session dealt with sedimentary iron ore deposits. J. Petránek described the evidence for shallow-water origin of the oolitic ores of the Lower Palæozoic. H. Harder drew a comparison between ores of the Minette type to which the iron was supplied by normal weathering processes and those of the Lahn-Dill type in which it is believed to have come from volcanic sources. L. Bubenicek considered the conditions of deposition of the Lorraine ores stressing the importance of diagenesis. A final session dealt with the origin of phosphorites (M.Slansky), of strontiobarytes from the German Zechstein (H. Puchelt and G. Müller) and of the Arkansas barytes deposits (R. A. Zimmermann and G. C. Amstutz). The papers and the ensuing discussion, ably summarized at the close by P. Routhier, are to be published as a separate volume from the remainder of the Congress proceedings.

After the Congress, seven different excursions in Belgium offered opportunities for studying a variety of sedimentary formations. At the general assembly of the International Association of Sedimentologists a proposal to hold the seventh congress in Great Britain in 1967 was unanimously approved.

J. H. Taylor

CONFERENCE OF THE UNIVERSITIES OF THE UNITED KINGDOM

THE report of proceedings of the Conference of the Universities of the United Kingdom*, held in London during December 14–15, 1962, includes the text of the speeches of Dr. E. G. Edwards and Prof. S. Wiseman in opening the discussion on alternatives to university education, and of Sir Noel Hall and Prof. A. G. Quarrel in opening the discussion on relations between the universities and industry, as well as of that of contributions to both discussions.

Dr. Edwards was concerned more especially with the deliberate interaction of college and industry attempted

* Conference of the Universities of the United Kingdom 1962: Report of Proceedings—Alternatives to University Education; Relations Between the Universities and Industry. Pp. 92. (London: Association of Universities of the British Commonwealth, 1963.) 5s.

by the colleges of advanced technology and with its implications for postgraduate study and research, education and industrial training generally, while Prof. Wiseman examined the part played by teacher training colleges as 'alternatives' to a university education. Both speakers insisted on the need to provide for some cross-over or transfer from college of technology or teacher training college to university and vice versa and on the importance of a broadly based education. Dr. P. F. R. (now Sir Percy) Venables referred to the necessity for all institutions of higher education to think in terms of university quality in determining their conditions, and in particular there should be adequate opportunities for higher study and research and professional contacts by way of travel, sabbatical leave and attendance at conferences. Prof. Wiseman's contention that the provision of teaching should be under the ægis of the universities was fully supported, but sandwich courses were not generally accepted as advantageous.

Sir Noel Hall thought that up to 1939 contacts between the university world and industry generally were much more limited in number and scope than those in other advanced countries and quite inadequate for the post-war demand, although in finance and public administration a pattern of relations had developed where practitioners welcomed discussion with university men and women on the problems of their own day-to-day responsibilities. Much re-thinking was now taking place in industry, and probably among university appointments committees, on the placement of graduates in industry, leading to developments which were steadily improving contacts between universities and industry in this respect. He thought that research by qualified university people into the activities of industry itself was likely to continue to be difficult to organize and pursue, though he was not particularly concerned about the problem of secrecy. A greater difficulty was that of deciding the point in time at which important decisions had in fact been taken. This difficulty appeared to be diminishing and much activity was also being devoted to studying the tools of management.

Prof. Quarrell was concerned more with the conduct of research by universities on behalf of industry, discussing more particularly the research contract but emphasizing the value of formal opportunities for personal contacts between industrialists and members of a university through the university court, council and committees of council. In this discussion also the importance of a wider basic training for professional students was emphasized. However, the main stress was laid on the importance of personal contacts, including participation by industrialists in university government at the executive level. The desirability of avoiding the use of the term 'management' was recognized and of referring rather to 'business studies'.

THE BRITISH COAL UTILIZATION RESEARCH ASSOCIATION

THE annual report for 1962 of the British Coal Utilization Research Association* includes a summary of the year's activities and lists of publications and lectures by the staff, information circulars, and membership of advisory committees and of research staff. The total staff of 310 at the end of the year comprised 63 in scientist grades, 115 in research officer and research assistant grades, 46 in clerical and administrative grades and 86 in industrial grades.

The bulk of the report is occupied by an account of research activities from which the following points are selected. A two-year programme on coal-fired boilers fitted with automatically controlled combustion equipment and ash-handling and coal-handling gear indicated that from the point of view of cost of steam generated as well as technically this assembly of coal-fired equipment can be recommended for industrial use in many localities and circumstances. Eight commercially produced solid-fuel boilers with outputs from 33,000 to 60,000 B.Th.U./h have been tested using anthracite and coal under varying conditions to provide basic information for developing more efficient and automatic appliances with high amenity.

* The British Coal Utilization Research Association. Annual Report, 1962. Pp. 67. (Leatherhead: The British Coal Utilization Research Association, 1963.)

Work on deposits and corrosion in large water-tube boilers has already indicated that the composition and structure of deposits are independent of time, and has led to the devising of an infra-red spectrometric method for determining sulphur trioxide and to the investigation of laboratory methods for assessing quantitatively the effects of the nature of the ash and the composition of the flue gases on the corrosion of austenitic steels.

A systematic study of the slagging properties of British coals now nearing completion should assist in the choice of coals for slagging combustion, and basic studies of combustion and gasification have sought to provide basic information on the behaviour and decomposition of coals while being heated. The apparatus for studying pre-ignition and devolatilization of small particles of coal has been further developed, enabling the amount and rate of decomposition at known particle temperatures to be determined under effectively isothermal conditions. Among instrumental advances during the year were the further improvement of the venturi pneumatic pyrometer, the use of a nuclear magnetic resonance method, a microwave method for continuous measurement of moisture in coal, and the use of ciné-photography for studying the operation of the open-screw feeder and the quenching of slag discharged from a gasifier.

TRIBOPHYSICS RESEARCH IN AUSTRALIA

THE Division of Tribophysics of the Commonwealth Scientific and Industrial Research Organization of Australia is a major centre of research on the physics and chemistry of surfaces of solids and on metal physics. The annual report for 1961–62 records that work continued on the friction and lubrication of surfaces*. Molybdenum disulphide has been shown to be superior in lubricating ability to many other inorganic compounds of comparable structure, and the effect of atmospheric humidity and water absorbed on the lubricant on burnishing and friction has been examined. Friction is highest at 100 per cent humidity. The theoretical study of how models of the atomic configurations of flat surfaces could, in principle, be constructed has now been applied to a number of different crystal structures and a set of photographs of models of about twenty of the simplest surfaces in each of the face-centred-cubic, body-centred-cubic, sodium chlor-

* Commonwealth of Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tribophysics for the year ending June 30, 1962. Pp. i+19. (Melbourne: Division of Tribophysics, C.S.I.R.O., 1962.)

ide, diamond and hexagonal closed-packed structures has been produced. The preparation of surfaces in high vacuum, the examination by electron microscopy of metal films evaporated on to glass, and the effects of bombarding gold with inert gas atoms have been studied. The oxygen adsorption on, and possible solution in, a clean silver surface at about 200° C, in the range 10^{-3}–5×10^{-2} mm of mercury pressure was measured and the quantity related to the surface area of the silver determined by the adsorption of krypton at −195° C. The quantity of oxygen sorbed per unit area and the shape of the isotherm depended markedly on the surface to volume ratio of the evaporated film. The surface appeared to be covered with a monolayer at approximately 5×10^{-3} mm of mercury pressure and the amount of oxygen adsorbed at that pressure corresponded to one oxygen atom for every krypton atom absorbed.

For the preparation of metal specimens for X-ray and electron microscope measurements a spark erosion machine was developed and the amount and depth of the damage produced by the machine on nickel was studied. Other investigations included the effects of surface, temperature and strain rate on the Bauschinger effect and cyclic work-hardening in copper single crystals; the measurement of the electrical resistivity of dislocations; the formation of point defects in plastically deformed metals and the dislocation rearrangements during recovery; and martensite transformations in steel. For the Divisions of Textile Physics and Protein Chemistry proton resonance studies of the kinetics of the absorption of water by wool and the exchange of deuterium for hydrogen in a particular wool–water system were made, and in conjunction with the Division of Applied Mineralogy the properties of interlayer water in vermiculite crystals were investigated.

The annual report includes details of the publications by the members of staff of the Division and a list of the names of the senior members of staff. Dr. L. M. Clareborough, Dr. M. E. Hargreaves and Mr. M. H. Loretto received the David Syme Research Prize for their work on imperfections in metal crystals and Dr. A. K. Head was visiting professor at Brown University, Rhode Island. Prof. P. P. Ewald, president of the International Union of Crystallography, visited the Division in October 1961.

S. Weintroub

CLEAN SURFACES AND SURFACE PHENOMENA IN SEMICONDUCTORS

A RECENT issue of the *Annals of the New York Academy of Sciences* contains the series of papers presented at the conference entitled "Clean Surfaces" held by the Academy during April 5–7, 1962, and, as a supplement, the papers of the symposium on "Surface Phenomena in Semiconductors" held by the American Chemical Society during March 21–29, 1962*.

The conference was divided into four parts, the first of six papers dealing mainly with electron diffraction and the electronic properties of clean surfaces; the second of six papers with cleaved and etched surfaces; the third of three papers with adsorption; and the fourth of six papers with nucleation. E. W. Müller gave a survey of field emission microscopy of clean surfaces with electrons and positive ions. He stressed that the field ion microscope is a research tool for the investigation of clean surfaces, and of the defect structure in the interior of metal crystals exposed at the surface by field evaporation. More detailed information on adsorption can be expected by the operation of the microscope with gases of lower ionization energy, but this will require ultra-pure imaging gases, and the lower field strength will reduce the resolution of the microscope. L. H. Germer discussed the very recent applications of the diffraction of slow electrons to the investigation of surfaces and the new science of two-dimensional crystallography. Experiments described by H. E. Farnsworth of low-energy electron diffraction from a cleaved germanium surface have established that crystal faces other than that desired by cleavage were present, and that a cleaved surface had a larger area of undesired crystal facets than a surface prepared by cutting the crystal with a diamond wheel, ground, etched chemically, and cleaned *in vacuo* by argon ion bombardment and subsequent annealing.

The symposium on "Surface Phenomena in Semiconductors" was suggested and organized by H. van Olphen, the chairman of the division of colloid and surface chemistry of the American Chemical Society. The object was to stimulate discussion among surface chemists and research scientists in the semiconductor surface field. The symposium was tutorial in character and the fourteen papers presented comprise a series of review or tutorial papers each devoted to a particular area of specialization.

* *Annals of the New York Academy of Sciences*, **101**, Article 3: *Conference on Clean Surfaces*, with Supplement: *Surface Phenomena in Semiconductors (Symposium)*. By F. G. Allen *et al.* Pp. 583–1014. (New York: New York Academy of Sciences, 1963).

The introductory review by W. R. Savage (Texas Instruments Inc.) covers the general field of the properties of semiconductor surfaces. J. N. Zemel (United States Naval Ordnance Laboratory) describes several fundamental physical measurements used in semiconductor surface research and their connexion with some of the main streams of contemporary work. He mentions that the properties of surface states on only five materials (germanium, silicon, zinc oxide, lead sulphide and indium antimonide) have been studied to any extent. The investigation of a clean semiconductor surface is a difficult and challenging pursuit, because whereas a metal surface with a hundredth of a monolayer is considered acceptably clean for thermionic work, such a density of contamination on a semiconductor would produce, if diffused in 10^{-4} cm, a degenerate layer of width equal to the surface space charge region. It is not surprising, therefore, that he concludes that though there is a great deal known about the surfaces of semiconductors, there is little understanding. Field emission as a tool, and the grain boundary as a system, for the study of surfaces are discussed by F. G. Allen (Bell Telephone Laboratories Inc.) and P. Handler (University of Illinois) respectively. Lifetime and conductivity measurements on clean germanium surfaces are reviewed by Y. Margoninski (Raytheon Research Division) and the pulsed field effect method of investigation of surface states on semiconductors by G. Rupprecht (Tyco Laboratories Inc.).

Several papers deal with aspects of surface chemistry and electrochemistry. M. Green (University of Pennsylvania), in a paper entitled "The Configuration of Complexes on Semiconductor Surfaces", discusses a new approach to the problem of the equilibrium configuration of surface layers which should be useful in problems of chemisorption, film nucleation and related phenomena. The reaction between heated germanium and oxygen, and between water and methyl alcohol is reported on by M. J. Sparnay (Philips Research Laboratories), and H. C. Gatos and M. C. Lavine (Massachusetts Institute of Technology) discuss the process of formation of thermal etch figures on germanium single crystals when heated to temperatures of 700°–925° C in the presence of argon–oxygen mixtures.

An interesting and instructive paper of the series, though it did not produce any comment in the final panel discussion session of the symposium, was that of N. J.

Harrick (Philips Laboratories) on total internal reflexion and its application to surface studies. The absorption of infra-red radiation in semiconductors has been used successfully to study bulk properties of semiconductors, but through the use of multiple total internal reflexion, the space-charge region can be probed and the properties of the carriers in the space charge region can be measured. Because of the special characteristics peculiar to total internal reflexion, both the outside and the inside of the surface can be studied. After a clear description of the process of internal reflexion and of some of the well-known, but nowadays rather neglected, beautiful optical demonstrations of fundamental phenomena associated with internal reflexion, Harrick discusses the application to surface studies. The experimental arrangement and preparation of the samples are described, and details are given of the results of investigations by various workers on chemisorbed molecules, oxide growth and thin films. Finally, various possible experimental studies, using infra-red radiation to obtain information concerning the space charge region on semiconductors are suggested and outlined.

S. WEINTROUB

ELECTRON-PROBE MICROANALYSIS OF ANODIC OXIDE FILMS ON ALUMINIUM ALLOYS

By DR. G. C. WOOD, V. J. J. MARRON and B. W. LAMBERT

Department of Chemical Engineering, Faculty of Technology, University of Manchester

IN view of the successful application of electron-probe microanalysis to the qualitative and quantitative analysis of oxide scales on alloys[1,2], it appeared desirable to examine the possibilities of the technique when applied to the examination of corrosion products obtained in aqueous media. In particular, the field of the formation, transformation and dissolution of anodic films, and indeed of the reactions between all types of semi-conducting films and electrolytes, is extremely important and likely to receive increasing attention. Anodic oxide films on aluminium alloys were chosen for preliminary studies because they are readily reproducible, their general properties are sufficiently well documented to enable comparisons to be made and metallographic preparation for the microanalyser is simple. In addition, it was felt that further useful information about their formation, dyeing, sealing and corrosion properties could be obtained.

Consequently, six small sheet specimens of an aluminium–magnesium alloy (magnesium 5·00, manganese 0·25, copper 0·01, iron 0·24, silicon 0·01, wt. per cent) were chemically polished and anodized at 25 m.amp/cm² in stirred 15 per cent w/v sulphuric acid at 15° C for 60 min. Individual specimens were then subjected to one of the following treatments: (1) dabbed with filter paper to remove surplus surface acid; (2) washed for 1 min in running distilled water; (3) washed for 24 h by rotation in distilled water; (4) sealed in potassium dichromate solution (50 g/l.) at 95° C for 1 h; (5) sealed in a conventional nickel acetate solution (nickel acetate 6 g/l., boric acid 8 g/l.) at 95° C followed by smudge removal with a clean dry rag; (6) sealed in concentrated nickel acetate solution (nickel acetate 60 g/l., boric acid 80 g/l.) at 95° C with subsequent smudge removal.

A further specimen was prepared under similar anodizing conditions at 30° C and washed for 1 min.

The specimens were then set in small electrically conducting mounts and cross-sections prepared metallographically in a conventional manner, finishing on 1μ diamond suspension. Optical examination showed that films formed at 15° C were 45–48μ thick, indicating very little dissolution at the outer surface during formation, whereas the film produced at 30° C was only 30μ thick because a third of it had been completely dissolved. Static probe measurements of aluminium, magnesium and sulphur concentrations, and of nickel and chromium concentrations where appropriate, were made at short intervals in the films along lines of traverse normal to the metal/oxide interface using a Cambridge 'Microscan' microanalyser. The apparent percentages of the elements were determined by comparison with appropriate standards, the results being corrected for background radiation and dead-time but not generally for absorption because of the complex and uncertain nature of the X-ray source in the specimens. Some of the more important trends are illustrated in Figs. 1–3.

The distributions of aluminium across films formed at 15° C and 30° C respectively, followed by washing for 1 min, are compared in Fig. 1. For the former specimen the aluminium concentration is virtually constant with a slight hint of lower values towards the outer interface, although this trend is masked by the scatter. Clearly there is little extra dissolution in the pores or at the outer surface in this case. The aluminium content of the unwashed specimen (not shown on the diagram) was slightly higher, probably due to Al^{3+} ions in the liquid in the pores, but for the other washed and sealed specimens was very similar. On the other hand, the partly dissolved film formed at 30° C shows a pronounced reduction of apparent aluminium content from 30 wt. per cent near the alloy to 11 wt. per cent near the solution, indicating extra dissolution in the pores. These results support the view that the truncated-cone pore model[3] must be largely due to extra dissolution rather than inherent in the mechanism of film growth. The apparent aluminium contents are somewhat low when compared with the figure of 37 per cent aluminium given for a typical anodic film[4]. This is

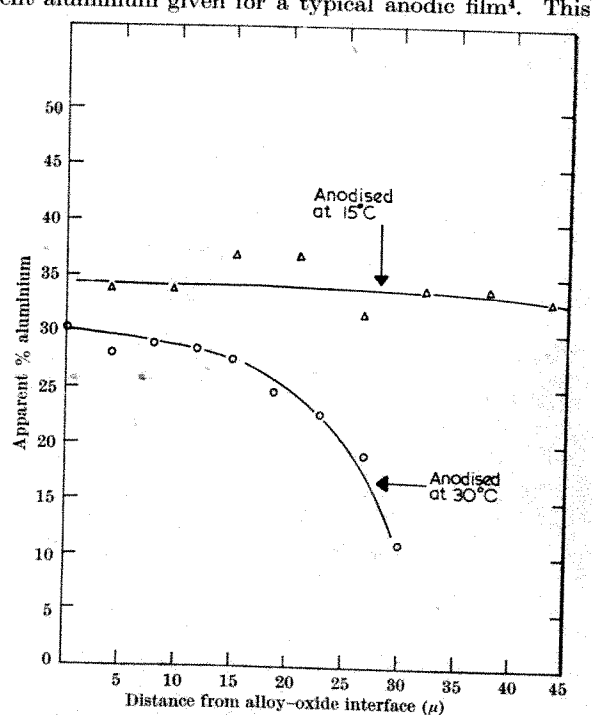


Fig. 1. Influence of anodizing temperature on aluminium distribution.

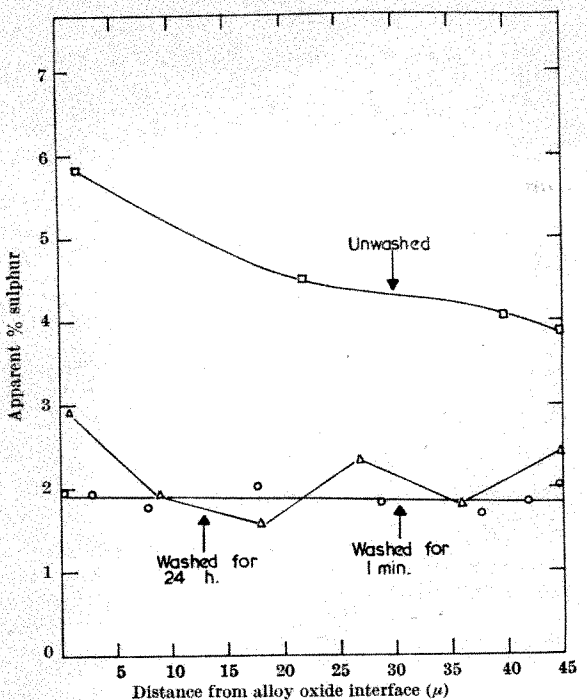


Fig. 2. Influence of post-anodizing treatments on sulphur distribution

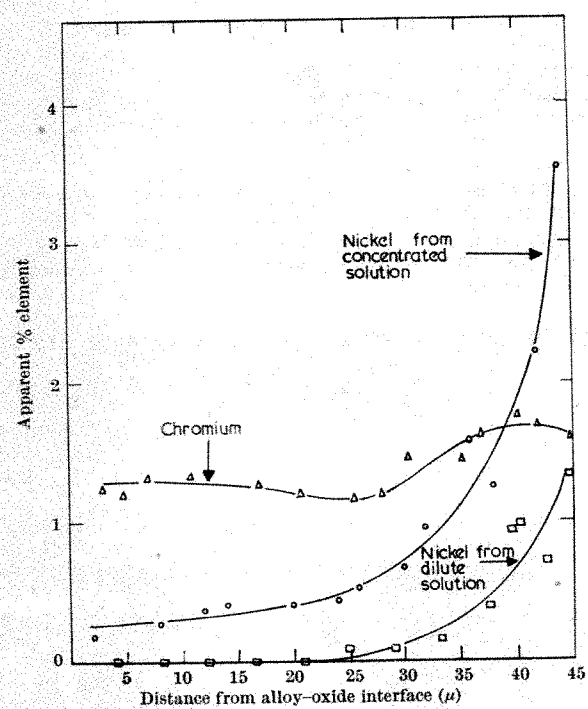


Fig. 3. Distribution of chromium or nickel produced on sealing

partly accounted for by the magnesium in the film, but greater errors are probably incurred due to the uncertain nature of the X-ray source and by absorption effects. A crude estimate of the absorption of aluminium radiation shows that the true figure near the alloy/oxide interface of the 15° C specimen should be nearer 48 per cent aluminium. This figure seems somewhat high and, bearing in mind the uncertainties in the corrections, it appears desirable to quote the measured values only.

Magnesium was detected in the films but with insufficient accuracy to show any trends. The distribution of a heavy alloying element such as copper would be measured easily and accurately.

Fig. 2 illustrates the sulphur distribution in washed and unwashed specimens. The reduction in sulphur content towards the outer interface of the unwashed specimen is almost certainly due to desorption of acid from the pores by the filter paper used for drying or by the mounting material. Washing readily removes more than half the sulphur in the film, and indeed washing for 1 min appears as effective as for 24 h, a point which was made on other evidence elsewhere[5]. The sulphur is readily removed from the pores but not from the bulk of the pore walls. If prolonged washing is really important for some post-treatments, as is sometimes indicated, it must be because of the removal of final traces of acid from microfissures or because of reconditioning of the pore sides. The undulations in sulphur content in the washed specimens are probably not genuine but are due to relief polishing or the difficulty in accurately measuring low concentrations of a light element. The scatter may be very little as for the specimen washed for 1 min, or considerable as for the specimen washed for 24 h. The apparent sulphur contents before and after washing are in good agreement with the literature[6].

The distributions of chromium and nickel obtained on sealing in the appropriate solutions are shown in Fig. 3. There is little variation in the chromium content, the measured values lying between 1·15 and 1·75 per cent. The enrichment near the outer surface is probably genuine due to the slightly greater porosity and readier replenishment of electrolyte in this region, but the slight fall in concentration near the centre of the film is not significant. The chromium concentration is about ten times as great as would be explained by the mere replacement of liquid in the pores by dichromate solution. Clearly, as the metal-containing anions are incorporated into the pore walls, fresh ones enter the pores, as previously demonstrated by Edwards[7]. This view is in harmony with impedance measurements which show that pore closure due to simultaneous hydration is slow in dichromate sealing during the early stages[5].

No nickel was detected in the inner 20μ of the film sealed in dilute nickel acetate solution, but the content then rose gradually and finally sharply to 1·3 wt. per cent near the outer interface. This nickel-rich plug, probably consisting of nickel hydroxide, is a feature often assumed to explain sealing in nickel acetate but has rarely, if ever, been demonstrated in detail. The nickel concentration is insufficient to indicate complete blocking by nickel hydroxide so this is partly accomplished by simultaneous hydration in all regions of the pores[5]. It might be anticipated that in the first few moments of immersion nickel ions succeed in penetrating the pores completely but are not measured in the final analysis due to their low concentration. That this is probably the case is shown by the results for the concentrated solution. Here nickel was detected throughout the film although the plug appeared to occupy about the same distance. The plug is richer in nickel but the nickel content is not proportional to the solution concentration. It should be noted that although the concentrated nickel acetate solution and the dichromate solution were of roughly comparable concentration, there is no comparison in metal contents in the inner regions of the pores. This is due to the more rapid pore closure in the nickel acetate solution preventing extensive replenishment. It was demonstrated that the nickel readings were not affected by the smearing of 'surface smudge' over the section by repetition after prolonged swabbing treatments and by showing that correct aluminium values could also be obtained. Chromium and nickel can be measured with great precision and reproducibility despite their low concentrations because this is a particularly favourable situation where heavy elements are being detected in a matrix of low atomic number.

The implications of the work should be noted. Evidently the microanalyser can provide useful information about the

composition and porosity of anodic films, and about their reaction with corrosive environments, provided they are of suitable thickness. The precision depends on the element being detected and the matrix. Clearly a particularly favourable situation is the determination of heavy elements in alumina, magnesia and silica. Regarding anodic films on aluminium alloys, determination of the aluminium content gives a measure of the porosity and should enable the concept of 'coating ratio' to be placed on a more precise basis. The influence of anodizing variables, alloy and heat treatment should now be investigated in detail. The depth of penetration and concentration of inorganic pigments containing heavy metals and possibly of metal-containing organic dyestuffs may be measured. Observations of the development of the nickel-rich plug during sealing and its subsequent removal during corrosion should throw considerable light on the mechanisms of these processes. The technique may also find some application in the study of hard anodized films and plating over anodic films. Some of these investigations are now in progress.

We thank Prof. T. K. Ross and Prof. F. Morton for providing facilities and for encouraging this work and the Department of Scientific and Industrial Research for the provision of the microanalyser and of a maintenance grant to one of us (V. J. J. M.).

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ELECTRON SPIN RESONANCE OF CRYSTALLINE CHLOROPHYLL *A* AND CRUDE MIXTURES OF CHLOROPHYLL *A* WITH NORMALLY ASSOCIATED PIGMENTS

By A. F. H. ANDERSON and Prof. MELVIN CALVIN, For.Mem.R.S.
Lawrence Radiation Laboratory and Department of Chemistry, University of California, Berkeley

IN a previous communication[1] we described the preparation of a highly purified crystalline chlorophyll *a*, on which we have made some preliminary electron spin resonance observations. The electronic properties of organic solids are known to be dependent on the purity of and order in the crystals. Therefore, we shall compare the simple characteristics of the electron spin resonance signals observed in the crystalline chlorophyll *a* with those obtained from crude mixtures of chlorophyll *a* with other pigments in the form of films.

We have no detailed knowledge of the crystal structure of chlorophyll *a*, but we present two pictures to show the form of the small microcrystals (Fig. 1) and the larger microcrystals (Fig. 2). The small microcrystals which are approximately 20 Å thick are probably two monolayers interacting by their phytyl chains or by the chlorin groups and the larger crystals well ordered composites of the small microcrystals stacked together.

The electron spin resonance was carried out on an iso-octane suspension which contains a distribution of both these crystallites the optical properties of which will be described elsewhere[2]. About 0·25 ml. of a heavy suspension of chlorophyll *a* crystals in iso-octane was smeared on a small quartz plate to give a 1·5 × 0·5 cm thin film of chlorophyll *a* when the iso-octane evaporated off. The crystallinity of the chlorophyll *a* was still intact as shown by X-ray diffractometry. Illumination of the sample was carried out by passing light from a 1,000-W projection lamp through 4 cm of water to remove infrared radiation and then through a Corning 2030 filter which transmits red light from 648 to 750 mμ. The chlorophyll *a* crystals were at the same conditions as the cavity of the spectrometer. The chlorophyll *a* crystals showed a small symmetrical dark signal in the region of $g \sim 2$, with a line width of 15 gauss. Shining red light on the sample induced a symmetrical signal of $g \sim 2$ and similar line width to the dark signal, which took about 15 min to reach a steady-state intensity of about four times that of the dark signal. When the light was turned off there was a slow decay of the signal which after 10 h had not returned to the intensity of the dark signal. The shapes of the electron spin resonance signals in the dark and light are compared in Fig. 3 and the kinetics of the production and decay of the signal in Fig. 4. Two different preparations of chlorophyll *a* showed similar behaviour.

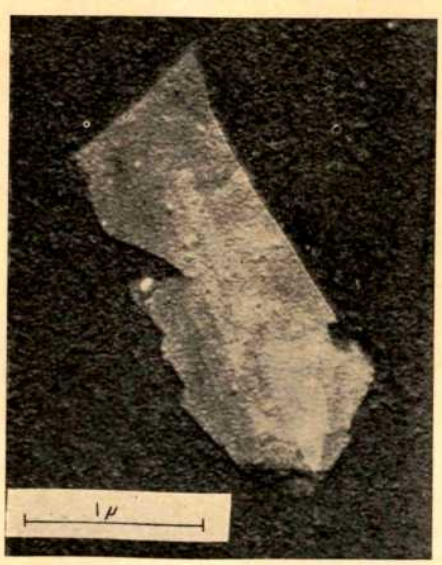


Fig. 1. Electron micrograph of chlorophyll *a* crystals (photograph: Terry Trosper and R. B. Park)

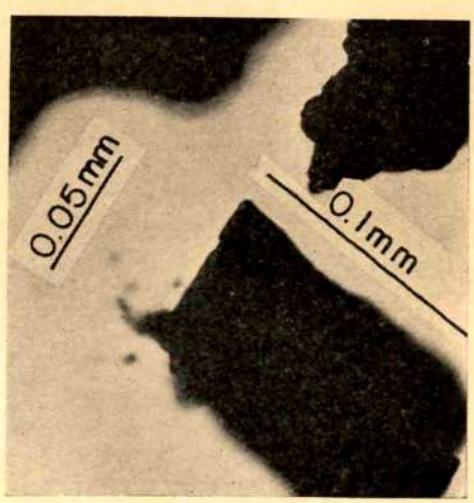


Fig. 2. Optical micrograph of chlorophyll *a* crystals

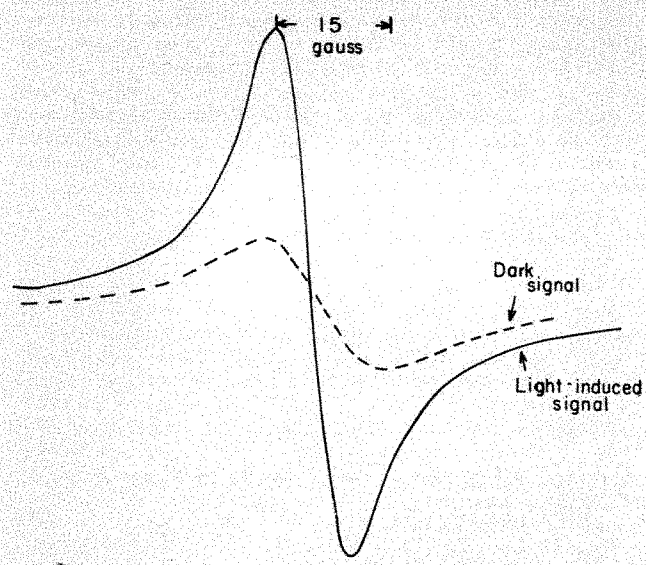


Fig. 3. Electron spin resonance signals in chlorophyll *a* crystals

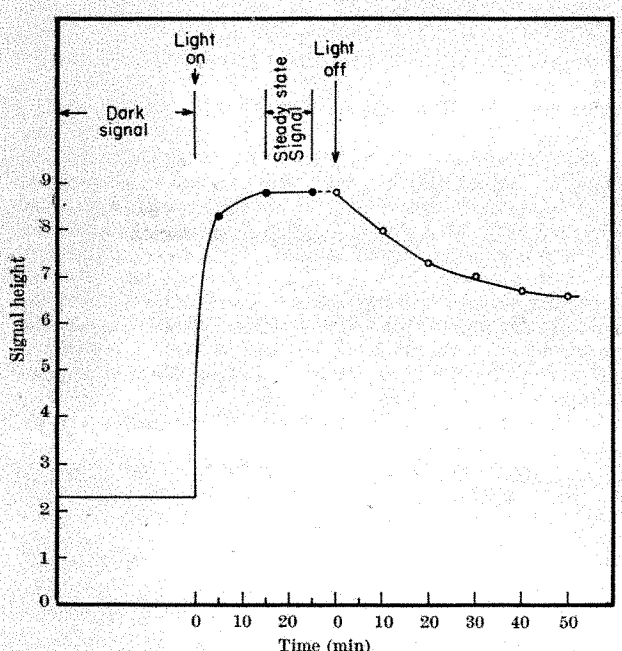


Fig. 4. Time dependence of electron spin resonance signals in chlorophyll *a* crystals

Sogo, Jost and Calvin[3] made preliminary experiments on methanol extracts of *Chlorella* algae which showed that adsorbed vapours (wet air versus dry nitrogen) on the surface of the extract were required for the production of light-induced electron spin resonance signals. We have extended these observations with the following experiment. *Chlorella* algae were washed twice with distilled water and then extracted with 100 per cent methanol at room temperature. The extract was made into samples suitable for testing in the electron spin resonance spectrometer in three ways: (1) the solvent was removed from the extract and the resultant sticky mass of pigments (chlorophyll *a* associated with chlorophyll *b*, carotenoids and quinones) was applied to a silvered copper rod; (2) the solid mass of pigments obtained in (1) was treated with 2 ml. of petroleum ether, which was then evaporated off in a cylindrical (8 mm O.D.) quartz tube to give a 0·5-mm thick and 1·5-cm long film of pigments on the bottom of the tube; (3) a phase separation of the methanol extract was carried out with petroleum ether which took up the chlorophylls and carotenes, leaving the bulk of the xanthophylls in the methanol. The petroleum ether phase was then made into a film as in (2).

Films prepared by method (1) gave only small dark signals when dry, but when treated with water vapour gave white light-induced signals; these were irreproducible in their intensities and decay times but had a constant line width of about 6 gauss.

Films prepared by method (2), where the preparation was carried out in dim light and the film had been degassed

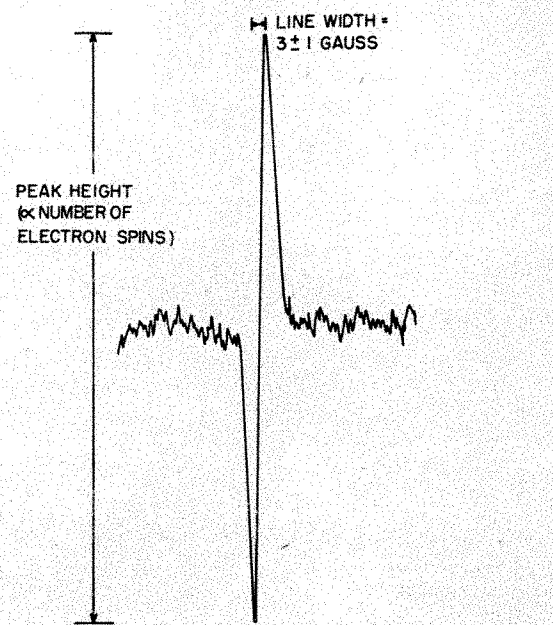


Fig. 5. Typical electron spin resonance signal from plant extract (petroleum ether)

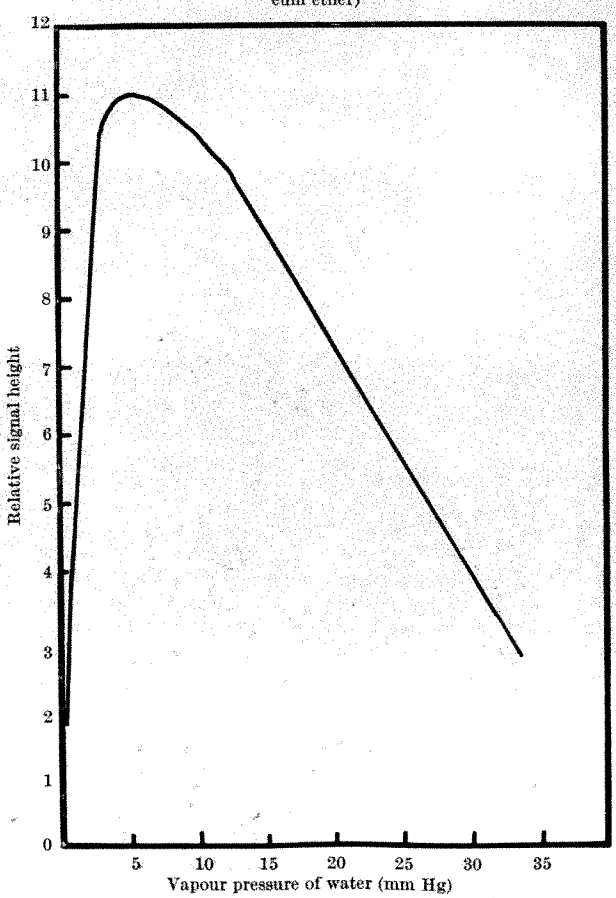


Fig. 6. Concentration of spins in an extract as a function of water vapour pressure

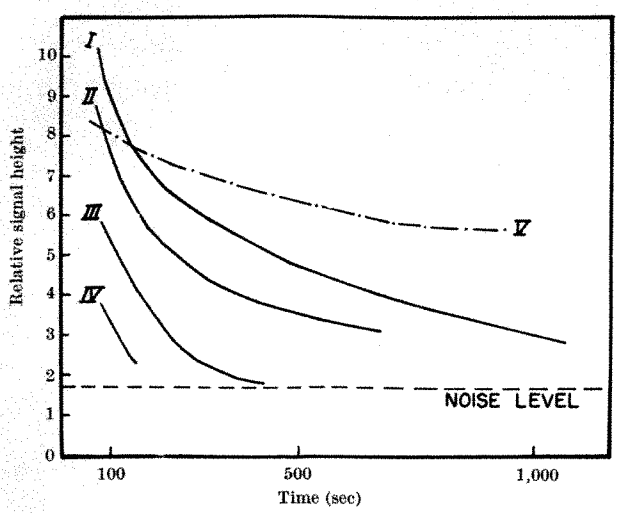


Fig. 7. Rates of decay of spins in an extract at a given water vapour pressure. I, 4·67 mm Hg H_2O; II, 14·5 mm Hg H_2O; III, 19·8 mm Hg H_2O; IV, 31·8 mm Hg H_2O

for 6 h at 10μ, gave small dark signals and white light-induced signals could not be obtained. When the same film was exposed to varying vapour pressures of deoxygenated water vapour, white light electron spin resonance signals were induced. Each addition of water vapour was carried out after the sample had been degassed and shown not to have light-induced signals. Since the line width of the signals remained constant as they decayed from their steady-state value, the concentration of spins was taken to be proportional to the signal height. A typical white light-induced signal is shown in Fig. 5; the steady-state concentration of photo-induced spins as a function of water vapour pressure in Fig. 6; and the rates of decay of the spins at given water vapour pressures shown in Fig. 7. The steady-state population of unpaired spins in the film goes through a maximum when the ambient water vapour pressure is 4·7 mm. For a given method (2) of producing the sample films, the decay rates, as shown by the slope of the curves in Fig. 7, are relatively independent of the different vapour pressures. However, a sample prepared by method (3) and with ambient water vapour pressure equal to 4·7 mm mercury, showed the equilibrium amplitude (value at $t = 0$) and the rate of decay to be markedly different from signals produced by the film (2) as shown by the curve V in Fig. 7. A solution of the extracted pigments in petroleum ether did not give light-induced signals.

It would be presumptuous to come to any definite conclusions from the two experiments described here; but they have clearly demonstrated the importance of two variables in the electron spin resonance properties of chlorophyll *a*. First, that water is necessary for the production of signals from crude chlorophyll *a*, and, secondly, the effect of purity on the signals from chlorophyll *a*. Holmogorov *et al.*[4] have also shown that water is necessary for the production of spins in preparations of chlorophylls. Because we can induce signals in crystalline chlorophyll *a* without the addition of water, we feel that water may be making small crystallites or micelles of chlorophyll *a* in the films which give rise to the signals. A similar conclusion has recently been reached by Terenin[5] to interpret the effect of water vapour on what he reported to be originally crystalline chlorophyll. The possibility that a group of water molecules might act as an electron acceptor[6] is still with us, but should be investigated on samples of chlorophyll *a* the crystallinity of purity of which are established.

The character of the signals we obtained from our crystalline chlorophyll *a* differs from those obtained by Brody *et al.*[7] using crystals prepared by a different procedure. Whereas the shape of the dark- and light-induced signal are the same in our crystalline sample, indicating the presence of one type of electron, those reported by Brody are markedly different, suggesting the presence of an impurity in his preparation (possibly xanthophyll) or different crystallinity. The light-induced signal in our avowedly impure films is much narrower (~ 3 gauss) than that produced in the crystals. These signals in the impure sample more closely resemble those seen by the other workers.

Only investigations on highly pure crystalline forms of chlorophyll *a* can be deemed significant in determining the nature of photo-induced electrons in chlorophyll *a* itself and indeed all its electronic properties.

This work was supported by the U.S. Atomic Energy Commission.

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ELECTRON SPIN RESONANCE IN PHOTODYNAMIC DYES

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MANY organic compounds sensitize biological systems to visible light, a phenomenon known as 'photodynamic action'[1]. The photon energy is absorbed by the photosensitizer and presumably transferred by an unknown mechanism to an organic substrate, which is then destructively oxidized by molecular oxygen. Both light-induced and natural (dark) electron spin resonance signals in organic dyes and photosensitizers have been described[2–11]. The formation of unusual molecules by visible light, especially those with unpaired electrons, may be an important factor in the mechanism of photodynamic action. The properties of the dark signals are also pertinent, for the accepted chemical structures of the compounds investigated contain no unpaired electrons. There is some evidence[12,13], however, that dark signals in several cyanine, triarylmethane and phthalocyanine dyes are caused by impurities probably on the surfaces of crystals.

We have investigated the dark signals in polycrystalline methylene blue, neutral red and acriflavin (acid and neutral), in a single methylene blue crystal and in methylene blue adsorbed on activated alumina, incorporated in gelatin films and taken up on 'Amberlite *IR*-120' ion exchange resin. The effect of visible light on methylene blue was also examined.

The first derivative of electron spin resonance absorption curves was obtained using a simple spectrometer[14,15]. All but one dye were obtained from Allied Chemical Corp. Some methylene blue was obtained from the Hartman Leddon Co. and some from Matheson, Coleman and Bell. Pure 'certified' grade commercial dye was used in the initial examinations on the effects of temperature. Dye recrystallized several times in the dark from aqueous

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† Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

alcoholic solution was used to investigate the effects of temperature, the absorbed state, and light on the signals. An apparently single crystal of methylene blue was grown from aqueous solution. For work on the adsorption of methylene blue on 'Amberlite *IR*-120' ion exchange resin, the resin was purified by washings with sodium hydroxide, hydrochloric acid, and distilled water. Thin sheets of methylene blue incorporated in gelatin were obtained by dissolving the dye in gelatin solution and evaporating it to a film in air. The sheets were illuminated inside the electron spin resonance cavity with light from a 1,000-W tungsten filament lamp.

The polycrystalline dye in electron spin resonance sample tubes immersed in a water bath at 18° C was irradiated by exposure to a 200-W tungsten lamp at a distance of 6 cm. The exposure rate, determined by thermocouple measurements, was roughly 1,500 ergs mm^{-2} sec^{-1} and the effective area of 0·1-g sample about 50 mm^2.

In polycrystalline methylene blue, and also in neutral red, a singlet absorption line with a width (peak-to-peak derivative width) of about 18 gauss and a *g*-value approximately that of diphenylpicrylhydrazyl (DPPH) was observed. The methylene-blue signal remained after two recrystallizations of the dye. The dark signal in neutral red increased markedly on heating, a result also observed by Lagercrantz and Yhland[5] with methylene blue. A small electron spin resonance absorption was seen in polycrystalline acriflavin (hydrochloric acid and neutral). The signals in methylene blue and neutral red corresponded to unpaired spin concentrations of roughly $1{\cdot}2 \times 10^{18}$ and $2{\cdot}2 \times 10^{17}$ spins/*g* respectively, using DPPH as the reference material. The signals did not saturate at the micro-wave power used. The dark signal from the single methylene blue crystal showed no hyperfine splitting, although the amplitude and width of the resonance changed with orientation in the magnetic field. Some of the dark signals, but not those observed in recrystallized methylene blue, may have come from the impurities (roughly 10 per cent) in dyes as commercially prepared.

A normal dark signal appeared when methylene blue was adsorbed on activated alumina and ion exchange resin, or incorporated in gelatin. The purified resin alone showed a small signal. The appearance of the methylene blue dark signal when absorbed on the resin indicated that the cationic part of the methylene blue molecule was responsible for the signal.

The dark signal in polycrystalline methylene blue, heated or cooled in the electron spin resonance cavity, varied inversely with the absolute temperature from 84° to 423° K (Fig. 1). The non-linearity observed in this range is probably caused by changes in dielectric constant or other complications. Extensive departure from linearity was observed above 425° K, which corresponded to the melting and/or decomposition of the material. The separately measured signal at 121° K saturated at a lower micro-wave power than at 298° K. From the approach to $1/T$ dependence predicted by the Curie law, we conclude that the signal is not due to conduction band electrons, which should show no temperature dependence.

Visible light induces a signal in methylene blue adsorbed on fused silica in the absence of oxygen. This signal is quickly and reversibly quenched by oxygen[16]. We have found light induction in three other systems—polycrystalline methylene blue, methylene blue adsorbed on activated alumina and methylene blue incorporated in gelatin—and have shown that induction can occur in oxygen. Previously, light induction in oxygen was not observed, probably because of the dilute dye concentrations used.

For methylene blue adsorbed on activated alumina *in vacuo*, 4 h of illumination increased the dark signal amplitude about 25 per cent; re-admittance of dry oxygen reduced the signal to approximately its initial value. Illumination of a sheet of dyed gelatin in which no dark signal was observed elicited a small signal that saturated after about 10-min exposure, and decayed but slowly.

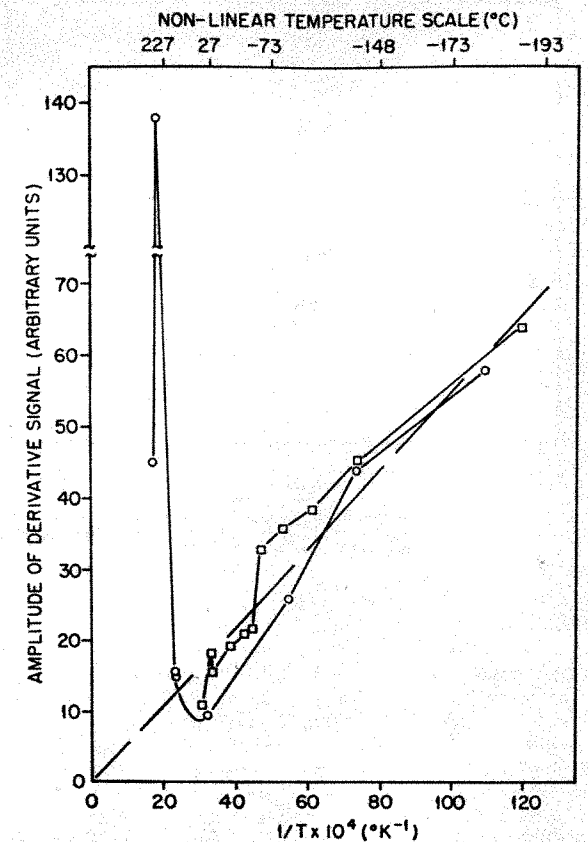


Fig. 1. Plot of polycrystalline methylene blue signal amplitudes for two samples arbitrarily normalized versus reciprocal of absolute temperature ($1/T$). The dashed lines give the $1/T$ dependence predicted by the Curie law

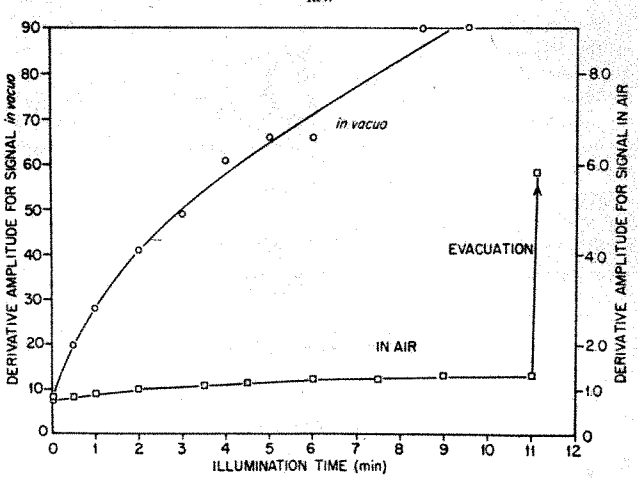


Fig. 2. Illumination of polycrystalline methylene blue in air and *in vacuo*. Note that the scale on the right for methylene blue in air is expanded ten times that on the left for methylene blue *in vacuo*

Evacuation for about 3 h multiplied the signal six times. Illumination for 13 min more *in vacuo* elicited a further increase of approximately 30 per cent. Exposure to dry oxygen produced no immediate change. Examination of separate controls revealed that no signal was produced by illumination of the alumina and gelatin alone in the *ESR* sample tubes. It was incidentally observed that visible illumination of the ion-exchange resin alone produced an increase in the small dark signal.

Results for the illumination of the dark-recrystallized dye in air and *in vacuo* are shown in Fig. 2. Evacuation of the sample illuminated in air produced about the same increase in signal as evacuation of the non-illuminated sample. The sample illuminated *in vacuo* was later

exposed to dry oxygen and no immediate effect noted. It was then kept at 96° C for 40 min in the dark: the signal increased in amplitude and decreased in line width. This heat effect thus overrides any possible annealing-out of the light-induced resonances.

Oxygen present during illumination interacts with methylene blue molecules to inhibit induction of long-lived resonance centres. If added after irradiation *in vacuo*, oxygen interacts with the abnormal molecules and quenches the apparent signal in several systems. The rate of diffusion of oxygen into the material is important for quenching and may explain the lack of immediate quenching in the gelatin and polycrystalline samples. Similar results were obtained by Kholmogorov and Terenin[21] with several triphenylmethane and xanthene dyes. However, a reversed action of oxygen has been observed for the dyes rose bengal, phloxine, and hæmatoporphyrin[7,17,21]. A possible explanation of the latter results has been proposed[21]. Some observed signals, especially in unirradiated dyes, may be those of biradicals since molecular weights are several hundred, and the accepted chemical structures have even numbers of electrons.

Our results suggest that the energy of the excited dye is transferred to oxygen, perhaps first in the photodynamic process, at least with methylene blue as the sensitizer. This conclusion can be fitted into the schemes of Schenck, *et al.*[18], Oster[19], and Kauzmann[20]. Because we have examined only the dye and oxygen in the solid-state rather than in solution with the oxidizable substrate, conclusions based on our results are tentative.

We thank Prof. H. F. Blum, of Princeton University, who suggested the original problem, and Prof. J. Turkevitch, also of Princeton for assistance. The work of one of us (L. P. S.) was supported by a grant from the U.S. National Science Foundation.

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USE OF INTERFERENCE MICROSCOPY FOR THE MINERALOGICAL ANALYSIS OF SAMPLES OF AIRBORNE DUST OBTAINED WITH THE THERMAL PRECIPITATOR

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MOST of the information on the composition of coal-mining dust in the respirable size range has been obtained by chemical or spectrographic analysis of bulk samples obtained by filtration or settling. These methods cannot be applied to thermal precipitator[1] samples because insufficient dust is precipitated. The National Coal Board's Pneumoconiosis Field Research[2] is particularly interested in a method of analysis which may be applied to samples taken with the thermal precipitator, since not only is this instrument used within the Research for the systematic measurement of respirable dust concentrations, but also the technique will allow all the samples taken and stored since the commencement of the Research in 1953 to be analysed. For this reason the possibility of using phase-contrast[3–6] and dark-ground[7] optical techniques was considered; but a method using the interference microscope manufactured by Messrs. C. Baker Instruments, Ltd. (now Vickers Instruments, Ltd.) has been developed and found to be preferable. Although interference microscopy is widely used in biological investigations, it does not appear to have been previously adopted for mineralogical analysis.

Interference microscopy, like phase contrast, depends on the conversion of the invisible phase changes produced by phase-changing objects into amplitude changes, resulting in a visible intensity contrasted image of the object. The Baker interference microscope is adequately described elsewhere[8]. Briefly, the contrast is produced by a double-beam interferometer system built within the microscope and, unlike phase contrast, it does not depend on diffraction by the object. The object modifies the phase relationship between the mutually interfering double waves by an amount dependent on its optical path length. The angular phase difference so produced determines the direction of vibration of the single plane polarized wave into which the double waves are converted. This enables the intensity of the transmitted wave to be controlled with a goniometer analyser.

The mineralogical analysis of thermal precipitator samples is based on the combination of interference microscopy and the well-known dispersion staining phenomenon[9]. The mineral particles are immersed in a highly dispersive fluid thickened with pyroxylin to avoid disturbance of the dust deposit on the cover-glass. If the embedding fluid is more highly dispersive than the mineral and the dispersion curves of fluid and mineral intersect, coloured images are formed in white light by interference microscopy. The order of interference is determined by the phase relationship between the double beams which, in these experiments, was adjusted (by inclusion of a unit retardation plate with the standard quarter wave-length birefringent compensating plate) to give colours similar to those normally experienced with phase contrast and dark-ground techniques. The colours themselves do not depend on the absolute values of refractive indices but mainly on the relative slopes of the dispersion curves of fluid and mineral and on their point of intersection, provided that the particles are not too thick. They may be varied by orientation of the analyser. This may be set to reduce the intensity of those wave-lengths producing either positive or negative angular phase differences between the fluid and mineral particles or for zero phase difference (that is, at the point of intersection of the dispersion curves); the angular phase difference, θ, is given by:

$$\theta = \frac{t\ (Nf - Np)\ 360}{\lambda}$$

where t is particle thickness, Nf and Np the respective refractive indices of fluid and particle and λ the wave-length of the illuminating light.

Fig. 1 represents the dispersion curves of quartz, kaolinite and muscovite in a fluid composed of orthophthalic acid dibutyl-ester and cinnamic aldehyde ($Nd = 1{\cdot}558$). In the range of wave-lengths where $Np > Nf$ the light is retarded by the particle and its intensity may be reduced by crossing the analyser for negative phase differences to produce positive contrast colours. The proportions of light of these wave-lengths in the particle image depend on the slope and point of intersection of the dispersion curves. Thus, for equal refraction at the red end (for example, quartz), the longer wave-lengths, where $Np > Nf$, are eliminated or reduced most and the blue-yellow wave-lengths, where $Nf > Np$, least. The result is a pale-blue particle. For equal refraction towards the violet end (for example, muscovite), light of all visible wave-lengths is reduced in intensity, with violet being reduced least, giving a dark blue particle. The opposite applies if the analyser is crossed to reduce the intensity of the wave-lengths causing positive angular phase differences (that is, $Nf > Np$), yellow or red negative contrast colours then being obtained, due to the greater reduction in intensity of the blue or blue-yellow wave-lengths transmitted by the particle. Similarly, by setting the analyser for zero phase difference (that is, $Nf = Np$), a third series of colours is obtained on a dark background, the colours depending on the wave-length at which the dispersion curves intersect. The colours produced by quartz, kaolinite and muscovite in the foregoing fluid are shown for each contrast position in Table 1. Other silicates such as montmorillonite and illite give similar colours to muscovite and, generally speaking, cannot be optically distinguished from it. The presence of carbonates, pyrites and coal particles does not interfere with the analysis.

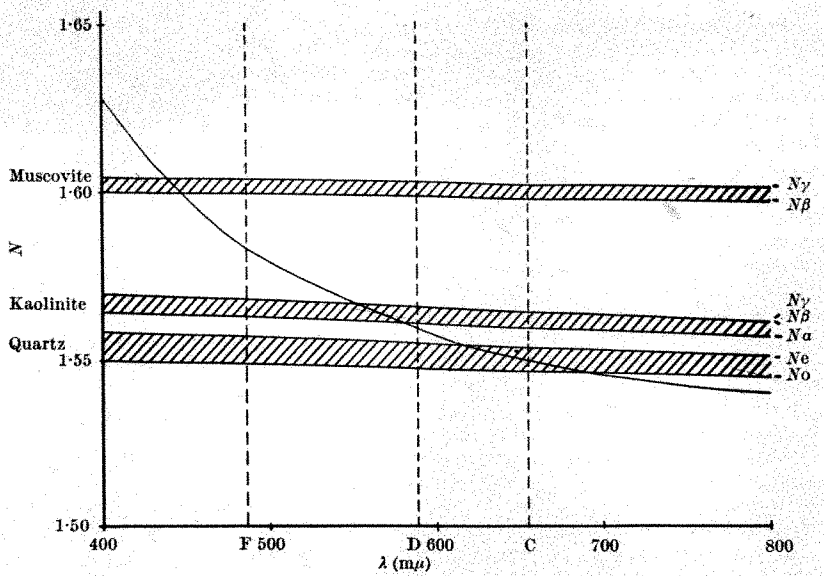


Fig. 1

Size analysis down to 1μ is possible if a 4-mm 'double focus' $N.A.$ 0·7 objective is used together with a low-voltage tungsten filament lamp, such as the Baker 'Intense Lamp', adjusted for Köhler illumination. A binocular attachment fitted with × 10 eyepieces ensures comfortable viewing conditions.

Table 1

Mineral		Interference colours when analyser set for :		
		Positive contrast	Central dark ground	Negative contrast
Quartz		Pale to light blue	Clear blue	Dark red
Kaolinite		Royal blue	Red-orange	Pale orange-yellow
Muscovite	Orientation of basal pinacoid parallel to support	Dark blue-grey	Yellow-orange	Yellow
	Orientation of basal pinacoid normal to support	Dark blue-grey to light blue	Blue to orange-red	Orange-yellow

Table 2

Sample No.	Sub-sample	Interference microscope (% by vol.)				Nominal composition (% by wt.)			Analysis (% quartz by wt.)	
		Quartz	Muscovite	Kaolinite	Other mineral	Quartz	Muscovite	Kaolinite	Chemical	X-ray diffraction*
X1072	1	96	< 1	< 1	3					
	2	98	< 1	< 1	1					
	Mean	97	< 1	< 1	2	100			90	84
X1876	1	< 1	0	93	7					
	2	2	0	89	9					
	Mean	1	0	91	8			100	4	1
X2431	1	< 1	98	0	2					
	2	< 1	99	0	1					
	Mean	< 1	98	0	1		100		5	0
X2043	1	58	6	33	2					
	2	61	6	31	2					
	Mean	60	6	32	2	67		33	60	56 (66)
X2479	1	7	84	8	1					
	2	8	76	14	1					
	Mean	8	80	11	1	14	61	25	14	12 (15)
X2480	1	32	56	10	2					
	2	33	53	13	2					
	Mean	32	54	12	2	48	37	15	44	39 (47)

* Figures in brackets are corrected mean values with Sample No. X1072 taken = 100 per cent.

The effect of thickness has been investigated empirically by the examination of pure mineral samples. Increasing thickness in particles of projected diameter of 1–20μ merely results in increased contrast and depth of colour, but the basic colour does not alter. Particles exceeding about 20μ projected diameter may show reversal of contrast due to the angular phase difference exceeding 180°. Changes in colour occur, but these are not sudden since only certain wave-lengths are affected at first. It is often possible to identify a particle of 50μ projected diameter; but, to reduce the risk of error, the method should be limited to particles of less than 20μ projected diameter.

Birefringence is unimportant in the case of quartz and kaolinite. Reversal of contrast may occur with small mineral particles of strong birefringence such as muscovite orientated with the basal pinacoid normal to the support. The effect is easily noticed and may be controlled to some extent by slight rotation of the analyser.

The most important advantage of interference microscopy is the ease with which one may switch from one contrast position to the other. This provides the equivalent facility to the use of both positive and negative phase contrast and dark-field microscopy, and is particularly useful where subjective assessment of colour difference is involved. Furthermore, the intensity of the interference colours in each contrast position may be controlled by the analyser, a valuable asset when studying particles of varying size. The design of the instrument permits use of the full aperture of the substage illuminator and so avoids the disturbing 'halo' or diffraction effects common to phase-contrast and dark-ground microscopy.

The assessment of the reliability of the method is made difficult by the need to compare number count data with the more usual mass units. Without accurate knowledge of the shape-density factor variations between different minerals, comparison of optical analytical methods with chemical or spectrographic analysis of similar samples cannot be very satisfactory. Nevertheless, mixed samples of 'pure' quartz, kaolinite and muscovite of known composition by weight, supplied by the Safety in Mines Research Establishment, and previously used by them to determine the accuracy of quartz evaluations of respirable dust[10], have been analysed for this purpose. The results are shown in Table 2, where the number/size counts obtained by the interference microscope have been converted to percentage volume, assuming no difference in shape-density factors between the minerals. The agreement is considered to be reasonably good, bearing in mind the limitation of this kind of comparison. It is worthy of note that the presence of an appreciable quantity of muscovite (samples *X*2479 and *X*2480) seems to depress the estimates of quartz and kaolinite. This suggests that the shape-density factor of muscovite may be materially different from those of quartz and kaolinite.

I thank Mr. F. N. Smith of Messrs. Baker Instruments, Ltd. for advice, and Dr. G. Nagelschmidt of the Safety in Mines Research Establishment for providing the mineral samples.

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MESOMORPHIC AND CRYSTALLINE STATES IN POLYVINYL CHLORIDE BY X-RAY DIFFRACTION

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THE presence of a large fraction of molecular chains in mesomorphic states inside stretched fibres of polyvinyl chloride, as for other polymers, increases the difficulties, always great, to determine within reasonable error the amount of the crystalline fraction by X-ray analysis. It is interesting, therefore, also in order to simplify these determinations and to make the results more reliable, to collect information on the molecular arrangement inside the non-crystalline fraction, the determination of the chain arrangement inside the crystallites having already been carried out[1].

The present analysis is based on the behaviour of the crystallites and of the free chains, that is, the chains not ordered in a three-dimensional network, during the shrinkage of previously oriented samples of polyvinyl chloride.

The behaviour was followed experimentally on the X-ray diffraction patterns from a completely oriented sample *A*, and from samples *B*, *C*, *D*, which were obtained by progressive shrinkages of *A*.

The typical diffraction spectra obtained (with copper $K\alpha$-radiation) from non-oriented samples of polyvinyl chloride (mean mol. wt. about 60,000; Montecatini production) are shown in Fig. 1*a*, for a completely amorphous sample—or at least with crystalline amount that should not exceed a few unit percentage, on the basis of its chemical preparation—and in Fig. 1*b* for a sample with rather large value of crystallinity (about 20–30 per cent in weight).

Two maxima appear in Fig. 1*a* which are usually indicated as amorphous diffraction[1]. The external maximum, due to a close arrangement of groups of atoms at Van der Waals distances, is at 3·6 Å, near the amorphous maximum present in most of the polymers at about 4·0 Å (ref. 2)—and the internal maximum, due to a free chain arrangement in which larger distances are preferred, is at 5·0 Å. In Fig. 1*b* considerable contributions of crystalline reflexions are present also in the peak at 3·6 Å.

Quite different spectra are obtained in the case of very strong orientations of free chains and crystallites along the fibre axis. The X-ray photograph from a 500 per cent stretched fibre (sample *A*) is given in Fig. 2*a* and the intensity on its meridional radius is shown by the photometer plot in Fig. 1*c*. The maximum at 5·0 Å is completely absent as are the equatorial (200) and (110) reflexions, while an external maximum appears centred at 3·8 Å. This value does not depend on any reasonable choice for the subtraction of the background. Since fibre is strongly oriented, no polar crystalline reflexions of this spacing may contribute on the basis of the assumed structure[1].

On the contrary, the photometer plot along the equator (Fig. 1*d*) shows strong reflexions (200) at 5·20 Å, (110) at 4·72 Å and (210) at 3·70 Å, besides others.

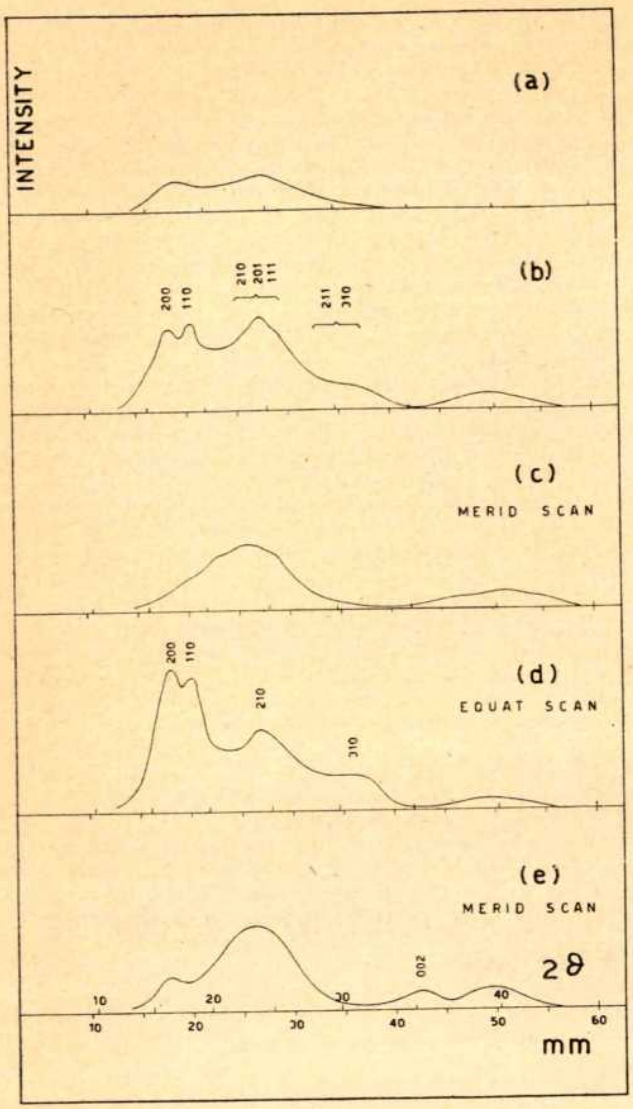


Fig. 1. X-ray diffraction spectra for samples of polyvinyl chloride. *a*, Geiger registration by amorphous polyvinyl chloride (ref. 1). The used angular scale is linear in tan 2θ. The intensity scale of (*a*) is not the same arbitrary scale of (*b*), (*c*), (*d*), (*e*), obtained by photometric registrations of films. *b*, Not-oriented crystalline sample. *c*, and *d*, 500 per cent stretched fibre (sample *A*). *e*, Shrunk fibre previously stretched (sample *C*)

A peculiarity of this oriented sample is the value I_{200} of the apparent intensity of the (200) reflexion, which is systematically larger than the corresponding value of I_{110}. This situation disagrees with the theoretical value $\frac{I_{200}}{I_{110}} = 0{\cdot}20$, and differs from that in Fig. 1*b* characteristic of non-oriented samples.

Interesting information can be obtained by analysing the X-ray patterns of fibres which are all initially stretched like sample *A* and afterwards shrunk in water for a fixed time at different temperatures T_s above the glassy transition point T_g. For $T_s = 85°$ C (sample *B*; reduction in sample length 31 per cent, Fig. 2*b*) the crystalline reflexions show an almost unchanged orientation, compared with Fig. 2*a*, and the equatorial arc (200) appears with the same length of the arc (110). Besides these strong reflexions a new complete ring, sharp enough to be compared with a crystalline reflexion, becomes clearly observable with a maximum of intensity at $5{\cdot}4_6$ Å (Fig. 2*b*). Sharpness, position and intensity of this ring were carefully evaluated by means of many radial scannings, each forming a different angle χ with the film polar radius. The intensity I_M of the maximum observed on these scannings between 4·0 and 6·0 Å (always taken as unresolved) versus increasing values of χ is shown in Fig. 3; the estimated position of this unresolved maximum is also indicated.

A remarkable fact revealed by these scannings is the sudden shift of the position of I_M from 5·4 to 5·0 Å at $\chi \simeq 40°$ and from 5·0 back to 5·4 Å at $\chi \simeq 140°$. As shown by Fig. 3, I_M is maximum on the equatorial radius ($\chi = 90°$) and decreases until χ reaches the limiting values of the interval 40°–140°. Outside this interval, I_M appears to be constant.

Since the high orientation of the crystallites, the main contribution to the intensity I_M for $40° \leqslant \chi \leqslant 140°$ certainly comes from the reflexions (200) and (110), even if some amorphous diffraction should be considered.

Other noteworthy facts that emerge from the analysis of shrunk samples are that for any value of $T_s > T_g$, the apparent value measured along the equator of I_{200} becomes smaller than I_{110}, and the arc-length of the (200) reflexion remains equal to the (110) arc-length at any stage of the disorientation, with unchanged spacings as in Figs. 1*b* and *d*.

Furthermore, the crystallite disorientation increases with T_s so that the reflexion condition $\varphi > \theta_{002}$ for (002) may be satisfied by many crystallites if T_s is large enough ($\theta_{002} = 17°\ 28'$ is the Bragg angle of this reflexion and φ the angle between the crystallite *c* axis and the fibre axis). For $T_s = 107°$ C (sample *C*: reduction in length 55 per cent) the reflexion (002), not yet observable for the sample *B*, reaches the maximum value of intensity. It appears as a polar arc, beside the maximum at 3·8 Å, the ring at 5·4 Å and other crystalline reflexions which are still oriented. The meridional scannings of the X-ray photograph obtained by sample *C* is shown in Fig. 1*e*.

For larger values of T_s the intensity of (002) decreases again. For a completely disoriented fibre (sample *D*, obtained by shrinkage at 150° with recovery of the sample length before stretching) the intensity is distributed on a complete ring and this reflexion is no longer detectable.

From the previous review of experimental data, it follows that the main points to be explained are: (1) the appearance above T_g of the continuous ring with spacing 5·4 Å; (2) the reasons, in terms of molecular arrangements, of the position of this ring, different from the maximum at 5·0 Å of Fig. 1*a*, and different with some evidence from the position of any classified crystalline reflexion from polyvinyl chloride[1,3].

By comparison of Fig. 1*a* with Fig. 1*c* it can be concluded that the chain arrangement responsible for the internal ring in Fig. 1*a* may be oriented like a crystalline arrangement. In order to explain the appearance of the complete ring at 5·4 Å for T_s immediately above T_g

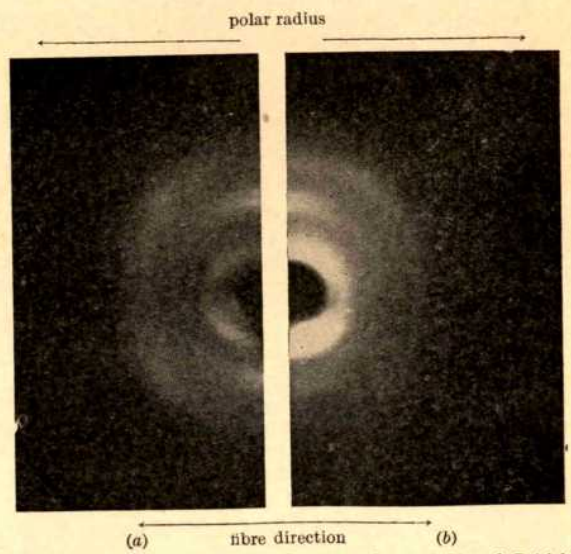


Fig. 2. X-ray patterns obtained from samples *A* (left) and *B* (right)

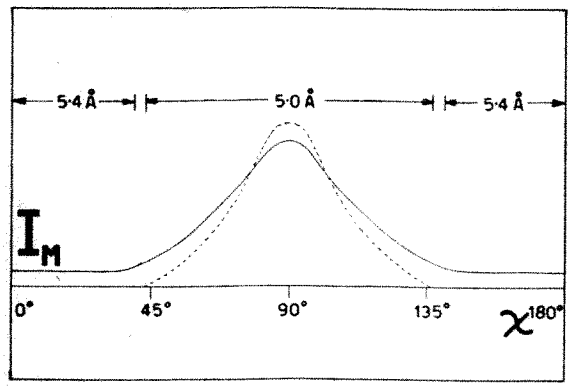


Fig. 3. Largest intensity value I_M for the unresolved maximum between 6·0 and 4·0 Å as function of χ. The spacings corresponding to the Bragg angles of I_M are also indicated. – – – spectrum obtained by sample *A*; —— by sample *B*

(sample *B*), a first picture could be based on a non-random disorientation of the crystallites of smallest dimensions apparently concerning only the (200) reflexion. If this is the case, one should assume, of course, a variation of the network spacing of 5·2–5·4 Å, due to very strong stresses or leaks in regularity of the polymer chains and tolerated by the network through dilatation of the cell.

A variation of 0·2 Å in the (200) spacing appears to be of the same order of magnitude as the differences in the experimental determinations reported in the literature[1,3]—also the value 5·39 Å instead of the theoretical value d_{200} = 5·20 Å has been observed[1]—but it appears extremely large if compared with the observed cell variations in other polymers; for example, not larger than 0·06 Å in polyethylene[4]. This variation in spacing, not dependent on any reasonable choice for background subtraction, must be considered particularly significant in our case, because it is also observed on the same X-ray photograph through different radial scannings. On the other hand, it largely exceeds any statistical deviation of the experimental determinations on many samples. Moreover, since the arc-length of the (200) and (110) reflexions from large-size crystallites are observed to increase both of the same amount by increasing T_s, one would also expect accordingly to observe in sample *B* (Fig. 2*b*) a second complete ring corresponding to the (110) reflexion.

To explain the absence of this ring, it should therefore be assumed that the modalities of this non-random disorientation vary with the size of crystallites.

It is worth noting that while the absence in sample *B* of a (002) reflexion from disoriented—small size—crystallites may be justified in the same way as for sample *D*, the same explanation cannot hold for the (110) reflexion.

We believe that a different explanation must be given to the appearance of the ring at $5{\cdot}4_0$ Å in the first stage of disorientation, avoiding the assumptions already mentioned.

It may be observed that the sudden shift of the position of the maximum intensity I_M at $\chi = 40°$ and at $\chi = 140°$ (Fig. 3) allows us to classify the oriented chains in the stretched fibre in two different groups. One group belongs to crystallites, with spacings independent of the orientation. These, even above T_g, become disoriented only with difficulty, thereby showing relevant friction forces associated with their displacement. A second group, having a very ordered arrangement, as the polar spectrum in Fig. 1*c* shows, becomes disoriented with the same facility that should characterize the oriented free chains which are certainly present in the fibre.

We classify these chains as being in a mesomorphic (nematic) state. The position of the ring at 5·4 Å can now be easily justified. In fact, a variation in the preferential spacing of mesomorphic chains with the state of the sample—for example, 5·0–5·4 Å as may be deduced from the comparison of the spectrum given by *B* or *C* with the one in Fig. 1*a*—becomes quite acceptable, since the intensity of the mean field of the forces acting on them is certainly less than the intensity of the crystalline field.

In the projection on the *ab* plane of the crystal cell, each chain appears[1] to be surrounded by four first nearest neighbours at a distance of 5·3 Å. It is therefore justifiable to hold that for iso-oriented chains not ordered in a three-dimensional network, the preferential distance is larger than 5·3 Å, with a small dispersion because of the strong C—Cl dipole–dipole interaction. This explains the rather sharp definition of the observed ring at $5{\cdot}4_0$ Å.

A second way to account for the differences between the position of this non-crystalline ring and the internal maximum in Fig. 1*a* is to consider an appreciable contribution in the last one of the (200) and (110) reflexions, both described by a very spread-out gaussian and superposed with an amorphous maximum, placed at 5·4 Å for any sample. In the last case, one should then admit even for the maximum at 3·6 Å in Fig. 1*a* an appreciable contribution of the crystalline reflexions (for example, 210, 201, 111), and the real position of the external amorphous maximum could therefore lie at a smaller value of θ. As required by the meridional scannings of the X-ray photographs (Figs. 1*c* and *e*) the correct position is 3·8 Å. This position is closer to diffraction maximum shown by the liquid monomer, which we have observed at 4·17 Å. An external amorphous maximum with near the same position of the corresponding liquid monomer maximum is in agreement with the known results of Katz concerning many different polymers[2]. From all the analysed spectra, this maximum does not show any orientation with fibre stretching. This interpretation of the spectrum in Fig. 1*a* that we consider the most reasonable requires, however, the presence of a crystalline percentage of about 10 per cent in the corresponding sample.

Experimental results so far obtained, completed by the shrunken samples technique reported here, does not give sufficient information to define with less uncertainty the chain arrangement inside polyvinyl chloride fibres. Improvements could perhaps be obtained by applying different techniques, like nuclear resonance or neutron diffraction by deuterated samples.

With the choice of the internal amorphous maximum at $5{\cdot}4_0$ Å, accurate measurements of variations in the crystalline amount for oriented unshrunk fibres are possible. On the basis both of this choice and of the assumed peak symmetry, one can separate the (200) and the (110) reflexions from the maximum due to the free chains without an extraordinary number of assumptions. By using the integral intensities of these reflexions, measured on the photometer equatorial plot, the variations of the quantity:

$$\frac{I_{200} + I_{110}}{I_{\text{mes}} + I_{200} + I_{110}}$$

give with good approximation the numerical values for the variations in crystalline fraction weight.

We have applied this method to fibres in which the crystalline fraction was increased by ultrasonic radiation (1 Mhz), keeping the orientation unaltered to the same degree for all the samples[5]. The variations determined in the crystalline fraction (even for a few unit per cent) were in agreement with the behaviour of the physical properties (for example, the values of breaking load, swelling, etc.) as required by well-known empirical relations[6].

Of course, the determination of absolute values needs a 'calibration' by means of a sample of known composition.

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AN INDICATION OF UNDERWATER SOUND PRODUCTION BY SQUID

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IN recent years there have been many investigations of underwater sound production by marine animals and of the role of such sounds in animal behaviour. Most of this work has been focused on fish and mammals, and to a lesser extent on invertebrates.

The purpose of this communication is to present additional evidence of underwater sound production by marine squids. Several authors[1-3] have reported sound production by squids which were out of the water, but Nishimura[4] was the first to report on sounds produced underwater by squids. These sounds were recorded near trap nets in coastal waters off Atami City, Japan, and were called 'swimming noises' by Nishimura.

The underwater sounds reported here were tape-recorded aboard the U.S. Bureau of Commercial Fisheries research vessel *Hugh M. Smith* on November 3, 1958, at a night-light station (2130–2230 h) at position 2° 02′ N., 150° 07′ W. There was no wind, the sea surface was completely calm except for an occasional gentle swell, and the moon was not shining. A 200-W light was submerged 60 cm below the sea surface. A directional microphone mounted in a waterproof metal container was attached to the end of an 8-ft. aluminium handle. The microphone had a frequency response flat of approximately 300–5,000 c/s and an impedance of 25–50 ohms; other details are unknown. The observer manipulated the handle to train the microphone on squids in the water. The microphone housing was submerged about 1 m. Wiring from the microphone passed through a watertight connexion linking the aluminium handle and the microphone housing, up the hollow handle, and to a 'Magnecord *PT6–BN*' recorder operating at a tape speed of 7·5 in/sec. Comments by an observer were included on the same tape. The recorded sounds were graphically portrayed by a Kay 'Vibralyzer' sound spectrograph. Fig. 1 shows the entire frequency spectrum recorded. The recording shown in Fig. 2 was first passed through an Allison model 2*A* band-pass filter, which eliminated frequencies below 300 and above 2,400 c/s. No attempt was made to measure loudness of the sounds when originally recorded.

The squids, in groups of 10–20 individuals, swam rapidly in and out of the area illuminated by the night-light. Individual squids approached within 1 m of the microphone, and groups often passed beneath the microphone at a depth of 3–5 m. Squids of three species were captured by dipnet during the night-light station: one *Thysanotheuthis rhombus* Troschel—dorsal mantle-length 154 mm; one *Symplectoteuthis oualaniensis* (Lesson)—dorsal mantle-length 151 mm; and three *Onychoteuthis banksii* (Leach)—dorsal mantle-lengths 44 mm, 45 mm, and 65 mm. Squids observed near the microphone during sound recording were estimated to be about 150 mm in dorsal mantle length and were probably either *T. rhombus* or *S. oualaniensis*. Other animals, such as myctophids and flying fish, were also attracted to the night-light, but the recorded sounds were not noted when they were nearby.

The recorded sound burst shown in Fig. 2 was approximately 1·2 sec in duration and included 33 individual sound pulses. Time-intervals between pulses increased from 31 to 48 msec during the sound burst. The number of pulses ranged from 28 to 47 in six sound bursts analysed in this manner. To the human ear, sounds of the squids were a faint, rapid popping sound. Bartsch[3] also reported hearing popping sounds produced by squids, but by squids the speed of which had carried them clear of the water.

The recorded sound energy was concentrated above 1,500 c/s (Fig. 2), but since the darkness of the spectrogram reflects the 'Vibralyzer' dial settings, comparison of the intensities is only relative. The lower frequencies of the pulses reach 500 c/s; but here they are largely masked by what is probably machinery noise from the vessel, centred at 500 c/s. Sounds of squids recorded by Nishimura[4], analysed with a panoramic sound analyser, contained frequencies of 1,000–4,000 c/s. He recorded peak intensities at approximately 1,500 c/s, with the intensities dropping sharply above 1,500 c/s.

Voice comments recorded concurrently with the underwater sounds indicate that in the majority of cases squids were near the microphone when the sounds in question were recorded. For example, during one 30-min recording period, the observer noted the presence of squids near the microphone in 10 of 16 instances when sounds were recorded. The sounds were not always picked up even while squids were near the microphone. This suggests that sounds were produced sporadically by individuals, perhaps incidentally to abrupt changes in speed or to radical manœuvres.

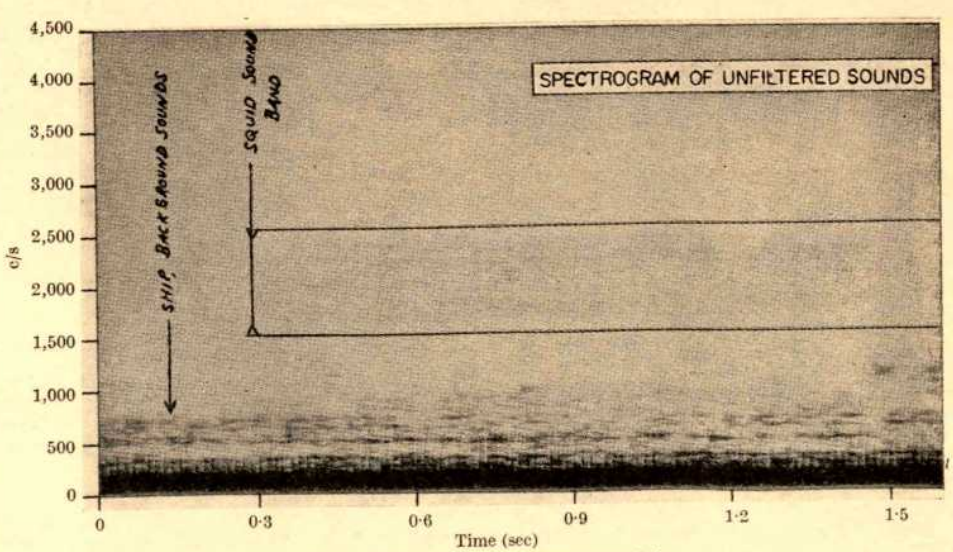


Fig. 1. Entire frequency spectrum recorded

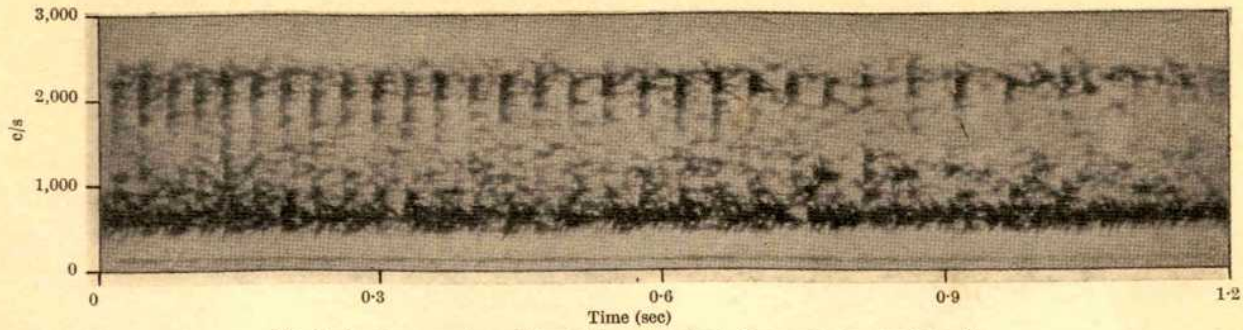


Fig. 2. Spectrogram of sound burst thought to have been made by oceanic squid

The following hypothesis is offered to explain how these sounds are produced. The sounds may result from fluttering of the thin external lips of a squid's siphon while water is being ejected through the siphon from the mantle cavity. When the expulsion (or ejection) of water begins, pressure in the siphon would be greatest and the lips would flutter more rapidly than at the end of the ejection, when the water pressure in the siphon would be lower. This would account for the increasing interval between sound pulses in Fig. 1. The process is analogous to that which occurs when a small rubber balloon is filled with air and then released. As the air escapes, the neck and lips of the balloon flutter and a series of sound pulses are produced, decreasing in frequency as the air pressure within the balloon decreases.

The foregoing hypothesis was suggested because of the lengthening intervals between pulses towards the end of the sound burst. It is possible, however, that the bursts are some other swimming sound of the squids. In this regard, Figs. 1 and 2 are similar to a spectrogram recorded by Moulton (reported in ref. 5) of the swimming sounds of several million anchovies (*Anchoviella choerostoma*). This spectrogram also depicts short pulses of sound as vertical bars, but the bars are more irregularly spaced and show frequencies approaching 5,000 c/s.

The hard beaks of the squids should also be mentioned as possible sound producers. On one occasion while squids were nearby, a faint snap was heard instead of the usual popping sound.

We thank Dr. William N. Tavolga for advice.

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CORRELATION OF WIDELY SPACED MAGNETIC PROFILES IN OCEANIC AREAS

By Dr. D. H. GRIFFITHS and R. P. RIDDIHOUGH
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DURING the interpretation of total field magnetometer observations from the Scotia Sea we were concerned with utilizing to the full the information from sets of more or less parallel traverses several hundred miles long, separated by distances of as much as 50 miles. Such separations clearly precluded the correlation of individual magnetic features between tracks, but visual inspection suggested that in places correlation on the basis of the general magnetic character of sections of traverse might be possible. A method was therefore devised by means of which it has been found possible to divide up traverses into sections of different character. In general these sections can be correlated from traverse to traverse, thus enabling a map of the region to be built up which it is believed may have some geological significance.

The regional gradient was removed and the choice made of magnetic features suitable for measurement. These were usually well-defined features with a total amplitude of greater than about 40γ, and of horizontal distance between positive and negative peaks of less than 16 miles. Three measurements were made on each feature. These are shown in Fig. 1. u is the peak to peak amplitude, v, half the horizontal distance between the points of half-maximum gradient, and w, the horizontal distance from the positive peak to the curve taken at half the vertical distance between peaks. The traverses to be discussed in detail have a north–south direction, and the measurements of v and w were always made on the south side of the positive peak.

For each traverse three separate graphs are drawn showing the variation of u, v and w with distance, and two others of the changes $\delta u/\delta v$ and $\delta w/\delta v$ with distance, where δu, δv and δw are the differences along the tracks between consecutive values of u, v and w. The use of a third possible relationship, the variation of $\delta u/\delta w$ with distance, has been found to be superfluous as in practice it provides no additional information of value.

The division of the traverses into sections is carried out by inspection in the following way. Over certain lengths of track there is an obvious similarity in the variation of the three indices; over other lengths only two indices may show any visible correlation, and there exist also stretches where all three are apparently unrelated. The points at which changes in the nature of these mutual relationships occur are taken as boundaries. Although most of the main divisions could be made by careful inspection of the graphs of u, v and w, the two subsidiary graphs are used to assist their recognition and make the process more objective.

The division into groups having been visually established as described, the probability levels of the correlation coefficients r_{uv}, r_{vw} and r_{uw} for each section of track are determined. These levels are used to classify the sections into the categories listed in Table 1.

The various traverses, plotted in their proper positions and divided into sections, are now inspected. Where appropriate, the boundaries are extended from track to track, thus defining regions of different magnetic type.

It must be emphasized that this method does not depend on the various correlation coefficients reaching a high level of significance. Probability levels merely serve to define magnetic types, and it is the various types which are

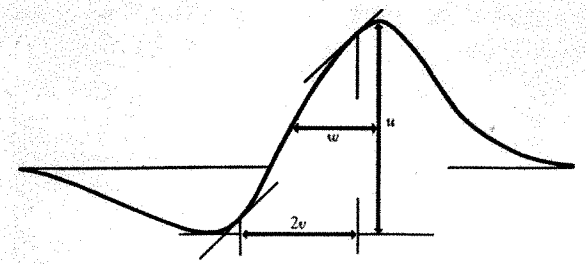


Fig. 1. Magnetic feature showing the indices u, v and w

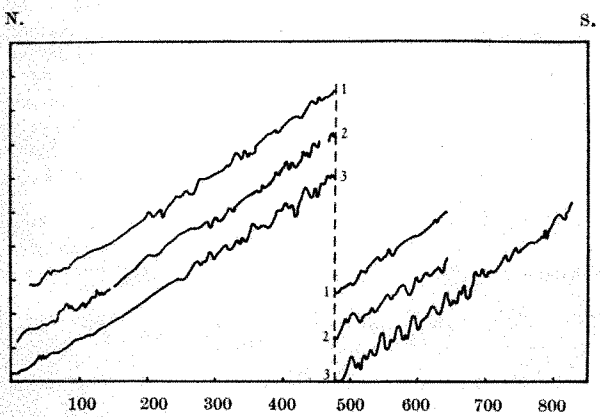


Fig. 2. Total magnetic field profiles across the Drake Passage. The vertical intervals are thousands of γ. The horizontal scale represents distances in nautical miles from Port Stanley

used in correlation from traverse to traverse. The numerical values of the levels, that is, 10 and 15 per cent, were chosen from observations so as to give a wide range of types in the area studied.

To illustrate the use of the technique three traverses from the Drake Passage area of the Scotia Sea, where the depth of water is fairly constant at about 2,000 fathoms, have been taken as examples. These are given in Fig. 2. They run approximately in a north-to-south direction and are numbered 1–3 from east to west. Apart from the magnetically undisturbed area in the north the traverses show no obvious features which can be used for correlation.

In Fig. 3 are plotted the values of u, v, w and $\delta u/\delta v$ and $\delta w/\delta v$ for Track 3. On this track the most obvious feature of the upper three graphs is a section of type A between 50 and 250 miles south of Port Stanley. South of this u, v and w show a recognizable similarity of variation until the vicinity of 550 miles is reached, where there is a sharp rise in all three values. The average rise in u at this point continues until 685 miles, and over this section there is no significant correlation of u with v or w. At 685 miles the value of u decreases again and the marked correlation between u, v and w reappears. From the lower two graphs it can be seen that there is also a change of character at 320 miles. The visually observed division in the vicinity of 550 miles can now be defined rather as a separate transition zone with limits at 510 and 580 miles. Calculation of the various correlation coefficients then determines the types of the various sections, these being marked on the diagram. Tracks 1 and 2 can be similarly divided, the sequence of sections being similar,

Table 1

Category	Probability levels of correlation coefficients (%)		
	< 10	< 15	> 15
B_1	$r_{uv}\ r_{vw}\ r_{uw}$		
B_2		$r_{uv}\ r_{vw}\ r_{uw}$	
or	any two		
C_1	r_{vw}		$r_{uv}\ r_{uw}$
C_2		r_{vw}	$r_{uv}\ r_{uw}$
D_1	r_{uv}		$r_{vw}\ r_{uw}$
D_2		r_{uv}	$r_{vw}\ r_{uw}$
E_1	r_{uw}		$r_{uv}\ r_{vw}$
E_2		r_{uw}	$r_{uv}\ r_{uw}$
F			$r_{uv}\ r_{vw}\ r_{uw}$

A_1, no magnetic features; A_2, less than 1 feature per 75 miles; G, areas of very narrow magnetic features of high frequency per mile (confined to shallow water areas).

but differing in some respects. Correlation of the three tracks is shown diagrammatically in Fig. 4. The area of type A_1 is well defined on all three, being bordered on the north side by an area, or areas, which are somewhat indeterminate owing to sparsity of data. To the south of the type A area all three tracks cross a B type area of considerable size, although there is a small region of type C_2 on Track 1 in the north-eastern corner. The southern margin of the B type area is clear, and beyond this lies a region which is marginally of F type and which grades into one of C_2 type in the east. The most southerly parts of all the tracks have very little in common, this being provisionally interpreted as indicating that the type boundaries have a trend roughly parallel to the traverses.

The correlation of only three traverses has been described, the purpose being to demonstrate the method, not to produce a structural map. Interpretation of other traverses crossing the Drake Passage region does in fact confirm and extend the main boundaries already defined. Five traverses, similarly widely spaced, have also been made in an east–west direction between the Falkland Islands and South Georgia. In this region it has also

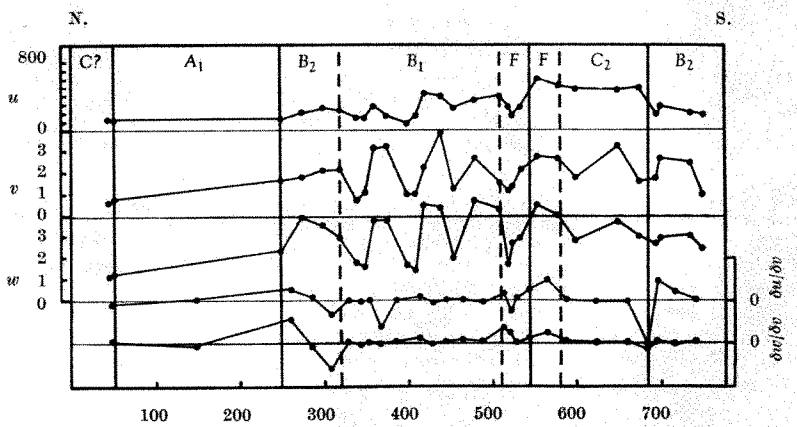


Fig. 3. Graphs of u, v, w, $\delta u/\delta v$ and $\delta w/\delta v$ against distance for Track 3 of the Drake Passage series. The horizontal scale represents distances in nautical miles from Port Stanley; u is measured in gamma, v and w in nautical miles. The vertical dividing lines are divided into two types, solid when obtained from inspection of u, v and w graphs and broken when obtained from inspection of the $\delta u/\delta v$ and $\delta w/\delta v$ graphs

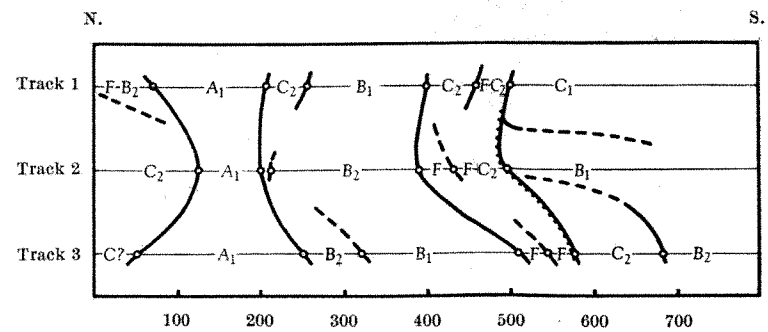


Fig. 4. Diagram showing correlations between Tracks 1, 2 and 3. The horizontal scale represents distances in hundreds of nautical miles from Port Stanley
—○—, Division of type; — –○– —, division of degree within one type or type division of uncertain position; ....., tentative correlation of magnetic feature

been possible to define the various type areas described, though, because of the difference in direction of the traverses in the two regions, it is not possible to be sure that any particular type has the same geological significance in both.

Correlation between widely spaced but parallel traverses in the way described presumably depends on the existence of considerable areas which are geologically similar to the extent that the magnetic anomalies produced have certain characteristics in common. If this is correct, the divisions between areas of different magnetic type are likely to be important geological boundaries of which some may be faults. A more detailed analysis of the data than can be described here has suggested that in a large part of the area under discussion the anomalies may be mainly caused by the sub-bottom topography of basic igneous rocks, changes in magnetic type being produced by variations in the form and relief of the topography, which is itself likely to be related to rock type and structure.

Using a seismic profiler it should be possible to show fairly easily whether or not there is any relation between the concealed topography and the magnetic anomalies, and together with detailed seismic refraction surveys the two methods should confirm or disprove the geological significance of the magnetic type boundaries. If the method is found to give geologically useful information it will be worth developing further.

We thank the Director of the British Antarctic Survey for providing facilities for carrying out the field work on which this analysis is based and for the assistance given by his staff. The magnetic measurements were made by P. Kennett and H. A. D. Cameron.

DOSE-EFFECT RELATIONSHIPS FOR RADIATION DAMAGE TO ORGANIZED TISSUES

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WHEN a population of cells is exposed to ionizing radiation its ability to give rise to viable daughter cells is impaired, fewer and fewer cells retaining this ability (that is, 'surviving') as the dose is increased. It is well established that the relationship between dose and the survival of mammalian cells is of the pattern illustrated by the curve *OAB* in Fig. 1. This type of survival curve has been observed for cells irradiated and cultured *in vitro* and *in vivo*[1-3]. Although some authors assume that the extrapolation number, N, is usually near 2 for mammalian cells[4], observations made at the cellular level have demonstrated that higher values of N are frequently observed[5-7]. The actual value of N for the cells in a given tissue is of great importance, both for theoretical reasons and for the practice of radiotherapy, and this article summarizes evidence from which it may be deduced that, if cell survival in organized tissues follows the pattern illustrated in Fig. 1, the relevant extrapolation number N may often be very much greater than 2.

The evidence is provided by animal experiments, and by clinical observations, in which a given biological end-point is attained either by a single dose of radiation or by two or more doses separated in time ('fractionated doses'). In general, the total dose when fractionated has to be greater than when given within a few minutes. A rationale for this effect was put forward theoretically[8] on the basis of Gray and Scholes's experiments[9], and was demonstrated experimentally with mammalian cells by Elkind and Sutton[2] from observations *in vitro*. Cells given a dose $\frac{1}{2}D_2$ (Fig. 1) and then allowed to metabolize would reach the survival level *BC* after a further dose $\frac{1}{2}D_2$, whereas if a single dose had been given the same level of survival would have been reached by dose D_1. This is true provided the interval between the fractions is several hours[2]. The difference between D_2 and D_1 is equal to the intercept of *BA* on the 100 per cent survival abscissa, and has been designated D_Q (ref. 10). For two fractions $D_2 - D_1 = D_Q$, and if n equal fractions D_n/n are given, each being large enough to bring the survival to the straight part of the semilog curve in Fig. 1, then $D_n - D_1 = (n - 1)D_Q$. Thus D_Q may be determined by comparing the total fractionated dose with the single dose necessary to give the same effect. It should be pointed out that if the fractions are so small that the cell survival after each dose lies on the shoulder of the curve in Fig. 1, then $D_n - D_1$ will be less than $(n - 1)D_Q$ so that spuriously low values of D_Q would be found by this calculation.

Table 1 shows a number of values of D_Q obtained by observations on mammalian tissues. Pig skin has been found to respond to radiation in a way similar to human skin, and is therefore a useful experimental system[11,12]. The results on human skin were taken from five publications used in a survey of the literature on dose-fractionation[13]. The clinical observations, although less accurate than the results of the experiments on pig skin, are in good agreement with them.

In the experiments in which death of mice within 4 days was studied[14], the lethal effect of radiation was presumed to be due to the lethal effects on epithelial cells lining the crypts of Lieberkühn[15]. We have calculated D_Q for mouse bone-marrow from the data of Brown,

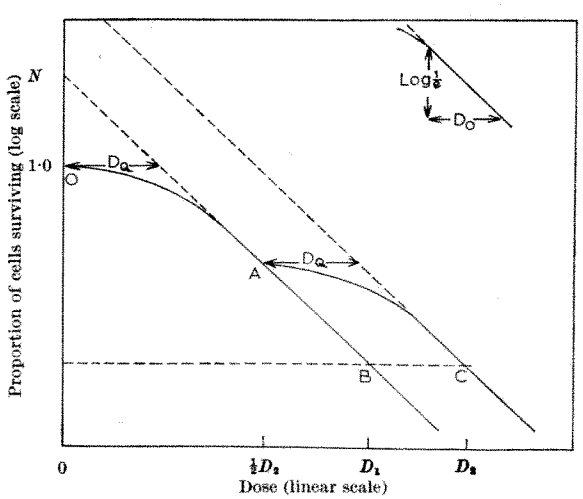


Fig. 1

Table 1

Biological end-point	No. of fractions	Overall time (days)	Dose per fraction = D_n/n	$D_Q = \frac{D_n - D_1}{n-1}$	Calculated extrapolation number N if D_0 = 100 rads	D_0 = 150 rads	D_0 = 200 rads	Ref.
Pig skin: given level of reaction	2	2	1,280	520 ± 50	300	50	18	—
	2	2*	1,310	600 ± 50 (577)				—
	2	5*	1,310	610 ± 20				—
	3	3*	1,060	580 ± 35	300	50	18	—
	5	5*	990	500 ± 20				—
	5	5†	890	400 ± 70 (450)	100	20	10	12
	5	29†	990	500 ± 50				12
	5	29†	870	400 ± 50				12
	6	17‡	650	380 ± 40	55	13	7·5	11
Human skin: given level of response	2	2	1,280	550 ± 250	20–600	8–200	4–50	13
	5	5	950	450 ± 100	30–200	10–40	6–18	13
	6	6–8	670	400 ± 100	20–150	8–30	5–12	13
30-day death of mice	2	1	500	220 ± 270	9–15	4–6	3–4	16
4-day death of mice	2	0·25	400–1,000	400 ± 22	55	14	7	14

* Pig No. 2154. † Pig. No. 2166. ‡ Average of 4 pigs: 2076, 2077, 2078 and 2091.

Corp and Mole[16], on the assumption that the death of mice within 30 days is attributable to lethal effects on the stem cells of hæmopoietic tissue[15].

If the dose-effect relationship illustrated by Fig. 1 applies to the test systems cited in Table 1, then $D_Q = D_0 \log_e N$, where N is the extrapolation number and D_0 is the dose required to reduce a surviving fraction f to fe^{-1}, once the survival curve has become exponential. This relation is shown graphically in Fig. 2. D_0 cannot be calculated from the data of Table 1, since the level of cell survival required to give any of the biological end-points is not known. However, an assumed value for either D_0 or N will enable the other unknown constant to be calculated. The available data which establish both D_0 and N for various cell lines have shown a smaller range of values for D_0 (from 84 (ref. 21) to 280 rads (ref. 17)), for well-oxygenated cells, than for N (from 1 to 10 or more).

In Table 1 values for N have been calculated from the data on D_Q for values of D_0 of 100, 150 and 200 rads. It will be seen that all the values of N are greater than 2, some being more than 100. Possible reasons for these high values should be considered.

One possibility is that a fraction of cells in the normal tissues used were anoxic. In this case D_0 is unlikely to be higher than 450 rads, and N for pig skin irradiated in 2 or 3 fractions would still not be less than 4. However, there is no reason to suppose that normal tissues contain significant proportions of anoxic cells. Moreover, there is direct evidence on this point from the experiments on 4-day lethality in mice[14], since those which were irradiated while breathing oxygen were no more radiosensitive than those breathing air.

Another possibility is that systemic factors may play a part when organized tissues are irradiated *in vivo*. These factors might influence cell survival, or might influence the observed response of the animal to a given degree of cellular depopulation. Some support for this hypothesis is given by a comparison of the results of Brown, Corp and Mole[16] on 30-day lethality in mice, with those of Till and McCulloch[18,19], McCulloch and Till[20], and Smith and Vos[21] on the survival of mouse bone-marrow cells. The four latter reports give values of D_0 near to 100 rads with N near to 2, and this cannot be reconciled with the value of D_Q deduced from the data of Brown, Corp and Mole[16]. This value of D_Q between 220 and 270 rads would give N between 9 and 15 if D_0 was 100 rads. On the other hand, the difference may be due to differences between strains of mice. Evidence which supports the relevance of cell survival curves to tissue damage *in vivo* comes from timing studies, which were done in the experiments on pig skin and on 4-day lethality in mice. Elkind and Sutton[2], using split doses on an *in vitro* cell system, found that survival after the second dose varied with time between doses, rising to a maximum, then falling to a minimum, and finally rising to a plateau when the interval was 12 h or more. This pattern has been observed to hold in the two experimental systems quoted. Furthermore, observations *in vitro* have shown that the value of N decreases with increasing linear energy transfer of the radiation, and split dose experiments on 4-day lethality in mice after exposure to fast neutrons have shown that the ratio D_Q/LD_{50} is considerably less than after exposure to X-rays[22]. It seems plausible that the observations are due to events taking place within the cells, and unlikely that unknown systemic factors should operate differently on cells exposed to the different qualities of radiation.

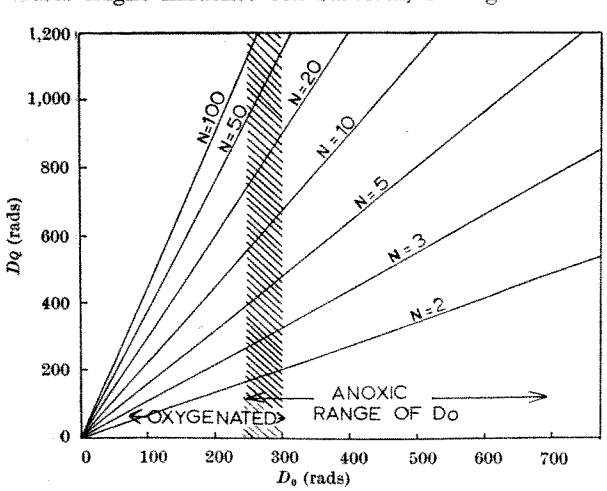


Fig. 2

Cellular re-population could not be a factor in increasing D_Q when the interval between doses was less than a day (the figures quoted in the gut-damage experiments are for an interval of 6 h between doses). In the pig-skin experiments it was found that, for a given degree of skin reaction, irradiation over a total period of 29 days required a dose only 15 per cent larger than that required when the total period was 5 days. The high D_Q values cannot therefore be explained on the grounds of increasing cell populations. Nor can they be explained on immunological grounds, which have been suggested[23] as a factor in explaining the high values of D_Q calculated from fractionation studies on solid tumours, since the experiments discussed here were made on normal tissues.

It is evident, therefore, that some normal tissues behave as though their constituent cells have survival curves with extrapolation numbers much greater than 2, which is sometimes regarded as a 'proper' value for all diploid mammalian cell lines. Although investigation of the possible influence of systemic factors is needed, the figures tabulated in this article have an important bearing on the rationale of dose fractionation in radiotherapy.

Note added in proof. The value of N for bone-marrow calculated from the data of Brown, Corp and Mole[16] was seen to be much higher than that obtained directly[19].

Recently, however, S. Hornsey carried out similar experiments using mice of strain *TO*. Intervals between doses varied from 1 to 24 h. A maximum in the curve of survival versus time between doses was found at 4–6 h, and at this time, as at 24 h, the estimated value of D_Q was about 100 rads. If 100 rads is adopted as the value of D_0 for bone-marrow cells[18–21], this value of D_Q gives $N = 2{\cdot}7$. This good agreement with the value $N = 3$ observed by Till and McCulloch[19] for bone-marrow irradiated *in situ* makes it seem unlikely that the high values of D_Q observed for other tissues are due to systemic effects.

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INFLUENCE OF ISOLATED COMPONENTS OF THE UTERINE SECRETION ON PHAGOCYTOSIS

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In an earlier article[1] it was shown that the uptake of starch particles by polymorphonuclear (PMN) leucocytes was inhibited in the uteri of pseudopregnant rabbits *in vivo* and in the presence of equivalent uterine flushings *in vitro*. No similar suppression of activity was observed with flushings from œstrous rabbits or in the uteri of such animals. It has been suggested that a polysaccharide material, shown to be present in uterine flushings obtained from rabbits during pseudopregnancy, but absent at œstrus, may be responsible for this phenomenon[2].

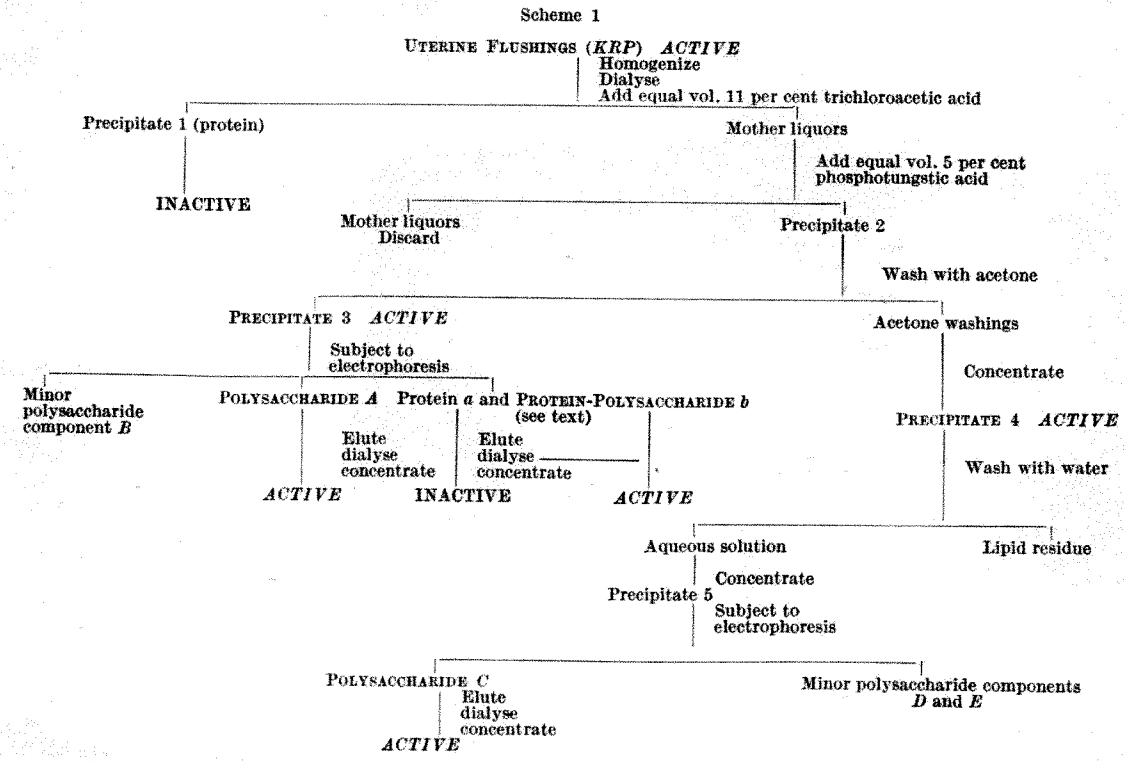
This article describes the fractionation of secretions obtained from the uterus of pseudopregnant rabbits, and an investigation into the ability of the major fractions of these secretions to inhibit the phagocytosis of starch particles by rabbit PMN leucocytes *in vitro*.

The preparation of experimental animals and collection of uterine secretions were carried out as described previously[1]. Prior to fractionation experiments, a preliminary test was performed to determine whether the activity was due to compounds of a heat-labile nature (the term 'active' is used throughout this article to denote a definite suppression of phagocytic activity relative to a control). No loss occurred on boiling an active secretion for 10 min before incubation with leucocytes and starch granules.

The fractionation procedure, together with activities of various fractions, is shown in Scheme 1.

This procedure was carried out several times, initially on flushings obtained from individual uterine horns and finally on a series of combined flushings from the uteri of five pseudopregnant rabbits. The fractions are designated

Scheme 1



protein, polysaccharide, and lipid by virtue of their staining properties only. The fractionation was carried out at room temperature except where solutions were concentrated (40° C at reduced pressure) and where components were eluted from paper. Precipitates were collected by centrifuging. Fractions were submitted to dialysis where stated, for 72 h, against several changes of distilled water. Electrophoresis was performed on 25-cm strips of Whatman No. 3 paper at 300 V for 3 h in various buffers, the material being applied in the same buffer as that used for the electrophoresis. Precipitate 4 was dissolved in 80 per cent aqueous acetone and applied to paper in order to demonstrate that the lipid fraction remained at the origin during electrophoresis. Preparative electrophoresis was carried out on 25 cm × 15 cm sheets of the paper already mentioned, the material being applied in streaks, detected by edge staining, and the portion of paper containing the required polysaccharide eluted with boiling water. The electrophoretic properties of these components were not affected by short periods in boiling water and a good and rapid recovery was obtained from the paper in this way. Portions of paper containing protein were eluted with water at room temperature. 0·2 M borate buffer, *p*H 7·5, was used throughout for preparative work apart from the separation of protein *a*. Electrophoretic mobilities and the staining properties of the fractions, together with those of heparin in various buffers, are shown in Table 1.

Table 1. Electrophoretic Mobilities and Staining Properties of Fractions from Uterine Flushings

	Electrophoretic mobilities (cm) (all migrating to anode)			Staining reactions		
	0·2 M Acetate *p*H 4·0	0·5 M Borate *p*H 10·1	0·2 M Borate *p*H 7·4	Toluidine blue	Bromophenol blue	Sudan black
Polysaccharide *A*	3·0	12·0	8·0	+ + (blue)	—	—
Polysaccharide *B*	2·0	9·5	—	+ (blue)	—	—
Protein *a*	Origin	5·0	—	—	+ +	—
Protein–polysaccharide *b*	—	—	4·5	+ (blue)	+ +	—
Polysaccharide *C*	8·5	13·5	11·0	+ + + (purple)	—	—
Polysaccharide *D*	3·5	9·0	8·0	+ (purple)	—	—
Polysaccharide *E*	2·0	6·0	6·5	+ (blue)	—	—
Precipitate 4 (lipid residue)	Origin	Origin	Origin	—	—	+ + +
Heparin	7·5	9·0	8·0	+ + + (purple)	—	—
Ppt 1 (protein)	—	—	—	—	+ +	—

After fractionation, activity determinations were carried out on the major polysaccharide and protein fractions from the flushings. The leucocytes were collected and phagocytic activity was measured as described in the previous article[1]. In the determination of activity a quantity of material approximating to that found in a single uterine horn was dissolved in 2 ml. of Krebs–Ringer phosphate solution (KRP). Leucocytes and starch particles suspended in this solution alone were used as a control. Other workers have reported that serum containing complement and antibody is necessary for the uptake of starch particles by guinea pig leucocytes[3,4]. However, in these experiments, although preliminary work showed that the uptake of starch particles increased in the presence of serum, leucocytes continued to show considerable phagocytic activity when no serum was present and this activity did not diminish when the leucocytes were washed with isotonic saline prior to incubation. Thus it was concluded that rabbit leucocytes differ from those obtained from guinea pigs in retaining their ability to engulf starch particles in the absence of serum. Phagocytic activity (per cent) of the leucocytes in the presence of the various fractions is shown in Table 2.

Activity equivalent to that found with the original flushings occurred with precipitate 3 and polysaccharide *A*, whereas precipitate 1 (protein) showed no activity.

Table 2. Phagocytic Activity (per cent) in the Presence of Various Fractions of the Uterine Flushing

	No. of starch particles per leucocyte			
	0	1–10	11–20	> 20
Control (Krebs–Ringer phosphate)	0·4	6·2	28·6	64·8
Untreated flushings	13·4	74·1	9·4	3·1
Precipitate 1	—	12·8	38·2	49·0
Precipitate 3	5·9	69·4	18·9	5·8
Precipitate 4	11·2	60·5	26·5	1·8
Polysaccharide *A*	0·3	60·0	30·3	9·4
Polysaccharide *C*	2·0	53·3	41·3	3·4
Protein *a*	3·0	22·0	40·0	35·0
Protein–polysaccharide *b*	21·0	63·0	15·0	1·0

Precipitate 4 and polysaccharide *C*, while inhibiting uptake of starch particles, also caused agglutination of PMN leucocytes, rendering the counting procedure less accurate in these cases. Protein–polysaccharide *b* obtained from precipitate 3 after electrophoresis in borate buffer *p*H 7·5 showed heavy staining for protein with bromophenol blue, slight polysaccharide staining with toluidine blue, and was active. On the other hand, protein *a* obtained from the same precipitate after electrophoresis in borate buffer *p*H 10·1 showed no polysaccharide staining and was inactive. It was not possible to determine activities of the dialysable material since concentrates caused degeneration of the PMN leucocytes, presumably due to the high inorganic content of these. The lipid residue was insoluble in Krebs–Ringer phosphate and was not investigated further.

These results indicate that the inhibitory effect of uterine flushings from pseudopregnant rabbits on phagocytosis can be repeated after fractionation. The final major components (excluding protein-polysaccharide *b*) which caused suppression of phagocytosis consisted of material staining only for polysaccharide. It is not known whether protein–polysaccharide *b* consists of protein and polysaccharide in chemical combination at *p*H 7·5, or of chemically distinct entities with the same electrophoretic mobility. It is reasonable to suppose, however, in view of the results from other fractions, that the polysaccharide portion of *b* is responsible for its activity. In the case of polysaccharide *C* and precipitate 4 it is possible that the inhibition may have been associated with the agglutination effect. This property does not appear to be a function of concentration since it persists when these fractions are diluted further before determinations of activity. None of the other components produced any noticeable agglutination effects.

The whole surface of the leucocytes, in solutions showing activity, stained heavily with 0·5 per cent toluidine blue. In the control treatment only the nuclei were lightly stained. This suggests that the polysaccharides may produce their effects by adsorption on the surface of the leucocytes. In solutions of the active components in higher concentration, the PMN leucocytes were observed to become more rounded and distinct, which again suggests a surface effect. It is thought that adsorption of a particle on the surface of a leucocyte is the first stage of phagocytosis[5,6]. Thus it seems probable that adsorption of a polysaccharide on the cell surface could interfere with this process and hence prevent the entry of the particle into the cell.

The presence of protein and lipid material at a late stage in the fractionation indicates that the active fractions studied are breakdown products from larger molecules which occur in uterine secretions and which contain protein, lipid and polysaccharide in some form of combination.

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MECHANISM OF REJECTION OF HOMOTRANSPLANTED KIDNEYS

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RENAL homotransplants in mammals usually cease to function within a few days or weeks[1]. The functional arrest of such kidneys is commonly attributed to immunological causes; but the exact mechanism has not been elucidated. Darmady *et al.*[2] demonstrated the evolution of histological changes within the homotransplanted kidney using stains for nucleic acids. The methyl-green-pyronine stain reveals stages in the evolution of the plasma cell within the homotransplant, similar to those described by Fagraeus[3].

With the notable exception of Dempster[4], workers in the field of transplantation biology have interpreted the plasma cell as a mobile effector of an immune response mounted by the host[5–7]. While it now seems certain that a portion of the pyronine positive cells are of host origin[8–10], little consideration has been given to the means by which these cells could bring about oliguria and disintegration of the homotransplanted kidney.

The work recorded here was designed to investigate the pathophysiological and anatomical changes within the homotransplanted kidney. Evidence will be presented to show that the destruction of renal homotransplants is an ischæmic process. This is brought about by progressive dissolution of small blood vessels by immature plasma cells, followed by the blockage of the intertubular spaces by plasma cells, red cells, leucocytes and rounded, isolated endothelial cells.

The experiments were performed on greyhound bitches with an average weight of 22–25 kg. In the first series of experiments a right nephrectomy was performed followed by auto- or homo-transplantation to the right iliac fossa. The technique was that described by Dempster[11], in which the renal artery was anastomosed end-to-side with the external iliac artery, and the renal vein end-to-end with the external iliac vein; and the ureter implanted into the bladder. Renal venous flow was determined prior to transplantation and during the life-history of the homotransplant, using a constant local indicator dilution method[12,13]. During placement of the catheters in the renal veins for measurements of venous flow, successive wedge biopsies of the cortex were taken. Small cubes from the centre of the biopsies were preserved in Palade's buffered osmium fixative for electron microscopy, and other portions fixed for light microscopy. Oliguria in the homotransplant was considered imminent when a significant fall in the serum antitrypsin-level occurred[14].

In a second series of experiments, the 'effective renal blood flow' and simultaneous '1 h percentage excretion' in the urine following a single intravenous dose of 20 μc. iodine-131-hippuran and 4 μc. of carbon-14-inulin were estimated daily on bilaterally nephrectomized, unanæsthetized dogs bearing an auto- or homo-transplanted kidney. Effective renal blood flow was measured by the rate of disappearance of the single intravenous dose of iodine-131-hippuran[15,16]. Iodine-131 and carbon-14 were assayed simultaneously in a Packard 'Tri-Carb' liquid scintillation counter, model 314 *EX*, using a gel scintillator. Oliguria of a homotransplant was taken to be the point when a sudden decrease in the daily urine volume and a poor response to an infusion of 500 ml. of 0·9 per cent saline and 300 ml. of 2 per cent saline occurred[17].

The variable survival times of renal homotransplants in dogs necessitates frequent observations on individual transplants. Single observations have little value. Renal venous flow was measured during the survival of 34 renal homotransplants and six autotransplants. The results of a typical homotransplant experiment are shown in Fig. 1. The usual period of ischæmia which is involved

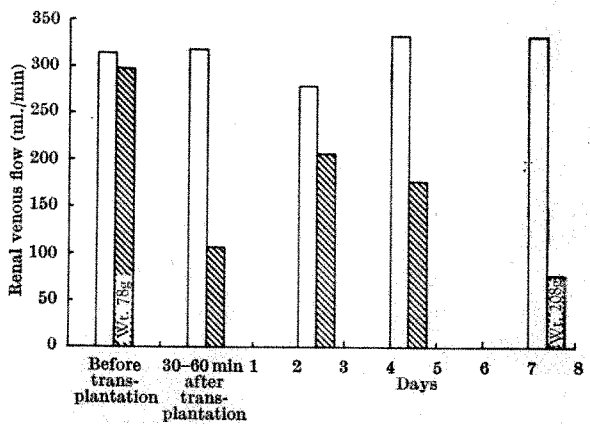


Fig. 1. Renal venous flow in a dog bearing a normal and a homotransplanted kidney
White, normal kidney; hatched, transplanted kidney

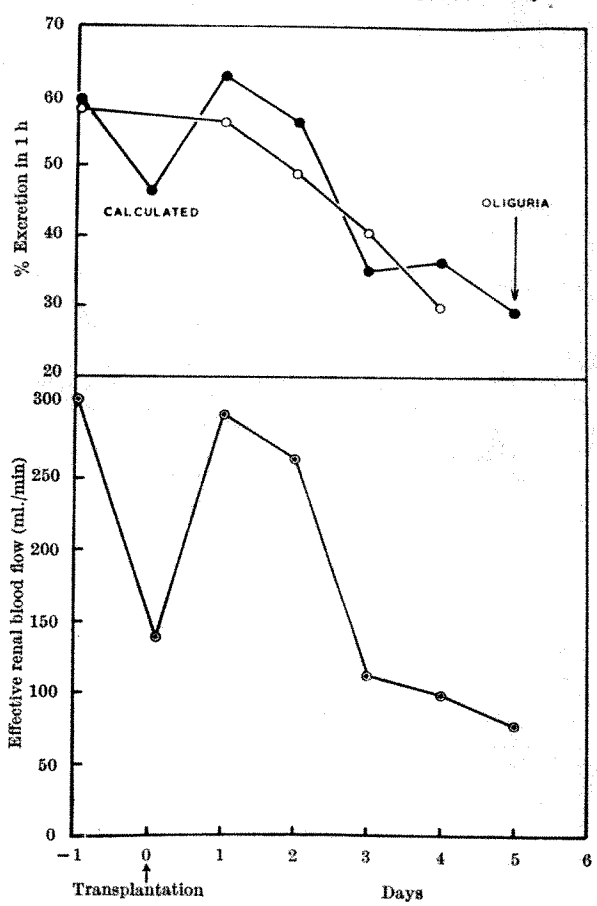


Fig. 2. Daily effective renal blood flow and the 1-h simultaneous percentage excretion in the urine of a single intravenous dose of 20 μc. of iodine-131-hippuran and 4 μc. of carbon-14-inulin in a dog bearing only a homotransplant
○, Carbon-14-inulin; ●, Iodine-131-hippuran

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Table 1. Balance Sheet showing the Percentage of the Injected Dose of Iodine-131-Hippuran accounted for in the Dog shown in Fig. 2

Days	−1	0	1	2	3	4	5
Calculated percentage of injected dose remaining after 1 h	58·8	—	63·0	56·6	35·6	36·5	29·6
Measured percentage excreted in urine in 1 h	35·4	—	35·4	43·3	61·0	64·2	62·4
Total	94·2	—	98·4	99·9	96·6	100·7	92·0

during transfer results in a post-transplantation decrease in flow, which returns to normal or near normal within 24 h. After 24–72 h there is a progressive decrease in venous flow until oliguria, after which it rapidly ceases. Except for the post-transplantation decrease in flow, venous flow was maintained at virtually normal levels in autotransplants.

In Fig. 2 a typical experiment shows the changes in the daily effective renal blood flow and the daily simultaneous 1 h percentage excretion in the urine of a single dose of 20 μc. of iodine-131-hippuran and 4 μc. of carbon-14-inulin by a homotransplanted kidney. The ischæmia during transfer again results in a post-transplantation decrease in flow, which returns to normal or near normal within 24 h. This was followed within 72 h by a progressive fall until oliguria. The percentage recovery of the 20 μc. of iodine-131-hippuran injected intravenously was determined by adding the amount measured in the urine to the calculated amount remaining in the animal after 1 h. The percentage of the dose remaining after 1 h was calculated by the following formula:

$$\frac{^{131}\text{I in 1 ml. plasma at 1 h} \times 100}{^{131}\text{I in dose}} \times V$$

where V is the thereotical volume of distribution. In Table 1 the results of the dog illustrated in Fig. 2 are shown. The 1-h percentage excretion in the urine of the injected dose also fell progressively after 24–72 h until oliguria, but not so rapidly as the effective renal blood flow.

The results of electron microscopy are summarized in Table 2. Although minor tubular changes are seen before oliguria, they are not progressive or serious in extent. No glomerular changes were observed (Fig. 3). In contrast to the foregoing, extensive and progressive vascular damage appeared as a major lesion. Oliguria occurred when the flow of blood was greatly reduced by blocked (Fig. 6) and damaged blood vessels. At this point tubular necrosis commences.

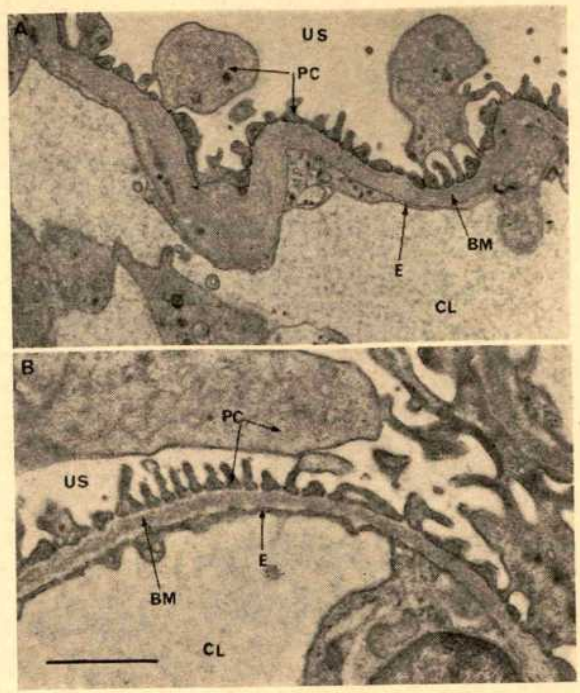


Fig. 3. *A*, Morphologically normal glomerular tuft in an anuric homotransplant of kidney; *US*, urinary space; *PC*, podocyte cytoplasm; *BM*, basement membrane; *E*, endothelium; *CL*, capillary lumen. *B*, Morphologically normal glomerular tuft in a functioning autotransplant. Labels as for *A*. Scale = 2μ

Table 2. Summary of Electron Microscopic Findings

Type and No. of specimens	Glomeruli	Proximal convoluted tubule	Distal convoluted tubule	Intertubular vasculature
Autotransplants (5)	Endothelium, basement membrane and podocytes normal.	Slight luminal shedding of cytoplasm, microvilli and free mitochondria. Slight increase in basal vacuolation.	Normal. Debris in lumen from proximal tubule.	Normal.
Functioning homotransplants (*a*) 24 h (2)	Normal, but contains refluxed mitochondria in urinary space.	Large numbers of small basal vacuoles. Luminal shedding more intense than in autotransplants.	Normal. Debris in lumen as above.	Erythrocyte sludging in a few vessels.
(*b*) 72 h (2)	Normal.	Marked luminal cytoplasmic shedding. Intertubular œdema. Many small basal vacuoles present.	Normal. Debris in lumen. Intertubular œdema.	Numerous intravascular and extravascular lymphocytes, immature and maturing plasma cells. Intimate cell membrane apposition of plasma cells and endothelial cells (Fig. 4)—patchy disappearance of intervening membranes with cytoplasmic continuity (Fig. 5). Endothelial cells separate from each other, round up and extrude cytoplasmic blebs.
(*c*) 96 h (1)	Normal.	As for 72 h.	Normal. Debris in lumen. Intertubular œdema.	Extravascular red cells, lymphocytes, plasma cells. Free mitochondria in lumen of vessels remaining intact. Many isolated masses of endothelial cytoplasm. Numerous rounded endothelial cells, some remaining in close apposition with plasma cells. Increase in intertubular collagen.
(*d*) 9 days (1)	Normal.	As for 72 h.	Normal.	Vessel area chaotic. Many free rounded endothelial cells, fewer plasma cells, much cytoplasmic debris and free red cells. Scattered masses of intertubular collagen.
Oliguric homotransplants (6)	Normal.	Shedding still occurring but large basal vacuoles present.	Normal. Debris in lumen.	Majority of intertubular vessels destroyed or blocked (Fig. 6). Intertubular spaces filled with red cells, rounded endothelial cells, collagen, and isolated plasma cells and phagocytes.
Anuric homotransplants (1)	Normal (Fig. 3).	Much cytoplasmic shedding. Large basal vacuoles in various stages of coalescence. Some tubules completely necrotic.	Most tubules normal. Some show early basal vacuolation.	Vasculature obliterated. Interstitium full of red cells, many active phagocytes and much scattered collagen (Fig. 7).



Fig. 4. Functioning homotransplant of kidney (72 h) showing intravascular cells
L, lymphocyte; *PC*, immature plasma cell; *EC*, endothelial cell; *BM*, basement membrane of neighbouring proximal tubule. Scale = 2μ

Measurements, by other workers, of blood flow in transplanted kidneys have been limited in the main to single observations[2,18,19]. Such observations did not permit a full assessment of the hæmodynamic changes occurring within the homotransplant. The observations reported here permit the formation of a theory for the rejection of first set renal homotransplants.

They indicate that the destruction of first set renal homotransplants is an immunological reaction mediated by host cells arriving via the blood stream. These cells are in the main immature plasma cells and appear to be directed specifically against the intertubular blood vessels. Following homotransplantation the resulting lesion becomes visible within 24–72 h. The initial point of attack is the vascular endothelium of the small intertubular vessels. Plasma cells enter into close cell membrane apposition with the endothelial cells over quite wide areas (Fig. 5). For the most part, a featureless 200 Å gap persists between the cells, but at scattered points there is a temporary dissolution of the adjacent membranes with the establishment of cytoplasmic continuity between the cells. In addition, scattered along the adjacent membranes, in a nodose fashion, are patches of increased density (about 0·1μ in length). This increased osmiophilia involves both the unit membranes themselves and also elliptical areas of the adjacent cytoplasm of the endothelial and plasma cell. The points of cytoplasmic continuity and of increased density may well be the sites of cytoplasmic interchange of materials between the cells. In this connexion it is interesting to note an apparent increase in the complement of free ribosome rosettes in endothelial cell cytoplasm at this stage.

During the apposition phase the attenuated outlying parts of the endothelial cells partially withdraw towards the nucleus and in part round up and become extruded as free, ribosome studded, anucleate masses.

The separation of adjacent endothelial cells results in the extravascular escape of red cells, and this is accompanied by a further accumulation of both intra- and extravascular plasma cells and lymphocytes. Concomitant with the vascular damage is a progressive decrease in total blood flow. The daily 1-h excretion of a single intravenous dose of inulin and hippuran reflect to a much smaller degree the vascular damage during the period of adequate function. The progressive attack on the smaller blood vessels by immature plasma cells culminates in: (1) the separation, rounding-up and final isolation of the endothelial cells; (2) the accumulation of plasma cells, lymphocytes, red cells, cell debris and ultimately phagocytes in the areas previously occupied by intertubular vessels (Fig. 7). The progressive damage to, and disappearance of intertubular vessels, the intertubular œdema and the massive interstitial cellular accumulation, together result in the total renal blood flow being severely reduced so that tubular perfusion becomes inadequate. At this point oliguria is initiated and tubular necrosis commences with massive vacuolation of the cells of the proximal convoluted tubules. Oliguria proceeds rapidly to anuria with physical destruction of larger blood vessels and hæmorrhage.

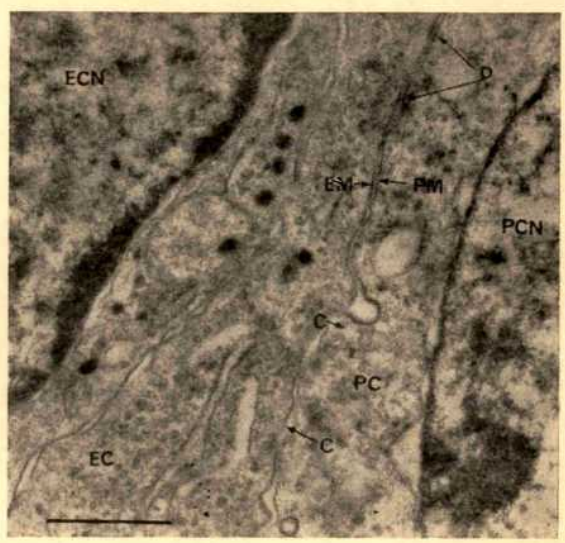


Fig. 5. Functioning homotransplant of kidney (72 h) showing intimate apposition of endothelial and plasma cells
ECN, endothelial cell nucleus; *PCN*, plasma cell nucleus; *EM*, surface unit membrane of endothelial cell; *PM*, surface unit membrane of plasma cell; *EC*, endothelial cytoplasm; *PC*, plasma cell cytoplasm; *C*, absence of membranes with cytoplasmic continuity; *O*, conjoined areas of increased osmiophilia Scale = 1μ

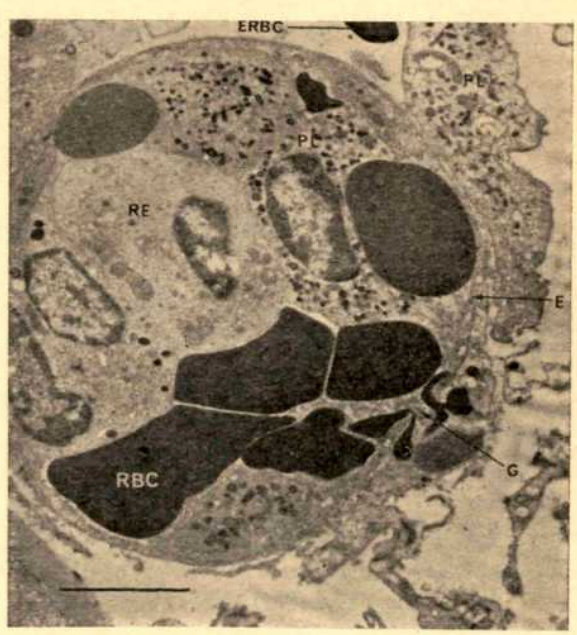


Fig. 6. Anuric homotransplant. Lumen of small vessels completely occluded with cells
ERBC, extravascular red cell; *RBC*, red cell; *PL*, polymorphonuclear leucocyte; *RE*, rounded endothelial cell; *E*, endothelial cytoplasm; *G*, endothelial gap with escaping red cells. Scale = 3μ

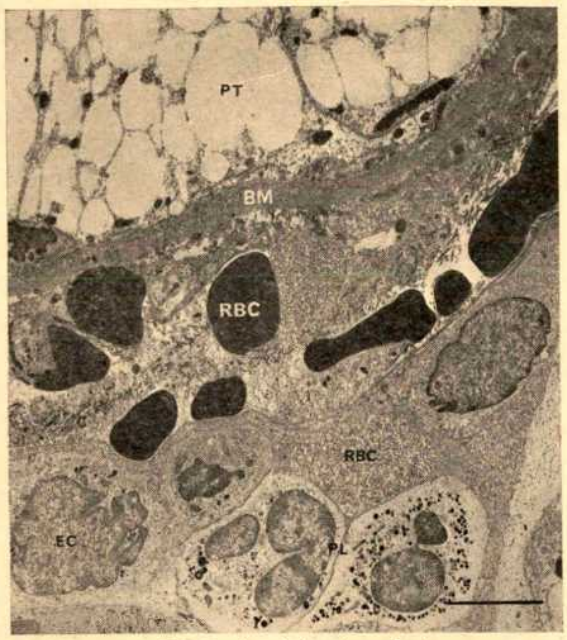


Fig. 7. Anuric homotransplant
PT, extreme vacuolation of necrotic proximal tubule; *BM*, tubular basement membrane; *RBC*, red cell; *EC*, rounded, isolated endothelial cell; *PL*, polymorphonuclear leucocyte; *C*, collagen Scale = 4μ

Although some shedding of proximal convoluted tubular cytoplasm occurs throughout the life history of renal homotransplants, it is not progressive in nature; while final tubular necrosis appears to be secondary to the primary immunological attack on the blood vessels. No evidence has been produced for an immunological attack on renal parenchymal cells.

The plasma cell attack is well under way by the fourth day and is probably initiated within 48 h after homotransplantation. While this is occurring, the host is apparently unable to mount a second set response[20]. One explanation may be that antigen is released in minute quantities only, which is insufficient to sensitize systemically the host, or alternatively antigen is a fixed surface component of the endothelial cell and stimulates the effector cell only during direct contact. The rapid response is probably effected by certain undefined but immunologically competent cells already circulating in the blood stream at the time of transplantation.

Vascular destruction has been incriminated as the cause of rejection of rat skin homografts but the effector cell would appear to be different[21].

This work was supported by grants from the U.S. National Institutes of Health, the U.K. Medical Research Council and the Wellcome Trust (to W. J. Dempster).

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EFFECTS OF PLANT GROWTH REGULATORS (AUXINS AND AUXIN ESTERS) ON THE SURVIVAL OF FREE CELLS OF THE EHRLICH ASCITES CARCINOMA

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RECENTLY[1] we reported a lethal effect of indolyl-3-acetic acid ethyl ester (IAEt)* and a protective effect of the natural plant growth regulator, indolyl-3-acetic acid (IAA), on the free cells of a diluted Ehrlich ascites carcinoma[1]. In this article, analogous effects for naphthyl-1-acetic acid methyl ester (NAMe) and for the synthetic auxin, naphthyl-1-acetic acid (NAA), are reported. Also reported is an unexpected effect resulting from the combined action of IAEt and IAA in suspension. This action is more lethal to the carcinoma cells than the action with IAEt alone. In view of the protective action of IAA when used alone, this result is surprising.

We now have evidence, obtained by paper chromatography, that the undiluted ascites tumour destroys IAEt rapidly both *in vitro* and *in vivo*. Such degradation within the tumour cells or in the ascitic fluid is an important factor which could limit or nullify the possible chemotherapeutic applications of auxin esters. For this reason we have been altering the chemical structure of these compounds in order to decrease their rate of degradation and, also, to determine specific structural requirements for optimum cytocidal activity. Some of the compounds have been tested against diluted ascites tumours ; the resultant data are reported here. Related results, obtained with undiluted tumours, will be reported in another communication.

As in our previous experiments[1], free ascites tumour cells were collected from male Webster white Swiss mice (initial body-weight 18–22 g) at 6–10 days post-transplantation by draining the ascitic fluid from the abdominal cavity. On the average, the fluid contained about 3×10^8 cells/ml. In the experiments recorded here, the ascitic fluids from a number of different animals were pooled and diluted by a factor of twenty with the Krebs–Ringer phosphate solution, *p*H 7·15. The final mixture contained glucose at 1 mg/ml., and the relatively insoluble auxins and/or esters (each at 0·5 or 2 mg/ml.) suspended with gum arabic at 1 mg/ml. Controls were

* We are now using the more descriptive symbol IAEt to represent the ethyl ester of indolyl-3-acetic acid, instead of the IAAEE symbol in our previous article (ref. 1).

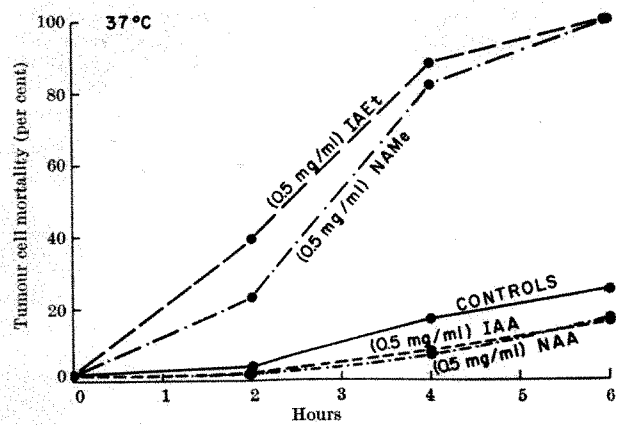


Fig. 1. Lethal effect of suspensions of ethyl indolyl-3-acetate (IAEt), or methyl naphthyl-1-acetate (NAMe), on the survival of free cells of a diluted Ehrlich ascites carcinoma, *in vitro*, at 37° C. Note, in contrast, the protective effect of indolyl-3-acetic acid (IAA), and naphthyl-1-acetic acid (NAA), under similar conditions

prepared in an identical manner, except for the omission of auxins and auxin esters. Incubation at 37° C and cell counting of samples withdrawn at various intervals were performed as described previously[1].

The Krebs–Ringer–glucose solution is a medium designed for short-term studies with isolated, live, animal tissues. The medium cannot support the life of the tumour cells indefinitely. After a 4-h incubation at 37° C, significant cell mortality could be observed in controls of some tumour preparations (Fig. 1); in other preparations, little mortality was observed even after 6 h. However, a 0·5 mg/ml. suspension of IAA or of NAA appreciably reduced the rate of cell mortality of a sensitive tumour preparation. In contrast, the corresponding esters, IAEt and NAMe, killed 39 and 23 per cent, respectively, of such tumour cells in 2 h, compared with less than a 4 per cent death in the untreated controls. After 6 h, these auxin esters had killed 100 per cent of the cells, compared with about a 25 per cent mortality of the controls, and only a 17 per cent death of those cells treated with the free auxins.

The difference between the effects of auxin esters and auxins on the tumour cells is evident. We attempted to use this difference to influence the kinetics of possible enzymatic processes related to the cytocidal action of the auxin ester. Accordingly, the tumour cells were exposed to a suspension of IAA (0·5 mg/ml.) plus IAEt (0·5 mg/ml.). In this way it was discovered that the auxin in conjunction with the auxin ester increased the rate of kill (Fig. 2). This result was unexpected because, as has been seen, auxin, when used alone, prolonged the life of the tumour cells. A similar test, with the ratio of IAA to IAEt increased to 4 to 1, conclusively demonstrated the decisive part that auxin plays in potentiating the cytocidal action of the auxin ester (Fig. 3). At the end of a 1-h incubation at 37° C, the mortality for the tumour cells treated with the auxin or auxin ester, separately, was 3·4 per cent or 7·3 per cent, respectively; in the same time the two agents together effected a 90 per cent kill.

Some recent results, obtained from paper chromatography, indicate that the natural auxin, IAA, inhibits the rate of degradation of its ethyl ester, IAEt, by the Ehrlich ascites tumour. This observation could be part of the explanation for the aforementioned potentiation of the cytocidal activity of IAEt by IAA.

The cytocidal activity of the ester of indolyl-3-acetic acid against the diluted Ehrlich ascites carcinoma, *in vitro*, is appreciably influenced by the structure of the alkyl group. From Table 1 it can be seen that the normal or tertiary butyl ester of IAA showed a rate of kill four times greater than the rate of IAEt. The normal pentyl ester killed only about twice as rapidly as IAEt. Surprisingly, however, the methyl, normal propyl and iso-

Table 1. Cytocidal Activity of Various Esters of Indolyl-3-acetic Acid against a Diluted Ehrlich Ascites Carcinoma

IAA ester	Concentration (mg/ml.)	Cell mortality after incubation at 37° C (%) (0 h)	(1 h)	(2 h)	(4 h)
Control	0·0	2·0	2·2	2·1	2·4
IAMe*	0·5		5·2	5·2	11·1
IAEt	0·5		6·4	41·5	91·6
IAPr(n)	0·5		2·5	8·0	7·9
IAPr(i)	0·5		1·1	2·6	2·8
IABu(n)	0·5		99·7		
IABu(t)	0·5		100·0		
IAPe(n)	0·5		21·7	83·9	100·0

* Me, methyl; Et, ethyl; Pr(n), normal propyl; Pr(i), isopropyl; Bu(n), normal butyl; Bu(t), tertiary butyl; Pe(n), normal pentyl.

propyl esters were markedly inferior in cytocidal activity to IAEt. Thus, these data indicate that there is no simple correlation of cytocidal activity with the size of the alkyl group. Also, the data do emphasize the advantages of rapid, inexpensive, *in vitro* screening procedures prior to detailed studies of the carcinolytic activity of auxin derivatives *in vivo**.

We share the opinion of Apffel, Tregier and Homburger[2] that synthetic plant growth regulators, particularly auxin esters, are a new family of compounds that deserve intensive study to determine the nature of their biochemical interaction with animal neoplasms. It is important to keep in mind that some of these compounds are closely related to selective herbicides, used commercially as weed killers, such as the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D). Audus[3] notes that plant roots are so sensitive to auxins that their growth can be stopped by the extremely small concentrations of natural auxins which normally occur in the shoot. However, for synthetic auxins to be effective as herbicides, considerably greater concentrations must be supplied. For example, a 2,4-D solution, 1 part in 100 million, will inhibit the growth of the seedling root of a susceptible plant such as garden

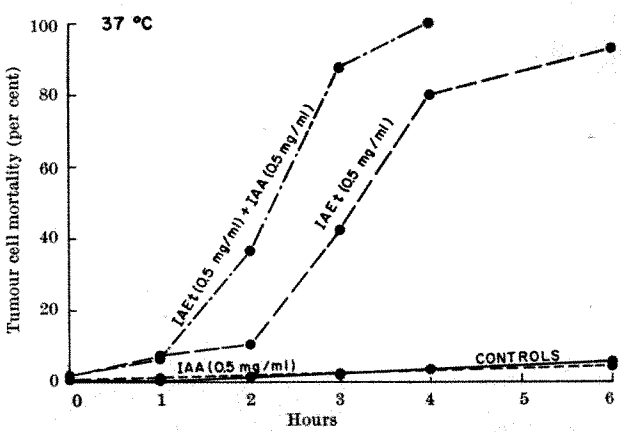


Fig. 2. Potentiation by IAA of the cytocidal activity of IAEt against a diluted Ehrlich ascites carcinoma, *in vitro*, at 37° C

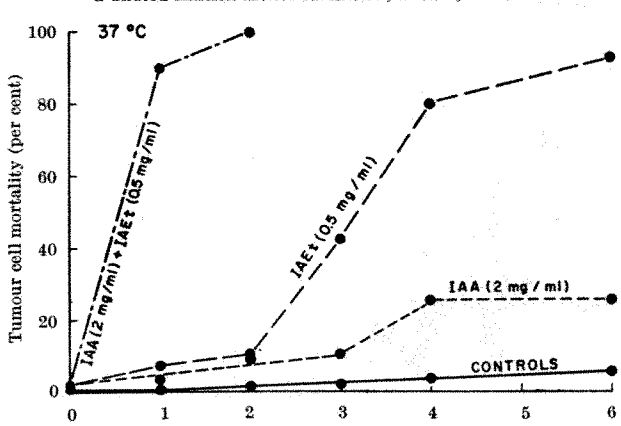


Fig. 3. Synergistic cytocidal activity of IAA plus IAEt, in a 4 : 1 ratio, against a diluted Ehrlich ascites carcinoma, *in vitro*, at 37° C. Note that, by itself, the higher dose of IAA in this test has a weak cytocidal action, instead of a protective action

cress. However, the concentration must be increased to 1 part in a million to stop all root growth and partially to inhibit germination. A leaf spray with a concentration of 100 p.p.m. or even 1 part per thousand must be used to kill the mature plant. It is the opinion of Audus[3] that, at such concentrations, one is no longer dealing with the normal physiological inhibition by the auxin. Instead, one has entered into a concentration region in which the auxin is acting as a cell poison. The growth processes, in which the natural auxin normally plays a fundamental part, are presumably clogged by a vast excess of the synthetic auxin and "a completely chaotic jumbling of the delicately interwoven pattern of normal growth processes is the result"[3].

Conceivably a synthetic auxin could be developed which would 'clog' the growth processes of an animal tumour cell without harming normal cells, somewhat in the manner that a selective herbicide kills weeds in a lawn of healthy grass. Of interest, therefore, is the report of Apffel *et al.*[2] that methyl esters of certain synthetic auxins inhibited the growth-rate of transplanted adenocarcinoma *H2717J*, and of spontaneous breast adenocarcinoma, in mice; this inhibition occurred at dose levels appreciably under the LD_{50}. However, under similar test conditions these particular esters showed little or no carcinostatic activity against the Ehrlich ascites carcinoma[2]. However, as noted earlier in this article, a lack of such activity might in some cases be due to the degradation of the auxin ester by the tumour.

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**Note added in proof.* Tests, at present in progress, indicate that daily intraperitoneal injections of IAPe(n) (the normal pentyl ester of IAA), 10 mg total, suspended in physiological saline, will triple the survival time of male Webster white Swiss mice bearing the Ehrlich ascites carcinoma. Treatment was started 1 day post-transplantation. Of special significance is the fact that normal healthy mice given similar daily injections of IAPe(n) have shown no gross evidence of acute or cumulative drug toxicity.

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KININ-INDUCED DIRECTED TRANSPORT AND SENESCENCE IN DETACHED OAT LEAVES

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KINETIN (6-furfurylaminopurine) is known to delay senescence in detached leaves of certain plant species[1-3]. Möthes *et al.* have associated this with another response to kinetin, namely, the accumulation and retention of metabolites in treated areas of leaves, which they have called "kinetin-induced directed transport".[2] The experiments summarized here are concerned with the way in which oat-leaf blades react to kinetin, and illustrate how the responses vary in leaves and leaf tissues of different ages.

The detached blades we have used are terminal 6-cm portions cut from first leaves of *Avena sativa* (var. Victory) seedlings grown under fluorescent lamps. These blades are convenient for 'floating' and 'drop' treatments. In the former, they are floated adaxial side up on test solutions, and in the latter, 0·01-ml. drops are placed on the adaxial surface. In all cases, the blades were kept in a water-saturated atmosphere in darkness during the period of treatment.

In experiments involving movement of labelled materials, the distribution of radioactivity in the blades was ascertained by passing them individually through a chromatogram scanner. Although it is possible to scan living blades repeatedly and thus follow movement during the course of treatment, we have generally dried the blades before scanning. This reduces self-absorption, and, in our conditions, does not bring about longitudinal movement of radioactive material in the blade tissues.

Fig. 1 shows some typical traces of the distribution of carbon-14 and phosphorus-32 in blades passed into the scanner at 6 in./h. A $\frac{1}{16}$ in. collimator slit under the detector provided sufficient resolution, and a twice magnified trace was obtained on a chart recorder running at 12 in./h. As indicated in Fig. 1, radioactive substrates were supplied to the tips of the blades, while the bases received 0·01-ml. drops containing 0·3 μg kinetin. Traces (*a*) to (*d*) illustrate accumulation of labelled material at the kinetin-treated area after 1, 2, 3, and 4 days, when the source at the tip of each blade was a 0·01-ml. drop containing 0·1 μc. glycine-2^{14}C. Dashed traces represent controls without kinetin. Traces (*e*) and (*g*) are controls for two other sources—a photosynthetically labelled tip region, and a 0·1 μc. drop of phosphate solution labelled with phosphorus-32 respectively. Traces (*f*) and (*h*) represent the corresponding kinetin-treated blades, which were dried (with the controls) 40 h after the commencement of the experiment.

With all three sources it is clear that radioactivity accumulates in the tissues under or close to the kinetin drop. In other experiments this effect has been measurable after as short a period as 12 h, and more sensitive methods would doubtless detect it earlier. The lowest active dose for kinetin 'drop' treatments is 0·001–0·003 μg. 6-Benzylaminopurine is as active as kinetin, while the 6-phenyl analogue is less active. Gibberellic acid, indolyl-3-acetic acid, benzimidazole, diphenylurea, and a selection of purines and pyrimidines and their anti-metabolites have not shown comparable activity. No movement occurs in a nitrogen atmosphere.

To obtain these traces, the scanner was adjusted to a sensitivity suitable for measuring radioactivity at the kinetin-treated part of the blades. In the case of glycine-2^{14}C this was less than 10 per cent of the total absorbed by the tip, so that (as with labelled phosphate) the trace went off-scale as the tip approached the collimator-slit. Much of this residual activity is extractable with 70 per cent ethanol, but it is also present in ethanol-insoluble molecules. Thus, stripping-film autoradiography using Kodak '*A.R.*10' emulsion shows heavy labelling in guard-cells and all underlying tissues near the glycine-2^{14}C drop. On the other hand, photosynthetic labelling (by illuminating blade-tips in an atmosphere containing $^{14}CO_2$) evidently provides labelled molecules in a much more available form, for in this case the 'kinetin' and 'tip' peaks on the traces are comparable in size. Movement is almost certainly through the vascular tissues, since in stripping-film autoradiograms of sections cut from the central region of blades provided with glycine-2^{14}C at the

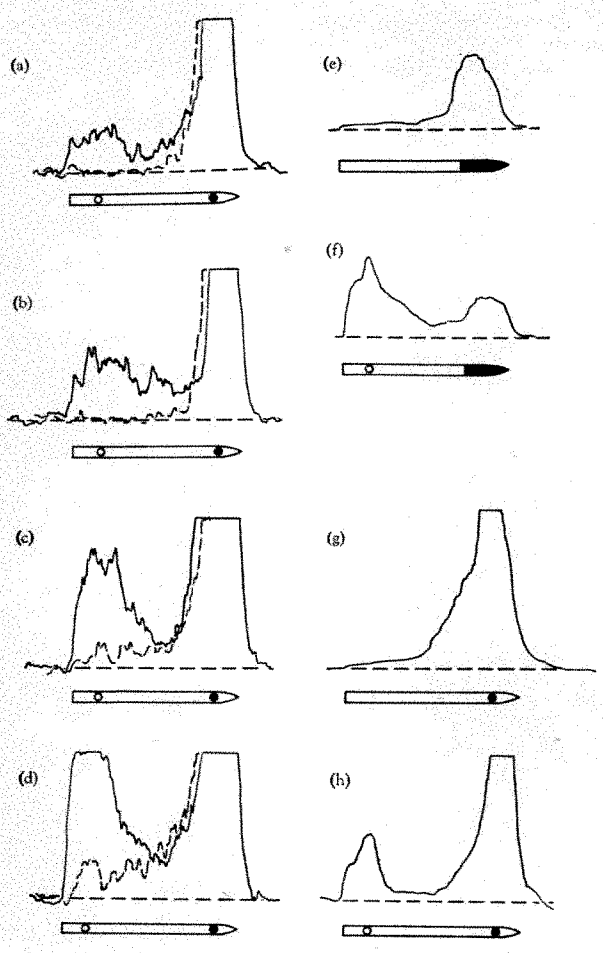


Fig. 1. Distribution of radioactivity in detached oat-leaf blades following application of labelled substrates to the tips (black circles and tips), and kinetin to the bases (open circles). In (*a*)–(*d*) dashed traces represent controls without kinetin. Horizontal lines show the level of background radioactivity

tips and kinetin at the bases, these are the only tissues that contain radioactive, ethanol-insoluble material. At the kinetin-treated area, however, labelling is general, indicating that all tissues receive transported molecules.

As in other species[3,4], carbon-14 becomes incorporated into protein and nucleic acid in kinetin-treated areas of oat leaves. Results of experiments in which such areas have been fractionated by grinding and differential centrifugation (largely according to the methods of Smillie[5]) indicate that the ribosomes play a key part in this aspect of kinetin action. The fractionation procedure we have used provides pellets of particles sedimenting over the ranges: 0–1,500*g*, 1,500–15,000*g* and 15,000–75,000*g*. The two low-speed fractions are washed and re-centrifuged before analysis, and the high speed (90 min centrifugation) pellet is analysed directly. Protein and nucleic acid are removed from the final supernatant by addition of 5 per cent trichloroacetic acid at 1° C.

Fig. 2 illustrates the time course of entry of carbon-14 from transported glycine-2^{14}C into these fractions. Fig. 1(*a*), (*b*), and (*c*) correspond to the 1-, 2-, and 3-day stages given in Fig. 2. Only the basal, that is, the kinetin-affected parts of the blades were fractionated.

Kinetin increases the radioactivity (Fig. 2(*a*)) and the nitrogen-content (Fig. 2(*b*)) of all fractions. The latter effect is not marked in the case of the 15,000–75,000*g* particles, but by contrast they do become highly labelled. This is best seen in Fig. 2(*c*), where changes in a form of specific activity, namely, radioactivity per unit nitrogen, are plotted. After one day of kinetin-influenced metabolism their 'specific activity' is more than twice that of any other fraction. Preferential labelling is maintained, although afterwards the soluble fraction does show higher values.

Similar results are obtained if labelled phosphate or $^{14}CO_2$ photosynthates are used as sources of label. In both cases, only the soluble fraction exceeds the 15,000–75,000*g* pellet in 'specific activity' after two days of kinetin treatment. Since phosphorus-32 and carbon-14 both enter these particles, it follows that their intense labelling is not simply a consequence of radioactive proteins forming on them. Electron microscopy of these mesophyll cells shows that the cytoplasmic ground-sub-

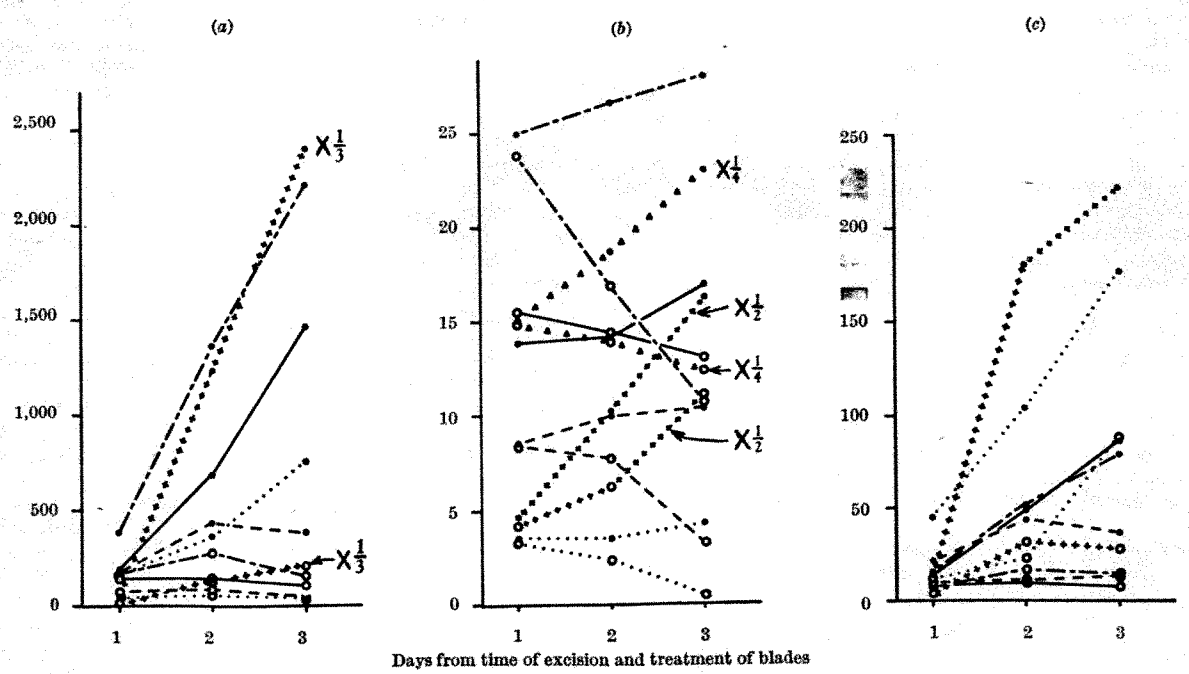


Fig. 2. (*a*) Counts per min, (*b*) μg nitrogen, (*c*) counts per min per μg nitrogen, in 2-cm basal portions of blades treated with glycine-2^{14}C at the tips and with (solid circles) or without (open circles) kinetin at the bases. Each sample, taken daily from the start of the experiment, consisted of 50 bases, and results are expressed per single base. Fractions: ——, 0–1,500*g*; – – –, 1,500–15,000*g*; ....., 15,000–75,000*g*; —·—·—, trichloroacetic acid-insoluble fraction from supernatant; + + + +, final 'soluble' fraction from supernatant; ▲▲▲, total nitrogen

stance and the chloroplast stroma are rich in ribosome-like particles (Gunning, in preparation). Assuming that protein synthesis in leaf cells occurs on such particles, and that they constitute a large part of the high-speed pellet, it seems likely that transported label becomes incorporated into their ribonucleoprotein as well as into protein that is afterwards freed. It is not yet known at what stage on the chain of events leading to protein synthesis kinetin is active, but the rapid entry of phosphorus-32 into ribosomes might suggest a primary effect on nucleic acid metabolism.

Additional evidence comes from experiments in which blade-bases are infiltrated with ribonuclease prior to treatment with kinetin. As compared with controls infiltrated with water, such bases receive much less nitrogen from the more apical regions. Glycine-2^{14}C transport is inhibited, and soluble (amino, acid, and neutral), protein, and nucleic acid fractions all show reduced labelling, as do all pellets and fractions obtained in the cell-fractionation mode of analysis.

The primary effect of kinetin could be to attract substrates; these could then enter metabolic pathways. Alternatively, kinetin could create a metabolic 'sink', with consequent attraction and utilization of substrates. In the first case, if ribonuclease were to inhibit ribonucleic acid-mediated pathways, it might be expected that transported soluble compounds would accumulate. This does not occur. In the second case, utilization of substrates, and hence the total amount transported, would be reduced. The results agree more with this interpretation; however, it is still possible that kinetin is active as a nucleic acid macromolecule[6], in which case the ribonuclease might inhibit by preventing formation of the active form. In this connexion it is of interest that ribonuclease preparations (L. Light and Co., "chromatographically purified, protease-free") have some directed transport effect on their own.

The foregoing results all refer to the behaviour of blades cut from 7–9-day-old seedlings. Such blades senesce basipetally, the basal tissues being the most recently formed, and the apical tissues the oldest. Evidence will now be summarized that shows that the effects of kinetin vary according to the stage of development of the treated tissue, and that metabolism is affected even though directed transport may not occur:

(*a*) *Directed transport.* When blades treated with kinetin at the tips and glycine-2^{14}C at the bases are scanned, no directed transport can be detected. Some acropetal movement does occur if the glycine-2^{14}C is placed in the central part of the blade, that is, closer to the kinetin.

(*b*) *Net movement of nitrogen.* There is only a slight inflow of nitrogen into the apical 2 cm of the blades in response to apical applications of kinetin. The scanning results (above) suggest that it is derived from the neighbouring tissue. Nevertheless, nitrogen metabolism at the tip is affected in that just as in bases, 'insoluble' nitrogen levels are maintained, even though the net inflow is less.

(*c*) *Age of leaf.* It is suggested that oat leaf tissue passes through a stage when it is capable of reacting to kinetin by exhibiting directed transport: thereafter, this property is lost. Fig. 3 compares the effects of apical and basal 'drop' treatments of kinetin in blades cut from 5-, 7-, 10-, and 12-day-old seedlings. None of the tip tissues reacts strongly. That this is due to the physiologically advanced age of the tissue is indicated by the fact that even the bases become less active as they age.

(*d*) *Respiration.* The respiratory drift of isolated bases (2 cm long), but not isolated tips, is influenced by kinetin. In these bases, kinetin prolongs a period of low, steady output of carbon dioxide by about three days. This occurs after an initial period of rapid respiration lasting for 12 h from the time of excision of the bases. The effect is another difference between tips and bases, and, in these isolated portions of leaf, it must be independent of the directed transport system.

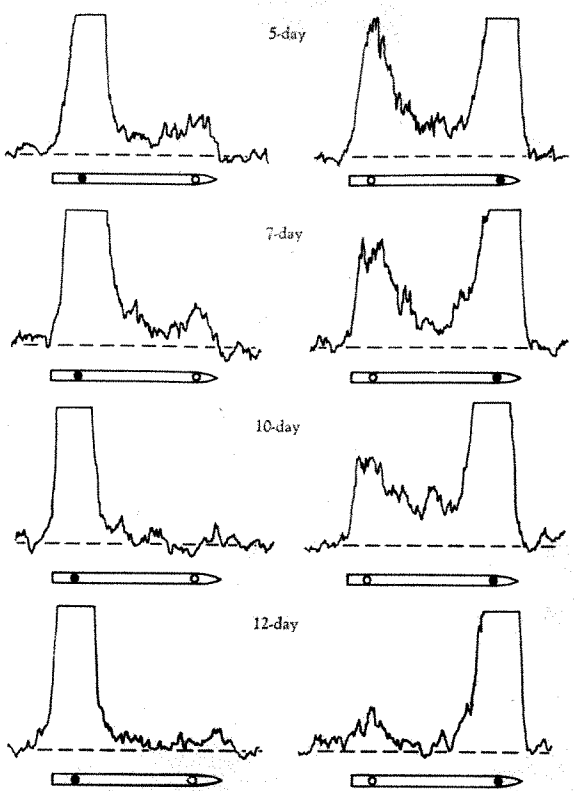


Fig. 3. Acropetal and basipetal transport in blades from 5-, 7-, 10- and 12-day-old seedlings. Open circles, kinetin (0·3 μg); solid circles, glycine-2^{14}C (0·1 μc.). No movement occurred in any minus-kinetin controls

(*e*) *Chloroplasts.* In contrast to the foregoing phenomena, kinetin affects the chloroplasts of both apical and basal parts of blades, and of different ages of leaves. Chlorophyll-levels (as determined by colorimetry of crude extracts) are maintained in treated tissues, while controls turn yellow. In 'floating' treatments, the threshold concentration is between 0·01 and 0·001 mg kinetin/l. 'Drop' treatments have been found to be effective at doses of as little as 0·0001 μg/0·01 ml. Maintenance of pigmentation reflects a general maintenance of chloroplast function, as infra-red gas analysis measurements show that so long as the pigment is present, carbon dioxide is fixed if the leaves are illuminated. The photosynthetic life of bases is prolonged by approximately the same length of time as is the period of low respiration rate.

In summary, kinetin postpones senescence in detached oat leaves. The younger the treated tissue, the more extreme are the effects. In comparatively mature apical regions of leaves, breakdown of chloroplasts is delayed, and 'insoluble' nitrogen-levels are maintained for some time. There is only a slight net gain of substrate from the neighbouring regions of the leaf. In younger tissues, such as are found closer to the basal meristem of the leaf, kinetin induces directed transport in addition to the foregoing effects. The inflowing substrate enters metabolic pathways leading to protein and nucleic acid synthesis, and small particles, probably ribosomes, are preferred receptors of carbon and phosphate derived from older parts of the leaf. Respiratory losses of carbon are reduced. As basal tissues age, they begin to react in the same way as tips, in at least some respects. Conversely, it is possible that leaf apices would show more of the responses demonstrated in bases if they were to be examined at a sufficiently early stage of development.

Since the responses described here occur in darkness, and since kinetin does not increase the respiration rate, the question of the energy source for the various reactions arises. If protein (and other) breakdown processes require

energy in leaf cells as they do in other tissues[7], and if kinetin prevents such breakdown, then the energy that would otherwise have been consumed in this way might become available to drive syntheses. It is not known whether kinetin induces syntheses directly, for example, by stimulating production of ribosomes, or indirectly, as by diverting energy from certain pathways into others. Again, it is not yet known whether the metabolic pathways stimulated under the influence of kinetin are similar to those found in normal cells, or whether certain pathways are favoured or even created. Finally, it remains to be seen whether the numerous responses to kinetin are independent, or whether they all derive from a single 'master' reaction.

We wish to thank Prof. D. J. Carr for obtaining facilities for the work, and for many stimulating discussions of the problems.

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TRANSPIRATION PHENOMENA IN DETACHED LEAVES

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MANY observations have been made[1–7] which indicate that pronounced changes in transpiration rate of leaves may occur when they are severed from the plant. Often there is a temporary increase in transpiration rate; but under some conditions there is a decrease in rate from the moment of excision.

Two contrasted explanations have been advanced to account for the increase in rate resulting from leaf excision: the first by Darwin and Pertz[2], who attribute the rise in transpiration rate to a temporary stomatal opening which precedes the closure in the later stages of wilting, and the second by Iwanoff[8], who regarded the release of water stress in the conducting system, a feature earlier noted by Dixon[9], as of primary importance, leading to an increase of water supply to the leaf. The experimental studies recently reported in *Nature* by Rufelt[7] have been interpreted by him as favouring the views of Iwanoff; but no direct information is reported regarding the behaviour of the stomata in these experiments.

Many investigations of the transpiration rate of detached leaves carried out in this laboratory indicate a close relationship of stomatal behaviour and transpiration in a range of plants under conditions resulting in a temporary widening of the stomatal apertures as well as under conditions when the leaves have a substantial water deficit and stomatal closure begins immediately the leaves are severed. Stomatal apertures have been studied by several techniques (Lloyd's strips[10], Knight porometer[11], Wheatstone bridge porometer[12]) and transpiration rates by means of a quick weighing procedure and by a hygrometer method. The observations made by the use of these various techniques were found to agree closely.

Two illustrative sets of results are quoted here. Both of the experiments cited relate to normally watered plants and the same procedure was followed. A fully expanded (but not senescent) leaf was enclosed in a 'Perspex' chamber through which was passed a constant stream of air containing 0·0377 per cent carbon dioxide (by vol.) and of 26 per cent relative humidity at 23° C. The outflowing stream (flow rate 1 l./min) was passed over the sensing element of a moisture meter (Shaw, Bradford) and the relative humidity assessed (the capacitance of the dielectric layer of the element varies with its moisture content, and this variation is indicated by the meter readings). Measurements of the carbon dioxide content of the outflowing air stream were also made, an infra-red gas analyser (Howard Grubb Parsons) being used. At the same time that records were taken of the moisture and carbon dioxide content of the outflowing-gas stream, measurements of the resistance of the leaf to gaseous flow were made by means of a Wheatstone bridge type porometer. A detachable porometer cup[13] allowed the surface of the leaf to be swept freely by the air stream between readings.

The results of an experiment on broad bean (*Vicia faba* var. Giant Seville Longpod) are shown in Fig. 1. After a period in darkness, when leaf resistance to gaseous flow was steady and high, and transpiration rate was low, the leaf, still attached to the plant, was illuminated by means of a water-cooled Osram reflector lamp giving an intensity

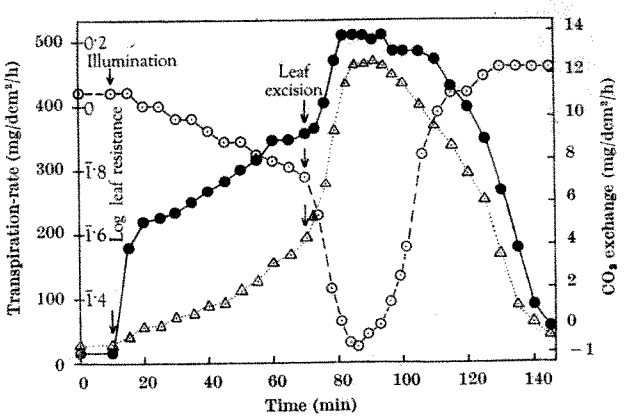


Fig. 1. Effect in *Vicia faba* of leaf excision on rates of transpiration and photosynthesis and on the leaf resistance to gaseous flow. Log. leaf resistance is expressed (as also in Figs. 2 and 3) in Gregory and Pearse units (ref. 14) and refers to an area of 38·5 mm²
△, Transpiration rate; ●, rate of carbon dioxide exchange; ⊙, leaf resistance. The times when the leaf was illuminated and severed are indicated by the arrows

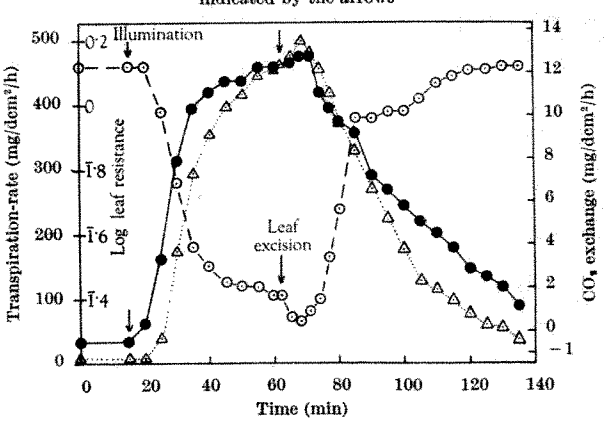


Fig. 2. Effect in *Pelargonium* × *hortorum* of leaf excision on the rates of transpiration and photosynthesis and on the leaf resistance to gaseous flow
See Fig. 1 for explanation of symbols

of approximately 1,650 ft.-candles. The results show that on illumination there was a progressive decrease in leaf resistance, a fairly steady rise in transpiration and an increasing rate of photosynthesis after a very quick initial rise. When the leaf had been illuminated for 1 h the petiole was severed. As shown (Fig. 1), excision of the leaf resulted in a sharp fall in leaf resistance, indicative of stomatal opening, and corresponding rises in both transpiration rate and rate of photosynthesis. After about 20 min from the time of cutting, the leaf resistance begins to increase and the transpiration rate to decline; after a further hour the leaf resistance and transpiration rate were fairly similar to those at the start of the experiment.

A comparable experiment on *Pelargonium* × *hortorum* gave results illustrated in Fig. 2. Here the trends of change were the same as for the broad bean, but the magnitude of the temporary decrease in leaf resistance on excision was neither so great nor was the decrease so prolonged. Nevertheless, there was a corresponding temporary increase in transpiration rate and in photosynthetic rate, results suggestive that the rates were both limited, under these experimental conditions, by the stomatal apertures.

In both the experiments described the effect of leaf excision was to accelerate very markedly the opening of stomata, which was already occurring as a result of illumination. It may be suggested that the basis of the mechanism of stomatal movement is the same under these different conditions, the stopping of the water supply to the leaves having a quicker effect on the balance of turgor of the epidermal and guard cells than a photo-induced change. The importance of the water relations of the leaf during normal stomatal opening in the light has already been noted[15].

One feature of considerable importance with regard to the occurrence, in detached leaves, of the temporary stomatal opening and increase in transpiration rate is the water deficit of the leaves at the time of excision. When the deficit is small the increase in transpiration rate is large, but when water stress is severe at the time of cutting there is a progressive decrease in transpiration rate and no evidence of any temporary opening of stomata. Studies made by Miss J. M. Marshall in this laboratory in 1954 on *Vicia faba* by means of a Knight porometer indicated that provided the leaf water deficit[15] was not greater than 11 per cent a temporary decrease in leaf resistance to gaseous flow was usually obtained on severance of the petiole; it was also found that the time taken for the stomata to open and then close to the degree of opening at the time of excision was longer the lower the water deficit. More recent investigations have confirmed these findings; representative results are shown in Fig. 3. Illuminated leaves of little deficit (approximately 2 per cent), moderate deficit (10·7 per cent) and considerable deficit (20 per cent) were severed from the plants after the leaf resistance had reached a fairly steady value (measurements made by Wheatstone bridge type porometer; an air-stream of approximately 33 per cent relative humidity at about 23° C was passed at 2 l./min over the leaves). Fig. 3 indicates that, on leaf excision, there was a substantial temporary stomatal opening in the leaf which was almost fully turgid, a small stomatal opening in the leaf of moderate water deficit but progressive closure of stomata in the leaf of high water deficit. Other experiments show that the changes in leaf resistance are paralleled by changes in transpiration rate, and that the phenomena reported occur also in leaves in darkness. It is of note that under conditions of high water stress the phenomenon of increased transpiration rate is least evident or not shown at all, whereas the Iwanoff effect might be expected to be greatest in these circumstances.

The changes reported by Rufelt[7] of the transpiration rate of leaves of aspen repeatedly taken from and brought into contact with water are explained by him on the basis of the Iwanoff theory. Under similar conditions, however,

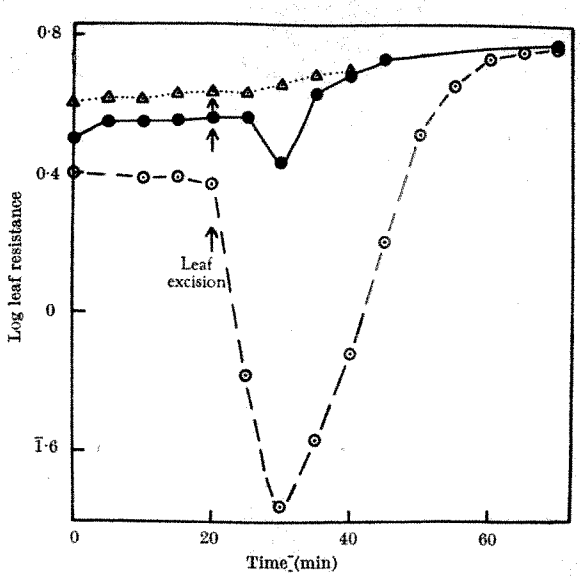


Fig. 3. Changes in resistance to gaseous flow in excised leaves of *Vicia faba* of different water deficits. Leaf of: ⊙, about 2 per cent deficit; ●, 10·7 per cent deficit; △, 20 per cent deficit

there is evidence[5] that in *Pelargonium* stomatal changes are related to changes in transpiration rate; furthermore, we have found that if water is made available to wilted leaves of *Pelargonium* that there is usually at first a decrease in transpiration rate, associated with increasing leaf resistance, and that later there is an increase in transpiration (and photosynthesis) associated with stomatal opening. It may also be noted that if leaves of, for example, *Vicia faba* are cut under water, normally a decreased transpiration rate is at first observed, a result at variance with the view that increased water supply to the leaf leads to an increased transpiration rate.

The results outlined above show that in many instances changes in transpiration rate of excised leaves are mediated by stomatal movements, doubtless arising from differential turgor changes in the epidermal and guard cells. Differences in quantity of osmotically active substances in these cells may to some extent result in differences in both uptake and loss of water, and changes in stomatal apertures are not unexpected when conditions of water supply to leaves are altered. Release of tension in the conducting system clearly occurs when leaves are severed, but the evidence briefly presented here indicates that it is not the release of water tension *per se* which results in changes in transpiration rate but the direct and indirect effect of this release on the turgor relations of the epidermal and guard cells, changes in turgor leading to changes in stomatal apertures and so influencing transpiration rate. The rapid change in the conditions of water supply to the leaf probably leads to a quick and pronounced displacement of the dynamic equilibria of water relations of the cells of the leaf, notably the guard cells and epidermal cells, so resulting in the phenomena observed.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Occultation Theory of Jovian Radio Outbursts

It has been suggested by Strom and Strom[1] that the sporadic decameter radio energy attributed to the planet Jupiter does not actually originate on that body, but comes rather from a distant background of discrete sources or 'radio stars'. According to the Stroms, the Jovian ionosphere could act as a gigantic spherical lens to focus energy on the Earth when these discrete sources are temporarily occulted by Jupiter. This theory is rendered more plausible by the work of Grossi, Strom and Strom[2], which shows that the ionosphere of the Earth itself may behave as such a lens in the frequency region of 5–30 Mc/s. The well-established correlation of the Jovian outbursts with the rotation of that planet is explained as a result of a magnetic field, tilted with respect to Jupiter's axis of rotation, which is presumed to introduce a periodic modulation in the focusing mechanism.

It is evident that if this theory is correct the observed durations of the noise storms should show a marked correlation with the apparent angular speed of Jupiter relative to the background of fixed stars. From an examination of 16 outbursts recorded by Douglas[3] during the period 1957–60, Strom and Strom concluded that such an effect did in fact exist. Recently, we have completed an analysis of our own observations of the 1961 apparition of Jupiter. Fig. 1 is a plot of the durations of 129 noise storms observed at a frequency of 18 Mc/s during this apparition. For comparison the angular speed of Jupiter on the celestial sphere is shown in the upper part of the figure. There does not in this case appear to be a systematic relationship between angular speed and noise storm duration, and this is particularly evident if one disregards the period of December 1–March 15, when the planet was too close to the Sun for reliable decametric observations to be made.

If the noise storms should result from occultations, it would further seem logical that the rate of occurrence of the storms should decrease as the angular speed of Jupiter decreases, perhaps approaching zero near the stationary points of May 26 and September 24, when the planet was motionless with respect to the celestial sphere. Fig. 1 shows that, on the contrary, the greatest concentration of noise storms occurred near the May 26 stationary point. There does not appear to be any systematic relationship between Jupiter's angular speed and the time-density of the radio noise storms.

Our flux measurements show that at frequencies greater than 17 Mc/s the Jovian radiation has a spectral index of less than -5 (that is, as the frequency, f, increases, the flux density falls faster than f^{-5}) (ref. 4). On the other hand, the great majority of the discrete sources have spectral indices ranging from $-0{\cdot}6$ to $-1{\cdot}2$. One might argue that the added steepness of the Jovian spectrum is due to frequency sensitivity of the focusing mechanism, analogous to the optical phenomenon of chromatic aberration, but in this event the relative intensities of the different frequencies should vary with the Earth–Jupiter distance, an effect that does not seem to be present in our data.

Our measurements also indicate that the Jovian flux density occasionally approaches 10^{-19} W/m²/c.p.s. at 18 Mc/s and that bursts having an intensity of 10^{-20} W/m²/c.p.s. are relatively common. Since Strom and Strom believe that the focusing mechanism yields a maximum power gain of about 1,000, their theory would seem to require the presence of a dense background of discrete sources producing individual 18 Mc/s flux densities of 10^{-22}–10^{-23} W/m²/c.p.s. During the time-interval shown in Fig. 1, the disk of Jupiter swept out an area of sky of approximately 0·37 square degree. If each of the noise storms in the figure represents an occultation, then the density of these rather strong sources must be of the order of 129/0·37 = 344 sources per square degree. (This figure is perhaps conservative, rather than otherwise, since it may well be that the occultations would have to be nearly central for reasonable power gains to be realized.) Actually, of the several thousand discrete sources that have been catalogued, relatively few are known to produce 18 Mc/s fluxes of the required intensity. If sources of large angular subtense are excluded, as the occultation theory requires,

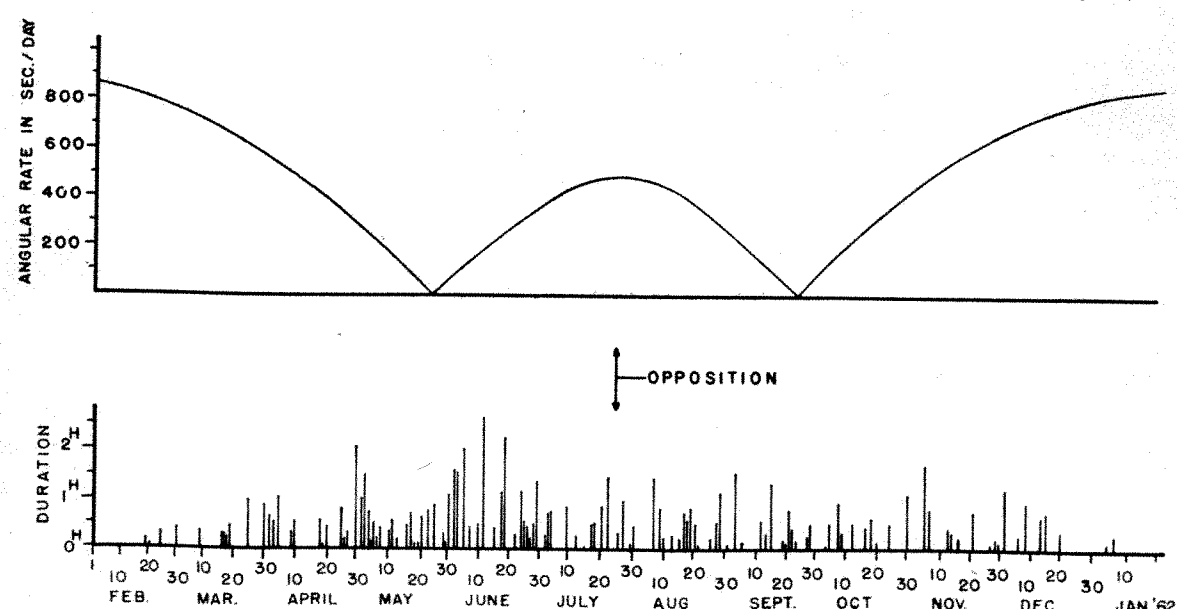


Fig. 1. Duration and time density of Jovian noise storms at 18 Mc/s during the 1961 apparition, compared with the planet's apparent angular speed

this number is still further reduced. By assuming a mean spectral index of $-1{\cdot}0$, a rough estimate of the 18 Mc/s source population can be obtained from the source density figures derived from Mills's 100 Mc/s survey[5]. The predicted density of sources having an 18 Mc/s flux greater than 10^{-23} W/m²/c.p.s. is only about two per steradian in the portion of the sky traversed by Jupiter during the 1961 apparition. While scintillation may affect somewhat the amplitudes of the Jovian bursts, it seems unlikely to alter in an essential way the orders of magnitude that are involved.

As a result of these discrepancies we have been led to conclude that our observations do not lend support to the occultation theory of the Jovian radio outbursts.

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Frequency Drift and Time Profile of 200 Mc/s Bursts of Type III and the Electron Temperature of the Corona

The most prominent feature of solar bursts of spectral type III is their frequency drift from higher to lower frequencies. Wild[1] has determined the drift velocity in the frequency region of 70–130 Mc/s, but until now there have been no satisfactory determinations of the drift rate on higher frequencies.

We therefore undertook to determine the frequency drift velocity of type III bursts from high-resolution swept frequency observations made in the 205–230 Mc/s range at the Oslo Solar Observatory between the middle of 1961 and late 1962.

Fig. 1 gives a histogram showing the distribution of bursts of different drift velocities. The curve has a maximum around -40 Mc/s² and a tail which extends to roughly -150 Mc/s². It is noteworthy that no bursts were observed with frequency drift velocities less than -20 Mc/s². The total number of bursts which were included in the analysis amounted to 68. The mean drift velocity as calculated from the histogram is $\mathrm{d}f/\mathrm{d}t = -60 \pm 10$ Mc/s².

In his observing range, Wild[1] found that the frequency drift velocity was approximately proportional to the frequency, and was given by $\mathrm{d}f/\mathrm{d}t = -f/k$, where k was a constant which varied from one burst to the next one. The mean value of k determined from 10 bursts was 4·5 sec. Inserting our observed mean value of $\mathrm{d}f/\mathrm{d}t$ in Wild's empirical equation, and using 215 mc/s as the centre frequency of the observing range, we find $k = 3{\cdot}6 \pm 0{\cdot}6$. This is relatively near the value found by Wild, but indicates that k decreases with increasing frequency. It is interesting to note that the observed variation of k agrees closely with that expected for an exciting disturbance propagating at constant speed in a corona in which the electron density follows Newkirk's[2] model for an active region.

The time profiles of the bursts in our observational material have been found from photometer tracings along the time axis of the records. For this purpose only distinct bursts, in which the maximum intensity did not saturate the records, were selected. The mean time profile of the bursts was slightly asymmetrical, and had a rising part which was steeper than the decaying portion.

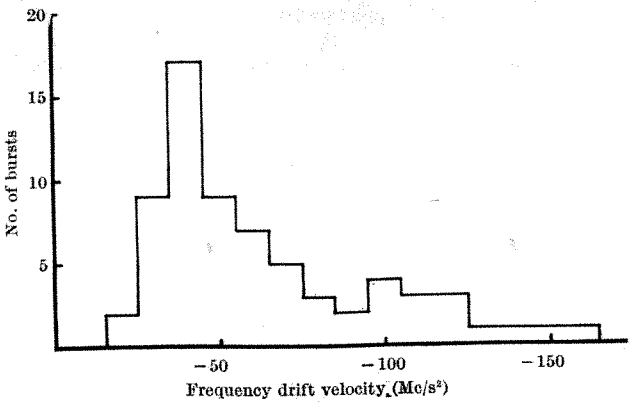


Fig. 1. Distribution of bursts of different frequency drift velocities

We now adopt the conventional model of type III bursts. The bursts are generated by a disturbance travelling at high speed through the corona, exciting oscillations with frequencies which are determined by the plasma frequencies at the appropriate heights. The oscillations are damped by collisions between electrons and ions. The time profile as observed on a single frequency can then be calculated in the manner outlined by de Jager[3]. The factors of importance for the calculated profile are the frequency drift velocity, the band-width of the excited oscillations and the collision frequency between electrons and ions. Using a frequency drift of -60 Mc/s², we found excellent agreement between the observed and the calculated time profile for a half-power band-width of 28 Mc/s and a collision frequency of 8 per sec. The large band-width agrees with the impression we get from a visual inspection of the records. The natural band-width of electron oscillations in the solar corona seems to be small, and it is therefore reasonable to suppose that the observed band-width is determined by the dimensions of the exciting disturbance. Using Newkirk's model of the variation of electron density with height in an active region, we find from our observations that the depth of the exciting disturbance at a height of $1{\cdot}15\ R_\odot$ is about $0{\cdot}05\ R_\odot$.

From a similar analysis of the time profiles of type III bursts observed by Weiss and Sheridan[4] at 40 Mc/s we find evidence for an expansion of the exciting disturbance as it travels out through the corona. If the disturbance is assumed to be a cloud of particles, expansion would occur if the particles were ejected with an initial velocity dispersion.

When the electron density and the collision frequency are known, the electron temperature can be determined. The value of 8 per sec for the collision frequency gives $2{\cdot}1 \times 10^6$ deg. K for the electron temperature at the height of $1{\cdot}15\ R_\odot$. This is in good agreement with a streamer temperature of $2{\cdot}3 \times 10^6$ deg. K as found by Hepburn[5] from optical eclipse observations.

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GEOPHYSICS

Particle Sampling from Noctilucent Clouds

PARTICLE sampling experiments have been conducted at the mesopause region of the atmosphere. The experiments utilized sounding rockets which were fired from the Kronogård range (66° N, 19° E) in northern Sweden, during the summer of 1962. Two successful flights out of a total of four firings were achieved: one (August 11, 0140 G.M.T.) in the presence of noctilucent clouds and one (August 7, 0047 G.M.T.) when no such clouds could be visually observed from the ground and from aircraft. The *Nike-Cajun* rockets carried identical instruments and in so far as can at present be determined the experimental conditions of the two successful flights were similar.

The experimental apparatus consisted of sampling surfaces mounted in two cylindrical containers symmetrically mounted about the longitudinal axis of the rocket at the forward end of the payload. Four high-purity collection surfaces: (1) nitrocellulose film with coating of aluminium; (2) nitrocellulose film coated with fuchsin dye; (3) indium-coated 'Lucite'; (4) calcium film with a thin protective coating of aluminium, paraffin and silicone oil, were mounted in each container. The different surfaces were intended to retain non-volatile particles or portions of particles for laboratory analysis as well as to indicate whether volatile particles or coatings had been deposited. To minimize contamination from terrestrial particles and to aid in the identification of such contaminants, techniques similar to those used in previous rocket samplings were used[1]. The primary controls consisted of areas which were shielded from particles moving in ballistic trajectories but not from laboratory particles.

From previous experience[2] the period August 1–20 was chosen as the most favourable time for observing noctilucent clouds at the firing-range location. During the twilight hours the sky was visually patrolled by trained observers (ground-based and airborne) 150 km to south where the conditions for viewing the phenomenon, if present over the rocket range, were optimum. Photographs were obtained of the clouds through which the second rocket was fired.

The collection surfaces were exposed at an altitude of approximately 75 km by the spring ejection of an outer rocket-nose tip. A second spring mechanism closed the sampling ports at the forward end of the nose cone and vacuum-sealed the containers which held the surfaces. This latter action occurred at an altitude of approximately 95 km while the rocket was still ascending. No attempt was made to sample during the descent of the rocket. The containers from the successful flights were recovered sealed and in excellent condition within an hour after the firings.

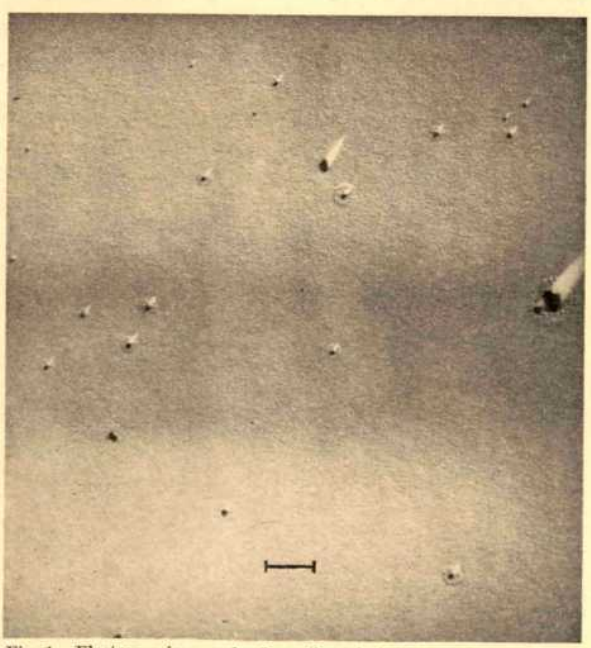


Fig. 1. Electron micrograph of noctilucent cloud particles on a nitrocellulose film which is about 200 Å thick and has had an evaporated coating of aluminium applied before collection and was shadow-cast with chromium afterwards (scale = 1μ)

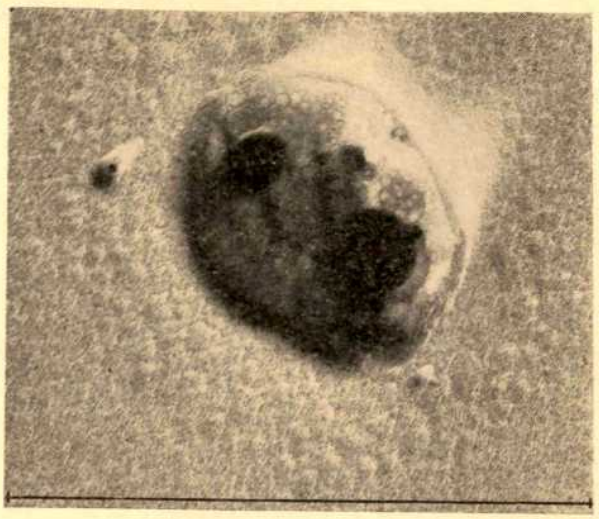


Fig. 2. Electron micrograph of a noctilucent cloud particle on a substrate prepared as in Fig. 1 (scale = 1μ)

After the flights, the gas in the containers was examined mass spectroscopically to determine if any volatile products foreign to the lower atmosphere had been obtained. Outgassing and leakage would be expected to mask slight concentration changes of gases or vapours normally found in the sea-level environment. Only traces of alcohol and acetone, which were both used in the cleaning operations, were detected by this technique.

The collection surfaces from the two flights show significant differences. The first and most apparent difference is that the nitrocellulose films from the cloud flight contain non-volatile particles the numbers per unit area of which are greater by two to three orders of magnitude than the surfaces from the non-cloud flight and the contamination level as determined from the control areas. These cloud particles are primarily in the size-range 0·05–0·5μ in diameter. Preliminary electron microscopic scanning indicates that in this size-range there exists an integral distribution function of the form $N \propto d^{-p}$, where p lies between 3 and 4. However, there appears to be a distinct cut-off in the power distribution for particles smaller than about 0·05μ. The cut-off size varies somewhat with sample position. The number of particles and the observed size distribution are in agreement with the observed light scattering properties of the clouds[3]. A significant percentage of the particles as seen on the nitrocellulose substrate are surrounded by a circular pattern. Some typical particles and patterns can be seen in the low-magnification electron micrograph shown in Fig. 1. The higher magnification electron micrograph (Fig. 2) shows the details of one large particle surrounded by such a pattern. Preliminary evidence indicates that this pattern was caused by a volatile coating evaporated from the particle after deposition and that this coating was ice. A number of the particles found on the surfaces recovered from the non-cloud flight show distinct similarities with those obtained from the cloud flight and from a previous rocket sampling at higher altitudes[1]. There is, though, no evidence of ring patterns surrounding any of the particles from these non-cloud flights. Fig. 2 also shows dark spherical inclusions in the particle which are opaque to 100-keV electrons. This feature is observed in a large number of the particles.

On prolonged intensive electron bombardment it is found that these opaque inclusions are by far more stable than other portions of the particles. Composition analysis using an electron beam microprobe has been started and yields evidence of iron and nickel. Further, some of the particles give a consistent electron diffraction pattern which has as yet not been identified.

Statistical and composition analysis is continuing. A more detailed description of these experiments and the quantitative information gained from them will be presented in the near future. A programme for similar experiments to measure other parameters (for example, particle concentration as a function of altitude) associated with the clouds is at present being formulated.

The rocket flights and analysis described here have been a joint undertaking of our institutions under the international sponsorship of the Swedish Space Committee and the U.S. National Aeronautics and Space Administration. The success of the programme is due to the co-operation of many American and Swedish individuals and organizations too numerous to fully acknowledge in this communication.

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Retention of Radiogenic Argon in Glauconites from Proterozoic Sediments, Northern Territory, Australia

Three glauconite concentrates from Proterozoic sediments of the Carpentaria region of the Northern Territory which have been dated by the potassium–argon method show that there is almost complete retention of radiogenic argon in these samples when compared with control ages by the potassium–argon and rubidium–strontium methods on micas and whole-rock samples.

Previous potassium–argon determinations on glauconites have shown that many of the measured ages are too young by amounts ranging up to, and in some cases exceeding, 20 per cent[1,2]. Evernden *et al.*[1] showed that there is generally, but not universally, an argon-loss pattern dependent on the depth of burial. On the basis of general agreement between potassium–argon and rubidium–strontium results on glauconites Hurley[2] concluded that argon leakage may not be wholly responsible for the young ages; but rather that some mechanism of slow structural development of the crystal lattice has occurred, which has resulted in an increase of potassium content with time. Russian workers have dated large numbers of Proterozoic glauconites, and, although independent control of these ages usually has not been possible, Polevaya *et al.*[3] regard glauconite dating as 'largely successful'. There have been no reports of potassium–argon dates older than the age of the sediments in which they occur; this provides further support for the accepted view that glauconites are of authigenic origin.

A report on the regional geology of the Carpentaria region is in preparation by officers of the Bureau of Mineral Resources[4]. Proterozoic sediments, ranging from 13,000 to 45,000 ft. thick, crop out over an area exceeding 50,000 square miles adjacent to the west coast of the Gulf of Carpentaria. The sediments are mainly well sorted, shallow water, shelf deposits, even in those areas where the maximum thickness is developed. The almost complete absence of detrital minerals, other than quartz, suggests that deposition occurred very slowly in a shallow, slowly subsiding basin. Volcanic rocks are present in minor amounts throughout the sequence. Subsequent folding over the whole area has been slight; dips commonly are less than 10°, but locally may be as high as 60°. Since the Precambrian, only a few hundred feet of flat-lying Cambrian(?) and Mesozoic sediments have been deposited.

The Proterozoic sediments are divided into three groups, which from oldest to youngest are the Tawallah, McArthur and Roper Groups. Two of the glauconite samples (*GA*753, *GA*754) are from the Aquarium Formation (Calvert Hills sheet 1 : 250,000 *SE* 53–8), a unit which is about 1,200 ft. below the top of the Tawallah Group in this area. The third sample (*GA*752) is from a locality, 120 miles to the north-west, in the Rosie Creek Sandstone, which is probably stratigraphically equivalent to the Aquarium Formation[5]. The estimated maximum overburden for *GA*753 and *GA*754 is about 2,500 ft., and that for *GA*752 is in excess of 15,000 ft.[5].

Control as to the maximum possible age of the sediments is provided by the age of 1,780 m.y. for the Norris Granite[6], which is unconformably below the Tawallah Group. A younger limit is given by the maximum age of 1,480 m.y. ($\lambda_{Rb} = 1{\cdot}39 \times 10^{-11}$ yr^{-1}) for the Packsaddle Microgranite[6], which intrudes the Tawallah Group and is believed to have been a feeder to a volcanic member (Hobblechain Rhyolite) in the uppermost formation of this Group.

All three concentrates consisted of discrete dark green pellets of glauconite in the size-range 0·1–0·3 mm. They were contaminated by up to 10 per cent of hæmatite-encrusted quartz with similar specific gravity and magnetic properties to the glauconite, thereby rendering beneficiation difficult. They were free from detrital micas and feldspars. An attempt was made to remove some of the impurities from *GA*754 by splitting the sample into two fractions, *A* and *B*. Fraction *A* was washed in warm 0·1 N hydrochloric acid. Potassium was determined by flame photometry as described by Cooper[7]; weighings were made from 1-g splits of the concentrates, which were pulverized. Argon determinations were made on splits of about 0·2 g by isotope dilution as previously described[8], the only modification being that the line was baked at 130°–160° C instead of the usual 270° C.

Table 1. Potassium–Argon Results on Glauconites from Proterozoic Sediments, Northern Territory

Sample No.	Potassium analyses (wt. per cent)	Average potassium (wt. per cent)	$^{40}Ar^*/^{40}K$	Atmos. ^{40}Ar (%)	Calculated Age (m.y.)
GA 752	7·16, 7·14	7·15	(1) 0·1369	1·4	1,523
	7·16, 7·14	7·15	(2) 0·1366	1·5	1,521
GA 753	5·81, 5·82	5·82	0·1304	1·8	1,473
GA 754	*A* 6·52, 6·51	6·52	(1) 0·1450	1·4	1,584
	A 6·52, 6·51	6·52	(2) 0·1411	1·1	1,555
GA 754	*B* 6·43, 6·43	6·43	(1) 0·1436	0·8	1,573
	B 6·43, 6·43	6·43	(2) 0·1473	1·7	1,601

$^{40}Ar^*$, radiogenic component. $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr^{-1}; $\lambda_k = 0{\cdot}584 \times 10^{-10}$ yr^{-1}; $^{40}K = 1{\cdot}22 \times 10^{-4}$ g/g K.

Sample localities:
GA 752: 15°55′ S, 135°40′ E, Rosie Creek Sandstone.
GA 753: 17°18′ S, 137°22′ E, Aquarium Formation.
GA 754: 17°28′ S, 137°39′ E, Aquarium Formation.

Results are given in Table 1. Duplicate potassium analyses agree to within 0·5 per cent, and duplicate argon results agree to better than 2 per cent. Polevaya *et al.*[3] suggested that because of the cation exchange properties of glauconites, a small amount of potassium in the mineral may be loosely adsorbed on the surface of the pellets. They showed that this potassium may be removed by treating the sample in dilute hydrochloric acid (0·05–0·1 N) without causing any loss of radiogenic argon. From Table 1 it is seen that the potassium content of *GA*754 actually increased, which suggests that removal of impurities occurred rather than removal of loosely held potassium. The results indicate that the acid washing

of sample *GA754 A* has had little or no effect on the measured age.

Glauconites *GA753* and *GA754*, from the Aquarium Formation, yield ages of 1,475 and 1,580 m.y. respectively, and sample *GA752* from the Rosie Creek Sandstone has a measured age of 1,525 m.y. It may have been expected that *GA753* and *GA754* would give similar ages, whereas *GA752* would yield a lower age because of its greater depth of burial. If it is assumed that the measured age for *GA754* is close to the true age of the Aquarium Formation then *GA753* has retained 93·5 per cent of its radiogenic argon, and *GA752* has an argon retentivity of 96·5 per cent, provided that the correlation of the Rosie Creek Sandstone with the Aquarium Formation is correct. The ages correspond to the lower part of the Middle Proterozoic according to the time-scale of the Geological Survey of Canada[9], and to the Lower Proterozoic according to the present Russian time-scale[10,11].

The measured ages of the glauconites are consistent with the control ages obtained on the underlying Norris Granite (1,780 m.y.) and the intrusive Packsaddle Microgranite (1,480 m.y.). Field observations indicate that the Packsaddle Microgranite probably acted as a feeder to the Hobblechain Rhyolite in the upper part of the Tawallah Group. There is no evidence of a hiatus between the deposition of the Aquarium Formation and the extrusion of the Hobblechain Rhyolite. Hence, the age of 1,580 m.y. for sample *GA754* may indicate that sedimentation in this area was extremely slow, which supports the conclusion reached independently from geological observations. The results show that under certain conditions it is possible to obtain reliable ages on Proterozoic glauconites. The discrepancies found by other workers[1,2] on glauconites of Palæozoic and younger age may be caused, in part, by other factors besides depth of burial and the slow increase of potassium with time. Many of these younger samples are from rocks which, although not actively involved in orogeny since their deposition, occur in generally unstable areas. Our samples, and much of the Russian material, come from very stable cratonic regions, and it is suggested that the tectonic history of the region may have an important bearing on the degree of argon retention in glauconites.

Note added in proof. Richards[12] has reported an isotopic analysis of lead from a galena (Pb-69) from the McArthur Group, which overlies conformably the Tawallah Group. This galena is thought to be of syngenetic origin. The preferred model age of the lead is about 1,560 m.y.; this is broadly consistent with the glauconite ages. Hence, now it is unnecessary to regard the lead as anomalous.

The samples were collected by geologists of the Bureau of Mineral Resources, and the age measurements were made in the Australian National University. The glauconite was separated by R. Rudowski. We thank H. G. Roberts for helpful discussions.

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PHYSICS

Simultaneous Sampling of Cosmic Radiation at Different Altitudes

A technique has been used with limited success to obtain simultaneous measurement of cosmic radiation at two different altitudes.

The method consists of suspending two packets of nuclear emulsion plates from the one balloon, while maintaining a constant vertical separation of 10,000–27,000 ft. between the packets. The separation has been maintained throughout level balloon flights of up to 4 h duration. This technique is of value in the investigation of the variation of cosmic radiation with height, as in this way the two packets do not separate in latitude and longitude—a factor which enters if two separate independent balloons are flown.

This has been achieved by carrying aloft a cone of nylon string which was allowed to unwind at a predetermined height, leaving one packet of plates suspended at the balloon while the other fell at the end of the string. Using 840 denier twisted nylon string of 10-lb. breaking strain, success has been limited to 'drop-off' loads of less than 300 g. The expansible balloons used were kept at a constant pressure-level for periods of up to 4 h by the use of a mechanical valve as described by Hopper and Laby[1].

The balloon train, as it leaves the ground, is as illustrated in Fig. 1, with the lower section (below the dotted line) being dropped off at the pre-determined height. Radar corner reflector targets at the balloon and on the released section have been necessary to obtain records of both emulsion packets, and to facilitate recovery of emulsions and nylon string. Tracking has been carried out using a 277 *Q* 10 cm radar set. A typical altitude-time record of a successful flight is shown in Fig. 2.

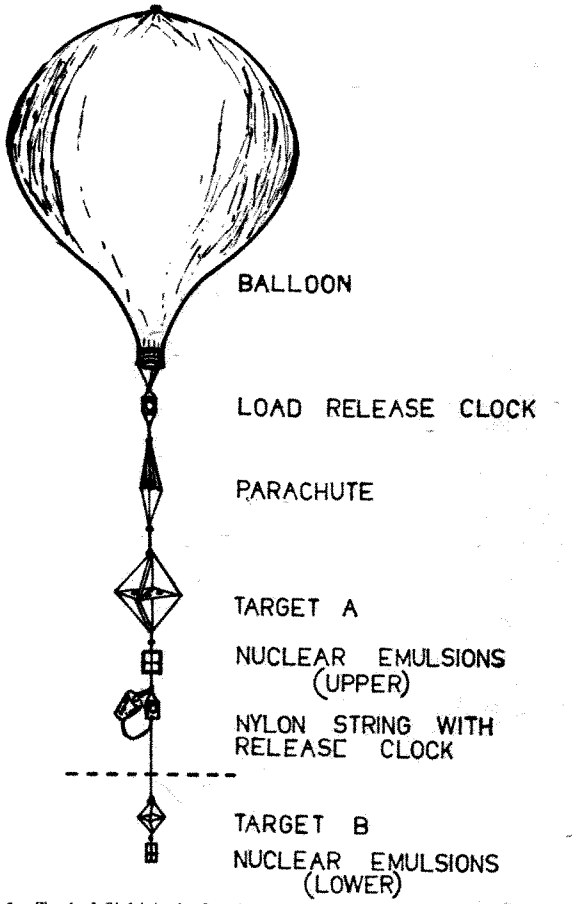


Fig. 1. Typical flight train for simultaneous cosmic ray measurements at different altitudes

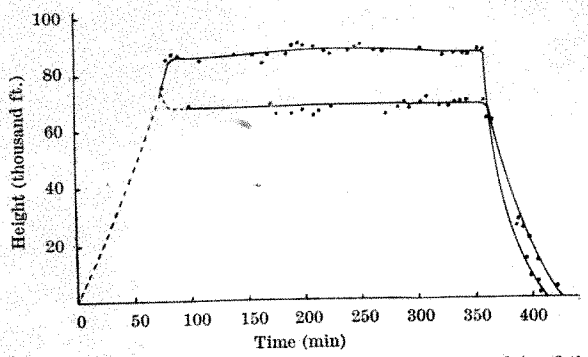


Fig. 2. Flight curve of March 29, 1962, showing radar plots of the two targets separated by 20,000 ft. 1,750 g balloon; released 9 a.m.

The nylon string is wrapped tightly in polythene sheeting to exclude moisture and prevent freezing during the ascent and also to prevent the nylon falling off the cone before it is released by its alarm clock, say 75 min after release of the balloon from the ground. As the alarm rings, the alarm winder releases the key (Fig. 3) holding the lower packet and target, which then fall away freely, unwinding the string in the process. Twenty thousand feet of string unwinds in approximately 10 min.

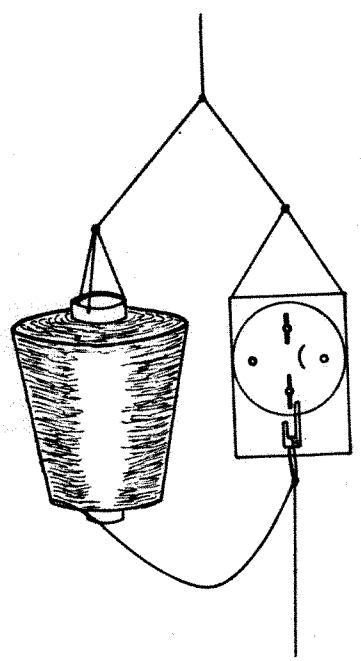


Fig. 3. Nylon string with release clock

A series of 30 flights, using nylon strings, undertaken to develop and test this technique has yielded 7 known successes. Of the remainder, only about 30 per cent are certainly failures of the string to hold the load during the fall or at the end of the fall. A further series of 10 flights using polyethylene monofilament string has yielded only two partial or limited successes and tests with this string have been discontinued.

Details of materials and conditions which have so far led to success with this technique are as follows: balloons used, 800, 1,750 and 2,400 g 'Darex'; string, nylon 840 denier, twisted; string-length, 10,000–27,000 ft.; total load dropped off, up to 300 g; height of release, 60,000–80,000 ft.; corner reflectors, target *A*, 30 in. double: target *B*, 12 in. double; wind at height of release, less than 15 knots.

One of the major difficulties encountered has been that of obtaining sufficient lengths of unbroken string and ensuring that, where knots are unavoidable, they are tied and glued in such a way that they achieve a high percentage of the strength of the unbroken string.

It is of interest to note that in several instances, although the string was intact during the level portion of the flight, it broke after the parachute was released and the two packets fell freely to the ground.

It seems highly probable that much greater loads could be dropped off with proportionally heavier string and the technique extended to sampling at three or more heights simultaneously. This would probably necessitate use in uninhabited regions where a greater breaking strain nylon would not become a hazard when it is eventually released from the balloon. Further applications might be found if heavier string permits the dropping of the necessary telemetry equipment to make simultaneous meteorological soundings.

We thank Mr. F. Alexander and Mr. W. Thornton, of British Nylon Spinners, Victoria, for their assistance, and the Australian Bureau of Meteorology, for use of radar facilities.

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Ball Lightning

Ball lightning has been described[1–3] as an approximately spherical mass of plasma about 20 cm diameter, moving at 2 m sec^{-1} or less, sometimes rotating, emitting bright radiation, having a lifetime of a few seconds or less and sometimes disappearing explosively.

Various explanations of these phenomena have been put forward[4,5], and I now suggest that the lightning ball consists of a plasma vortex ring. Vortex rings are hydrodynamically stable[6] and are easily produced, for example, as smoke rings. Plasma vortex rings have been observed in the laboratory for a few microseconds[7,8] and rotating plasmas[9] have been studied as a means of storing electrical energy in a "hydromagnetic capacitor".

If the plasma possesses vorticity $^6\zeta$, a vortex of radius r_0 has a strength k of $\zeta r_0^2/2$. Then a vortex ring of minor radius r_0, major radius α moves with velocity $k/(2\ \alpha)$ parallel to its major axis. The mean angular velocity about the minor axis is k/r_0^2, giving a kinetic energy of the order of $\pi^2\rho\alpha k^2$ for a plasma of density ρ. If the vortex ring is produced from a plasma in a magnetic field, the centrifugal force due to the vorticity is balanced by a magnetostatic pressure[10]. The trapped magnetic field prevents expansion of the ring.

A plasma vortex ring with a translational velocity of 2 m sec^{-1} and major radius of 10 cm has a vortex strength of 4×10^3 cm^2 sec^{-1}. The lightning channel may produce a shock velocity of 10^6 cm sec^{-1}, the resulting gas density being many hundred times that of air at N.T.P. Consequently, the kinetic energy associated with the vorticity can attain a value of a few hundred joules. This energy would produce an appreciable explosion if the field and current distributions were suddenly disrupted, as they might be on collision with a metallic object.

The lifetime of the plasma vortex is controlled by loss of thermal energy and dissipation of the magnetic field. If the plasma temperature is 10^4 °K, a value reported[10] for lightning flashes, the magnetic diffusivity D_m is of the order of 10^{-5} cm^2 sec^{-1} and the time r_0^2/D_m for the field to diffuse out of the ring is greater than 10^5 sec. In addition to kinetic energy, the plasma possesses thermal

and ionization energies amounting to several megajoules. This energy is mainly lost by radiation, at a rate which is initially of the order of 10^7 W. Consequently, the lifetime of the plasma must be a few seconds or less. Viscous dissipation and thermal conduction to the surrounding atmosphere increase the major radius of the plasma vortex ring and reduce its translational velocity.

The vorticity of the lightning ball could be produced impulsively by asymmetric expansion of a lightning stroke, particularly if the stroke occurred in the vicinity of a solid object having an aperture. Reports of ball lightning describe its arrival down chimneys or through a doorway. It is not easy to see how the appropriate field distributions could be set up by such an event but this may be a factor contributing to the rarity of the lightning ball.

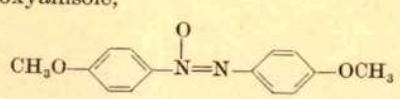
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Liquid Crystals in an Electric Field

It has long been known that nematic liquid crystals show remarkable orientation effects in electric fields as well as in magnetic fields[1]. We have observed the formation of domains in thin layers of the liquid crystal phase of several organic compounds. Typical results were obtained with *p*-azoxyanisole,



The domains form in electric fields of about 3,000 V/cm. Their appearance and behaviour suggest several properties of liquid crystals which have not previously been reported.

The properties reported here may be observed at any temperature within the range 116°–134° C, over which *p*-azoxyanisole forms a nematic liquid crystal phase. Samples of the material are melted between glass plates the inner faces of which have a transparent conductive coating of tin oxide. Samples 25–100μ thick are observed by unpolarized transmitted light while a voltage is applied between the conductive coatings on the plates. As the voltage is increased from zero, nothing is observed until a critical field strength is reached where parallel domains form as illustrated in Fig. 1. The ragged vertical line near the centre of the illustration is the edge of the conductive coating on one of the glass plates. To the left of this line there is an electric field perpendicular to the plane of the page, and to the right of the line there is no field. The domains are seen in the left half of the picture. They form within a time of about 2 msec after applying the field and disappear in about 20 msec after the field is removed.

Substantially identical domains are observed with either an a.c. or a d.c. field. With a.c. fields the effect is observed for all frequencies below an upper limit which ranges from 15 to 100 kc/s in different specimens.

If the domains are observed with monochromatic light their appearance does not change with changes in wavelength of the light, showing that they are not due to an interference effect. Because the resistivity of the liquid is about 10^9 ohm-cm, the domains could not result from electrolysis or mass transport due to current flow. Since they are seen by transmitted light which is unpolarized, the domains must be regions of differing refractive index. It may be seen from Fig. 1 that there are several sharply bounded areas within which the domains are parallel. At the boundary the orientation changes discontinuously and, in the adjoining area, the domains are still parallel but with the new orientation. The lines separating these areas are present without the field and remain unchanged when the field is applied. Several such lines may be seen in the right half of the picture where they form small closed loops. These lines are the 'threads' which are always observed in nematic liquid crystals and from which the name 'nematic' is derived[2]. The parallel domains within these bounded areas and the sharp changes of orientation on crossing a boundary suggest clearly that the threads are analogous to the crystal boundaries in an ordinary solid polycrystalline aggregate.

This analogy is further illustrated by the behaviour when the liquid is heated above the transition temperature to the isotropic liquid[2] and then cooled until the nematic liquid crystal phase again forms. When the nematic phase first forms there are many such small bounded areas, each with its own orientation as revealed by the domains. Over a period of minutes, these areas coalesce to a small number of larger areas having millimetre dimensions. Within each of these the domains are parallel and uniformly oriented throughout. In the isotropic liquid the individual molecules are randomly oriented, while in the nematic liquid there is a high degree of parallel ordering of the long molecules[3]. It would appear to be a natural consequence of the space-filling requirements of elongated molecules that the transition from the isotropic phase to the ordered nematic phase results in adjacent areas of differing orientation.

Experiments are still being carried out to elucidate the structure which gives rise to the domains. Tentative results indicate that the domains result from forces of the same general kind which give rise to domains in ferroelectric crystals, that is, cancellation of persistent internal polarization by polarization reversal in neighbouring

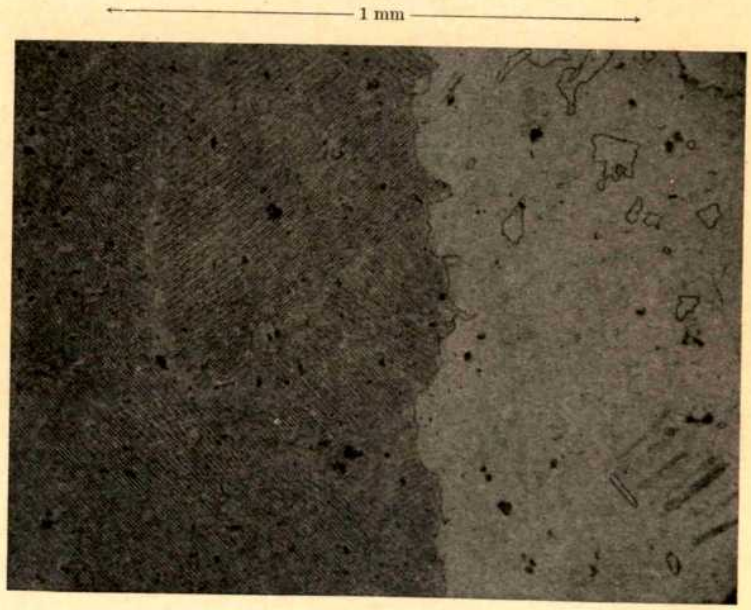


Fig. 1. Domains formed in liquid crystal phase of *p*-azoxyanisole by applying a 1 kc/s a.c. electric field of 3,000 V/cm in a direction perpendicular to the plane of the page. Further details of the experiment are given in the text

volume elements. Such polarization must necessarily be small since the liquids are not ferroelectric[3]. It could be brought about by the combined ordering effects of the walls and the intermolecular forces characteristic of nematic liquids.

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Secondary Electron Emission of Evaporated Titanium Films

The secondary electron emission coefficient δ of evaporated titanium films has been measured at target voltages between 0 and 1,875 V with electron current densities ranging between 2 and 50 μamp/cm². The bias voltage applied to the secondary electron collector was between 0 and +12 V. The experiments were done in an ultra-high vacuum system made of stainless steel, sealed with aluminium wire gaskets, and pumped by a fractionating oil diffusion pump. The diffusion pump was fitted with a liquid nitrogen trap and charged with silicone 704 fluid[1].

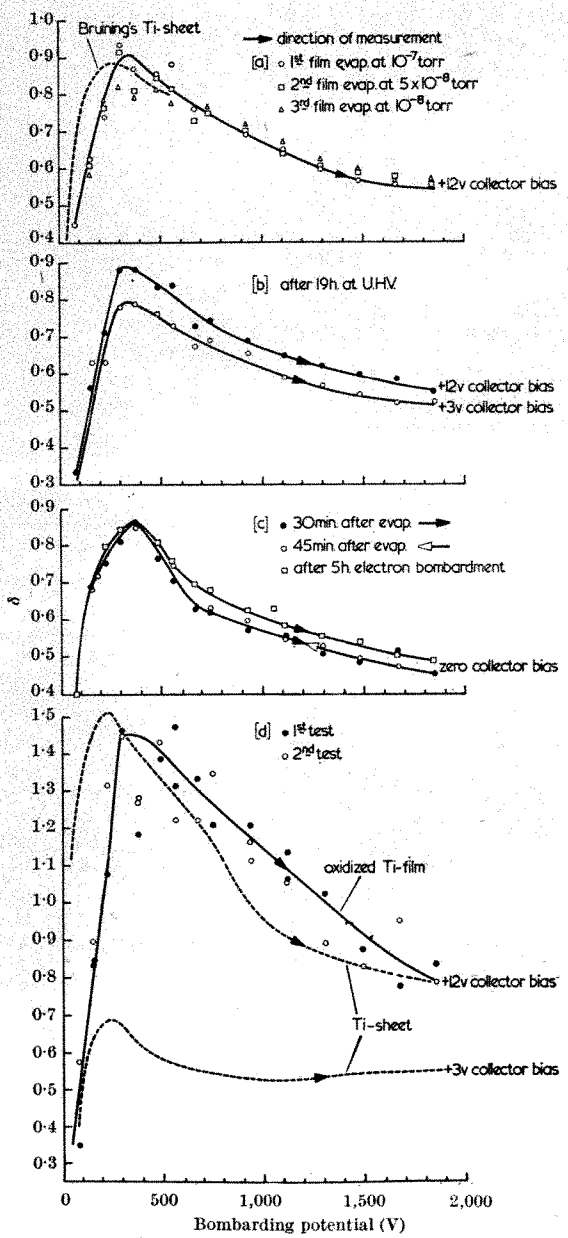


Fig. 1

Tests were made to ensure that the target would remain free of adsorbed oil molecules during the measurement of δ. The target was bombarded with electrons and the rate of growth on its surface of polymerized organic matter measured. Under ultra-high vacuum conditions (ultimate pressure 10^{-9} torr) the growth-rate of the organic deposit was < 5 Å/h (ref. 2); the period for measuring a δ/voltage curve was about 15 min.

Values of δ measured at a pressure of 8×10^{-10} torr are given in Fig. 1*a* for three titanium films deposited in sequence on to a glass substrate; each film was about 200 Å thick. Although the evaporant was thermally degassed during system bake-out the pressure still rose during evaporation, but the pressure rise was less on each successive evaporation. During the third evaporation the pressure rose to only 10^{-8} torr and fell to $< 8 \times 10^{-10}$ torr after the evaporation.

Values have been reported by Bruining[3] of δ for titanium sheet heated in vacuum to dissolve the surface oxide in the bulk metal and his results are shown in the dotted curve in Fig. 1*a*.

The secondary emission of the third titanium film was remeasured after 19 h in ultra-high vacuum (5×10^{-10} torr) and was found to be unchanged (Fig. 1*b*). Likewise continuously bombarding a thick titanium deposit (500 Å) with 30 μamp/cm² for 5 h under the same vacuum conditions caused only a small change in the δ/voltage curve (Fig. 1*c*).

A titanium film evaporated in ultra-high vacuum was oxidized by raising the pressure in the vacuum vessel with dry air to 0·1 torr for 24 h and the δ/voltage curve measured (Fig. 1*d*). The δ/voltage curve differed from that of the unoxidized metal and resembled the curve for oxidized titanium sheet as shown in the dotted curves (Fig. 1*d*). The target voltage for δ maximum of titanium sheet is lower than that for the oxidized titanium film. The rough surface of the oxidized titanium sheet prevents the primary electrons from striking the surface at normal incidence and it is known that varying the incident angle lowers the voltage at which δ maximum occurs.

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GEOLOGY

Potassium–Argon Age Determinations on Some Rocks from the Broken Hill Region of New South Wales

Age measurements have been made on a number of rocks from Broken Hill in Australia. The significance of these will be discussed in a forthcoming publication[1]. The following is a brief description of their geological settings together with those age measurements which were made using the isotope dilution technique of potassium–argon age determination.

The Precambrian rocks of the Willyama complex have been subjected to a series of metamorphic and intrusive episodes. A general outline of these events is as follows.

Metamorphism M1. Throughout most of the Willyama province the original rocks, which were largely detrital sediments, have been reconstituted into garnetiferous, feldspathic, siliceous and pyroxenic gneisses.

Retrograde metamorphism has occurred subsequent to *M1* (refs. 2–5).

Metamorphism M2. Pelitic rocks of the north-western Barrier Ranges are dominantly coarse-grained mica schists exhibiting evidence of further, mainly metasomatic metamorphism. They can be distinguished under the microscope from the products of *M1* by their textures.

Metamorphism M3. Pelitic rocks occupying a belt extending northwards from Lake's Grave along the line of the Mount Franks fault have been recrystallized to knotted quartz-mica schists bearing porphyroblasts of andalusite. Basic rocks have been recrystallized to coarse-grained amphibolites. Metamorphism *M3* was a period of strong deformation and extends in part into the zone occupied by *M2* rocks. Metamorphisms *M2* and *M3* may be related.

Metamorphism M4. Muscovite schists of the *M2* type in places have been modified to fine-grained chlorite–sericite schists, usually found in shear zones parallel to the *M1–M2* schistosity. The intensity of this dynamic metamorphism increases towards the Torrowangee unconformity and it is possible that *M4* is related to the folding and metamorphism of the Torrowangee rocks above the unconformity.

Mundi Mundi Granite. Two large bodies of this massive muscovite–microcline–oligoclase granite outcrop immediately to the east of the *M3* zone of knotted schist. Lack of foliation suggests a late stage of intrusion though its relationship to the knotted schists is not yet clear. Outcrops of the granite are mainly restricted to the *M2* zone.

Boulders of granite and of *M3* type schists have been reported in the basal tillites of the Torrowangee Series[6]. Hence both pre-date the deposition of these Proterozoic rocks.

Shattering and shearing[3,7] of the granite were probably caused by metamorphism *M4* during the folding of the Torrowangee rocks.

In an attempt to clarify the geological history of the region a large number of potassium/argon age measurements were made using both the total volume and isotope dilution techniques. In view of the possible unreliability of the former only the results yielded by the latter will be considered in this work. The samples used are listed here and the results given in Table 1.

Table 1. Results of Potassium/Argon Age Determinations

Specimen	Mineral	K_2O (%)	Atmospheric contam. (%)	Vol. of radiogenic ^{40}Ar (mm^3) N.T.P./g of sample	Apparent age and error m. yr.
84336	Hornblende	0·70	0·60	0·0300	987 ± 62
84332	Hornblende	0·45	0·8	0·0170	894 ± 57
84368	Muscovite	3·53	0·4	0·2017	1,226 ± 54
84367	Biotite	5·26	4·8	0·1633	762 ± 38
	Muscovite (*a*)	7·19	2·4	0·4138	1,233 ± 54
	Muscovite (*b*)	6·97	1·1	0·4526	1,345 ± 30
84359	Muscovite (i)	6·47	1·8	0·2440	892 ± 43
	Muscovite (ii)	6·47	0·3	0·2604	939 ± 22
84365	Muscovite	9·90	0·4	0·6173	1,307 ± 56
84391	Muscovite	9·61	0·6	0·4113	985 ± 38

$\lambda_e = 0{\cdot}584 \times 10^{-10}\ yr^{-1}$ $\lambda_\beta = 4{\cdot}72 \times 10^{-10}\ yr^{-1}$

Small Roman numerals denote repeated determinations on same mineral concentrate.
Small letters denote determinations performed on different mineral concentrates from same rock.

Attempts to date metamorphism M1. Mineral concentrates prepared from high-grade gneisses and amphibolites, products of first regional metamorphism showing little effects of subsequent metamorphisms (hornblendes 84336 and 84332).

Attempts to date metamorphism M2. Specimen 84368 from west of the Mt. Robe Mine containing biotite, undergoing shredding and reaction to sillimanite, being enclosed with its reaction products in large crystals of muscovite.

Attempts to date metamorphism M3. Specimen 84367, from the Silver King Mine, contained perfectly fresh andalusite porphyroblasts in a biotite–muscovite–quartz schist. Both micas were measured.

Attempts to date the folding and metamorphism of the Torrowangee Series (M4 ?). Concentrated muscovite was prepared from a fine-grained schist (specimen 84359) occurring within tillites near the unconformity at Cartwright's Creek.

Attempts to date the consolidation of the Mundi Mundi granite. Muscovite was separated from specimens 84365 from the large mass east of Mt. Robe, and 84391 from the type locality at Mundi Mundi Creek.

From the results listed in Table 1 it is clear that the influence of the final Torrowangee movements was widespread and that these movements took place about 916 million years ago. This compares well with the lead isotope age determinations on ore bodies of the 'Thackaringa type' ('younger than 1,000 million years'[8]).

Data from the Mundi Mundi Granite would suggest that it has an age of some 1,307 million years and was affected in part by the *M4* movements.

The apparent ages yielded by muscovites from samples 84368 and 84367 must be considered to be minimum values and not to date metamorphisms *M2* and *M3* with precision. They do, however, suggest a close relationship in time between *M2* and *M3* and the emplacement of the granite.

Metamorphism *M1* cannot be dated accurately with the information yielded by this investigation, though on geological evidence it must be older than 1,345 million years. Russell and Farquar[9] using the lead isotope method have, however, assigned an age of 1,600 million years to the Broken Hill ore body, which is considered to be either contemporaneous with[7] or post main phase[10] of the *M1* metamorphism.

The general conclusions are as follows:

Geochronological sequence in the Broken Hill District

Geological event	Approximate age
Metamorphism *M1*	1,600 m. yr. or older
Metamorphisms *M2* and *M3*	1,345 m. yr.
Closely followed by intrusion of Mundi Mundi Granite	1,307 m. yr.
Metamorphism and folding of Torrowangee *M4*	916 m. yr.

The Cambrian metamorphic episode in Willyama rocks dated at about 520 million years and reported by Evernden and Richards[11] was not detected in the present investigation.

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RADIOCHEMISTRY

Rapid Non-destructive Analysis of Chlorine by Neutron Activation

It is known that 1·0-sec chlorine-38*m* is formed by neutron irradiation of natural chlorine and that it emits 0·66-MeV γ-ray. So it seems that chlorine can be determined by short-time irradiation with neutrons followed by γ-ray spectrometry. Since the half-life appeared to be too short to bring about precise determinations, we tried at first to observe the precision of the use of ten samples of ammonium chloride, 150 mg each.

They were placed by turns in the nuclear reactor *JRR*-1 for 2·5 sec to be irradiated with neutrons at a flux of 3×10^{11} neutrons/cm²/sec, cooled for 5 sec, and counted for 1 sec. The counter used was a 2-in. thick, well-type sodium iodide crystal attached to a 256-channel pulse-height analyser. During the counting operation the sample was placed 1 cm above the crystal together with its containers made of polyethylene (inner capsule) and of polyester (outer capsule 'rabbit'). A 2,150-mg/cm² thick aluminium disk was used as an absorber for β-rays from the sample.

On the spectrum obtained the height and the 'width at half-maximum' of 0·66-MeV photopeak were measured, from which peak area was estimated by use of a formula presented by Connally and Leboeuf[1].

Thus, the coefficient of variation of single analyses (or relative standard deviation) was estimated at 14 per cent. Though the coefficient is rather large, it may still be sufficient for the purpose of rapid, non-destructive analysis for chlorine.

Then this method was modified to determine the half-life of chlorine-38*m* so as to confirm the identification of chlorine, and was applied to samples of bleaching-powder. Ammonium chloride was used as a chlorine standard. The results of the analyses are given in Table 1, and a typical spectrogram in Fig. 1.

Table 1. Results of Analyses of Bleaching Powder

Sample		Sample weight used (mg)	Chlorine found (%)
A	No. 1	393·1	30·1
	No. 2	429·2	31·9
B	No. 1	229·1	27·0
	No. 2	218·8	29·7

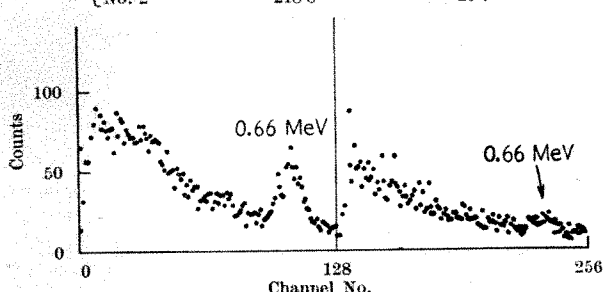


Fig. 1. γ-Ray spectrogram. Sample: 393·1 mg of bleaching powder (sample *A*, No. 1 in Table 1). Time of irradiation, transfer, first counting, interval, and of second counting are 2·5 sec, 5 sec, 1 sec, 1 sec, and 1 sec, respectively

It is deduced that the method is favourably applicable to various organic compounds in spite of the relatively low activation-cross-section of chlorine.

It was found that both sodium chloride and potassium chloride can be used as a chlorine standard.

We thank Prof. Shoji Makishima, Dr. Fumio Aoki and Dr. Toshio Nakai for helpful suggestions, and Mr. Shigeru Yamagishi for helpful assistance.

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CHEMISTRY

Nature and Reactions of the Reducing Species in Aqueous Solutions irradiated with γ-rays in the Presence of Oxygen

We have previously[1] demonstrated the existence of a dehydrogenating species in de-aerated neutral aqueous solutions of aliphatic alcohols, when these are irradiated with γ-rays. This particular species, produced in a yield of $G = 0{\cdot}5$–$0{\cdot}6$ (molecules/100 eV), was assumed to be a hydrogen atom, possibly formed from excited water molecules according to: $H_2O^* \rightarrow H + OH$. A similar conclusion has also been arrived at by Rabani and Stein[2,3], using 200-kV X-rays. The identity of the dehydrogenating species as a simple hydrogen atom appears to be supported by kinetic evidence[2,3] as well as by its reaction with OH^- ions[4,5]. Other authors have suggested that one may be dealing with a radical pair (H + OH) (ref. 6) or an excited water molecule reacting as such[6,7]. For this, and other, reasons we shall represent this dehydrogenating species as H^α. The major proportion of the reducing species consists of electrons, also described as negative polarons[8], and represented here as $(H_2O)^-$.

Recently, questions have arisen concerning the existence of H^α in systems containing oxygen[7,9,10]. In particular, Hummel and Allen[10], in an investigation of the yields of hydrogen on γ-irradiation of aqueous ethanol solutions containing oxygen, concluded that there was a competition between ethanol and oxygen for a reducing species, but were unable to decide whether this was $(H_2O)^-$ or a hydrogen atom. Somewhat similar effects in oxygenated lactate solutions, irradiated with 200-kV X-rays, have been observed by Johnson[11]. We have re-investigated this effect using oxygenated solutions of sodium formate. This particular organic solute was selected because: (*a*) its reaction with the negative polaron does not apparently lead to formation of hydrogen[12]; (*b*) the formate ion has a relatively high reactivity towards H^α (ref. 13) as well as to externally generated hydrogen atoms[14]. Solutions of formate of varying concentrations (10^{-3}–5×10^{-1} M) were saturated with oxygen (1 atm.) and irradiated with cobalt-60 γ-rays. The yields of hydrogen were determined mass spectrometrically, after removal of excess oxygen with yellow phosphorus, this method giving results accurate to about ±5 per cent. The results (Table 1) show that the yields of hydrogen increase with increasing formate concentration, indicating a reaction involving H^α and not $(H_2O)^-$. Furthermore, the results follow simple competition kinetics leading to a value of $G(H^\alpha) = 0{\cdot}75 \pm 0{\cdot}1$, in fairly good agreement with that obtained in de-aerated systems. Thus, both $(H_2O)^-$ and H_a can undergo reactions in the presence of oxygen, in disagreement with the statement of Hayon[7] that only $(H_2O)^-$ reacts under these conditions.

Over the range of formate concentration where $G(H_2)$ increases markedly, the yield of hydrogen peroxide changes relatively little (Table 1), a situation similar to that observed in the ethanol–oxygen system[10]. This would be expected if H^α is, in fact, a hydrogen atom, reacting competitively, according to: $H + HCOO^- \rightarrow H_2 + COO^-$ and $H + O_2 \rightarrow HO_2$, since the reaction with formate will be followed by, $COO^- + O_2 \rightarrow CO_2 + O^-$. Given the accepted mechanism for the radiolysis of aqueous oxygenated formate solutions[15] and assuming $G^w_{H_2} = 0{\cdot}39$, the value of $G(H_2)$ at the lowest formate concentrations, the results presented here lead to values of $G((H_2O)^-) = 2{\cdot}3 \pm 0{\cdot}1$ and $G(H^\alpha) = 0{\cdot}75 \pm 0{\cdot}1$. In certain other aqueous systems, for example, in solutions containing N_2O + alcohol[13], or CO_2 + alcohol[16], $G(H^\alpha)$ and $G((H_2O)^-)$ have been directly measured. The values obtained for $G(H^\alpha)$ are in agreement with that obtained here. However, values of $G((H_2O)^-)$ up to 2·9 have been observed.

Table 1. YIELDS OF HYDROGEN AND OF HYDROGEN PEROXIDE ON IRRADIATION, WITH COBALT-60 γ-RAYS, OF AQUEOUS SOLUTIONS OF SODIUM FORMATE IN THE PRESENCE OF OXYGEN (1 ATM.)

Concentration of sodium formate (mole/l.)	$G(H_2)$	$G(H_2O_2)$
10^{-3}	0·39	3·48
10^{-2}	0·39	3·58
10^{-1}	0·64	3·59
$1·5 \times 10^{-1}$	0·70	—
2×10^{-1}	0·78	—
5×10^{-1}	0·92	3·57

The conclusion that both H^{α} and $(H_2O)^-$ are present in irradiated oxygenated formate solutions is inconsistent with the findings of Czapski and Allen[9] that, in the γ-radiolysis of solutions of oxygen and hydrogen peroxide, the reducing radicals behave as if they are all $(H_2O)^-$. If the results of Czapski and Allen are accepted, then it is apparent that the reactions of H^{α} with either formate or with oxygen do not lead to hydrogen peroxide; in these circumstances, it may be necessary to consider other concepts of the identity of H^{α}. In order to settle this aspect we feel it important to have a further independent determination of the value of $G((H_2O)^-)$, in solutions irradiated in the presence of oxygen.

We thank Prof. J. J. Weiss for his interest in this work. We also thank Prof. G. Stein, Dr. G. Czapski and Dr. G. R. A. Johnson for useful discussions and to Mr. P. Kelly for mass spectrometric analysis. One of us (M. S.) is indebted to the U.S. Army for financial support.

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Infra-red Spectra of some Anhydrous Complex Cyanide Acids

AN appreciable number of acids derived from metal cyanide complexes are known, and many can be obtained anhydrous. However, very little information is available as to their structures in the solid state. Penneman *et al.*[1] measured the infra-red spectrum of solid anhydrous aurocyanic acid, $HAu(CN)_2$, and reported a single cyanide stretching frequency at 2,146 cm^{-1} and an Au—C stretch at 399 cm^{-1}. They found no absorption which could be attributed to a hydrogen stretching frequency.

This communication reports the detailed infra-red spectra of this acid, cobalticyanic acid and ferrocyanic acid in the region 5,000–400 cm^{-1}. The results obtained, together with those for related compounds, are given in Table 1 and some of the spectra are reproduced in Fig. 1.

The spectrum of ferrocyanic acid differs considerably from those of the other two acids. The strong broad absorption (with some structure) in the region 3,300–2,300 cm^{-1}, and the corresponding bands at 2,460 cm^{-1}, 2,270 cm^{-1}, and 1,908 cm^{-1} in the deutero-acid can plausibly be attributed to the stretching modes of N—H (or N—D) bonds; some, at least, of the hydrogen atoms are presumably

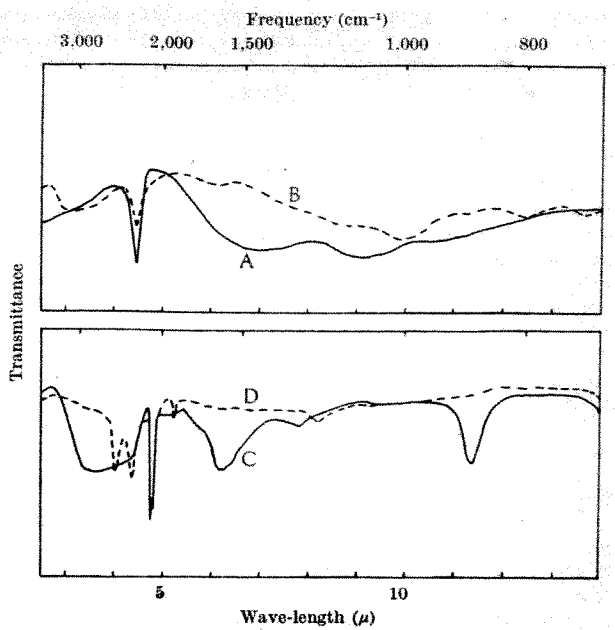


Fig. 1. Infra-red spectra of: *A*, $HAu(CN)_2$; *B*, $DAu(CN)_2$; *C*, $H_4Fe(CN)_6$; *D*, $D_4Fe(CN)_6$

Table 1
(cm^{-1})

C≡N Stretches

$K_3Co(CN)_6$, 2130*s*. $Ag_3Co(CN)_6$, 2188*s*. $(CH_3)_3Co(CN)_6$ (Hartley's (ref. 2) β-ester), 2280*s* (C≡N of C≡N—CH_3), 2154*s* ('free' C≡N). $H_3Co(CN)_6$, 2193*s*. $D_3Co(CN)_6$, 2193*s*. $K_4Fe(CN)_6$, 2140*s* (Fe—C at 580*s*). $H_4Fe(CN)_6$, 2110*s*, 2086*s*, 2028*w*. $D_4Fe(CN)_6$, 2095*s*. $KAu(CN)_2$, 2143*s*. $Au(CN)$, 2238*s*. $HAu(CN)_2$, 2212*s*. $DAu(CN)_2$, 2210*s*.

Other Bands

$H_4Fe(CN)_6$: 3300–2300*vs*, broad (N—H stretch), 1800–1400*vs*, broad, 1270*w*, 875*s* (N—H deformation), 686*w*, 659*w*, 580*s* (Fe—C stretch).
$D_4Fe(CN)_6$: 2460*s*, 2270*s*, 1908*w* (N—D stretches), 1209*m*, 1015*w*, 960*w*, broad, 634*m* (N—D deformation), 580*s* (Fe—C stretch), 500*w*, 430*w*.
$HAu(CN)_2$: 4200*w*, broad, 1400*s*, broad (N—H stretch ?), 1110*s*, broad (N—H deformation ?).
$DAu(CN)_2$: 3000*m*, broad, 1010*m*, broad (N—D stretch ?), 810*m*, broad (N—D deformation ?).

moderately strongly hydrogen-bonded to adjacent —C≡N groups. The bands at 1,600 cm^{-1} and 875 cm^{-1} (which shift appropriately on deuteration) may be hydrogen deformation modes, although it is possible that the 1,600 cm^{-1} band is a strongly hydrogen-bonded N—H stretching vibration. This band could also arise from small amounts of retained water, although it may be noted that the analysis of ferrocyanic acid indicates that it is anhydrous[3] and prolonged heating *in vacuo* did not diminish the intensity of this band. Two cyanide stretching frequencies are observed with $H_4Fe(CN)_6$ while $D_4Fe(CN)_6$ gives a broad asymmetric peak with a low-frequency shoulder.

Aurocyanic acid and cobalticyanic acid, on the other hand, give single cyanide stretching frequencies. The bands, although broader than in the corresponding potassium salts, are quite symmetrical. The cyanide-stretching frequency of aurocyanic acid found in this work does not agree with the value given by Penneman *et al.*[1]; their value is almost identical with that found for $KAu(CN)_2$.

Both $HAu(CN)_2$ and $H_3Co(CN)_6$ show only slight absorption around 3,000 cm^{-1}, but give very strong general absorption with some structure below about 1,900 cm^{-1}. In aurocyanic acid the bands at 1,400 cm^{-1} and 1,110 cm^{-1} shift to lower frequencies on deuteration. This broad absorption is very similar to that found in certain acid salts such as sodium hydrogen diacetate, where symmetrical O—H—O hydrogen bonds have been postulated[4–6]. It therefore seems plausible that in aurocyanic and cobalticyanic acid there are very strong and possibly symmetrical N—H—N hydrogen bonds. This is consistent with the single cyanide stretching frequencies. From the available data it is not possible to distinguish between a truly symmetrical hydrogen bond with a single potential

minimum and a very strong but asymmetrical hydrogen bond. Although hydrogen bonds between nitrogen atoms are normally rather weak, these acids seem to provide a favourable opportunity for strong hydrogen bonding. Symmetrical-type hydrogen bonding would result in reasonable structures for both compounds, that is, infinite chains for aurocyanic acid, and a three-dimensional lattice based on linked octahedra for cobalticyanic acid. Further work is in hand and it may be noted that preliminary infra-red data on a number of related anhydrous acids indicate that they fall into two classes which resemble ferrocyanic acid or cobalticyanic and aurocyanic acids, respectively.

Measurements were made using nujol and fluorolube mulls, a Perkin–Elmer model 21 spectrometer with calcium fluoride, sodium chloride and potassium bromide prisms, and Infracord and Unicam *SP*200 spectrometers.

The acids were prepared by standard procedures[1–3] and dried *in vacuo* at 80°–100° C. The deutero acids were obtained either directly, using deuterium chloride and deuterium oxide, or by repeated exchange with deuterium oxide, and the extent of deuteration (> 90 per cent) was checked from examination of their proton resonance spectra in deuterium oxide solution.

We thank Mr. P. Nicholson for samples of $H_4Fe(CN)_6$ and the Ethyl Corporation of America for financial support to one of us (D. J.).

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Far-infra-red Absorption Spectra of some Complex Halides of Molybdenum and Tungsten

In a previous publication[1] Adams and Gebbie reported the far-infra-red absorption spectra of some transition metal complex halides containing regular octahedral ions of the type MX_6^{n-}, X = halogen. We have now extended our observations to a series of halides of molybdenum and tungsten recently prepared by Edwards, Kennedy, Peacock and Said[2].

The spectra were obtained by means of a far-infra-red interferometer, the samples being in the form of 'Nujol' mulls sealed in polythene cells; details of techniques and procedure have already been published[1]. The results are given in Table 1.

Table 1. Far-Infra-red Absorption Frequencies (cm^{-1}) of Some Complex Halides of Mo^{IV} and W^{IV}

	ν_3	ν_4	Lattice mode (?)
K_2WCl_6	324	165	77
Rb_2WCl_6	306	160	66
Cs_2WCl_6	308	166	71
K_2MoCl_6	340	174	74
Rb_2MoCl_6	334	172	70
Cs_2MoCl_6	325	170	70
K_2WBr_6	229	74	—
Rb_2WBr_6	220	78	—
Cs_2WBr_6	214	60	—

Spectra of the halides $M_2'[MCl_6]$, M = molybdenum or tungsten, are analogous to those found for compounds such as K_2PtCl_6 and K_2IrCl_6, a three-band spectrum being observed[1]. The spectra of the molybdenum chlorides all had absorption near 230 cm^{-1}; but as this increased if the sample was exposed to air, we consider it to be due to a breakdown product. Weak absorption was also found in Rb_2WBr_6 and Cs_2WBr_6 at 116 and 114 cm^{-1} respectively. Absorption in the range 308–340 cm^{-1} is due to ν_3 (metal-chlorine stretching) and that at 160–174 to ν_4 (metal-chlorine bending). In the bromides, M_2WBr_6, ν_3 and ν_4 are correspondingly lower and compare closely with those observed for K_2PtBr_6, namely, 240 and 78 cm^{-1}. As expected ν_3 is lower for the tungsten than for the molybdenum compounds, and it is interesting to note that ν_4 follows this trend.

There is a marked dependence of ν_3 (and to a lesser extent, ν_4) on the nature of the cation. As the size of the cation is increased, cation–anion interactions decrease. The rise in ν_3—and hence in the M–X bond-strength—with increasing ionic interaction may be caused, at least in part, by varying amounts of $d\pi$–$d\pi$ bonding between metal and halogen in the anion. This dative π-bonding, which Woodward and Ware[3] have shown to be present in $PtCl_6^{2-}$ and $PtBr_6^{2-}$, will be favoured by the proximity of a relatively small cation such as K^+.

In $(NEt_4)W^{V}Cl_6$, ν_3 is at 329 cm^{-1} (ref. 4); in $Cs_2W^{IV}Cl_6$, ν_3 is at 308 cm^{-1} and would presumably be a little lower in $(NEt_4)_2W^{IV}Cl_6$. Thus a drop of some 25 cm^{-1} in ν_3 occurs as the oxidation state of tungsten is changed from +5 to +4, comparable with that of 37 cm^{-1} for the +4 to +3 change in K_2IrCl_6 and K_3IrCl_6 (ref. 1).

The third, and lowest, frequency band found for the chlorides (Table 1) is in addition to the predicted infra-red-active internal modes of an isolated MCl_6^{2-} ion. It was previously assigned to a lattice mode[1], largely on the basis of the large shift it suffered (45 cm^{-1}) in $M_2'IrCl_6$ when M' was changed from K^+ to $(NH_4)^+$. An alternative assignment is to ν_6 (f_{2u}), the inactive bending frequency, made active by the lower symmetry of the crystal lattice.

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Polymorphism in Silicon Carbide

Silicon carbide exists in two distinct crystalline polymorphs, the cubic or β-form, and the hexagonal or α-form[1–3]. Although the crystal structure and related physical properties of the two forms of silicon carbide have been extensively investigated, the chemical relationship between the two silicon carbide polymorphs has apparently failed to receive equivalent attention. The problem was attacked by Baumann[4], who investigated both the chemistry of silicon carbide formation, and the rate of conversion of β-silicon carbide to α-silicon carbide at several temperatures. Baumann observed that although β-silicon carbide began to transform to α-silicon carbide slowly at 2,100° C, the reaction was rapid at 2,300° C.

The suggestion that the transformation from the cubic form to the hexagonal form may proceed via the vapour phase has been made by Slack[5]. Thus, if a solid-phase transition occurs at all, it must be very slow as compared with the vapour phase process. Indeed, Kistler[6] summarized the problem when he stated, "It is probable, although I believe the subject has not been sufficiently

explored, that the cubic form of SiC can be maintained at higher temperatures (> 2,000° C) without conversion as long as *recrystallization does not take place*".

It is the purpose of this communication to show that polymorphism in silicon carbide may be reasonably interpreted as proceeding through the vapour phase. No attempt is made to prove that silicon carbide transforms through a vapour phase condensation, only that such an interpretation is not unreasonable in view of the available facts.

According to Buerger[7], the barrier which opposes and thus regulates the rate of transformation in solid–solid reactions should be represented by the structural bonds which it is necessary to disturb during the transformation. In the case of reconstructive transformations, defined as the disintegration of one structure into small units followed by reconstruction of a new crystalline modification from these units, Buerger has related the barrier to the heat of vaporization of such units. Buerger's transition barrier can be reasonably represented by the activation energy for the phase transition in question. In the case of silicon carbide an activation energy for the β-silicon carbide to α-silicon carbide reaction can be calculated from the rate-temperature curves for the transformation as measured by Baumann. A comparison, then, of the calculated activation energy with the energy required to bring about disruption of the chemical bonds in the silicon carbide lattice can lead to a reasonable interpretation of the path connecting the two silicon carbide polymorphs.

In his paper, Baumann plotted two time–temperature curves for the conversion of β-silicon carbide to α-silicon carbide, from which Table 1 was deduced.

Table 1. Rate Data on the Transformation. β-Silicon Carbide to α-Silicon Carbide

Time (sec)	Temp. (° C)	β-Silicon carbide (%)	α-Silicon carbide (%)	Time (sec)	Temp. (° C)	β-Silicon carbide (%)	α-Silicon carbide (%)
0	2,100	100	0	0	2,300	100	0
300	2,100	86	14	300	2,300	16	84
600	2,100	81	19	600	2,300	5	95
900	2,100	63	37	900	2,300	1	99
1,200	2,100	67	33	1,200	2,300	0	100

It should be noted that in his time-temperature studies, Baumann used mixtures of silica and carbon rather than crystals of β-silicon carbide. In work on the formation of silicon carbide from $SiO_2 + C$ he found that β-silicon carbide began to form at 1,400°–1,500° C, and that the reaction was complete at 1,700° C. By directly observing the reaction by means of the thermal microscope Baumann found that the reaction between silica and carbon at approximately 2,200° C was quite rapid, β-silicon carbide being formed in about a minute or less. (An excellent 16-mm colour film entitled, "Exploration with the High Temperature Microscope," is available from the Carborundum Co., Niagara Falls, New York.) For purposes of this discussion, therefore, it will be assumed that in the aforementioned time–temperature curves β-silicon carbide was present at 'time-zero'.

From the results shown in Table 1 it was found that first-order plots for the conversion of cubic to hexagonal silicon carbide could be calculated. These are shown in Fig. 1.

The average rate constant for the transformation was found to be $4{\cdot}22 \times 10^{-4}$ sec^{-1} (± 20 per cent) at 2,100° C, and $5{\cdot}40 \times 10^{-3}$ sec^{-1} (± 10 per cent) at 2,300° C. From the usual plot of log k versus $1/T$ the activation energy for the transformation was found to be 158 kcal mole^{-1} (± 20 per cent).

Drowart, DeMaria and Inghram[8] have recently measured the partial pressures of various silicon–carbon species over silicon carbide in the temperature range 2,149 to 2,316° C, and have calculated the heats of formation of the various gaseous species from hexagonal silicon carbide. From the work of Drowart *et al.*, an estimate of the heat of

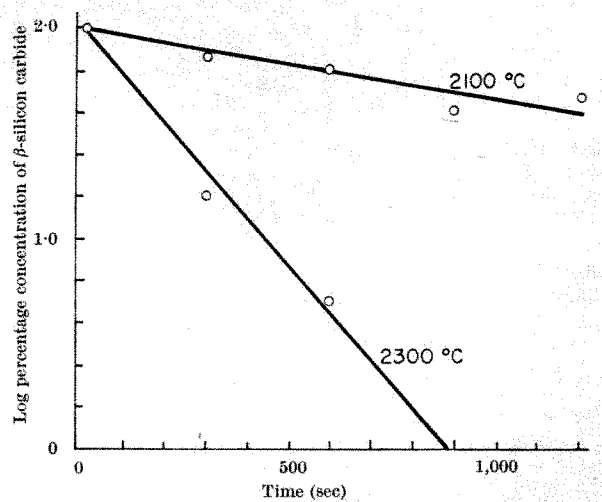


Fig. 1. First-order plot for the reaction. β-Silicon carbide → α-silicon carbide

vaporization of silicon carbide at 2,149° and at 2,316° C may be obtained by considering the partial molal equation for the total heat of reaction (ΔH) to form the various gaseous species from solid silicon carbide. The partial molal equation is[9]:

$$\Delta H = n_1 \Delta H_1 + n_2 \Delta H_2 + n_3 \Delta H_3 \qquad (1)$$

where n_1, n_2, and n_3 represent the mole fractions of the various gaseous species in equilibrium with solid SiC, and ΔH_n the corresponding heats of reaction to form the various species. The mole fractions are readily determined from Raoult's law.

From the preceding data, the total pressure over SiC was calculated to be $2{\cdot}43 \times 10^{-5}$ atm. at 2,149° C, and $2{\cdot}53 \times 10^{-4}$ atm. at 2,316° C. Carbon–silicon species with partial pressures less than $1{\cdot}0 \times 10^{-6}$ atm. were neglected. It was also assumed for purposes of this discussion that the heats of formation of gaseous species from silicon carbide are the same for both the cubic and hexagonal polymorphs. This is considered to be a valid assumption in light of recent work[10], in which it has been shown that the ΔH of formation of both cubic and hexagonal silicon carbide at 298° K is $-15{\cdot}0 \pm 2$ kcal mole^{-1}. From these data, the total heat of vaporization of the gaseous species in equilibrium with silicon carbide (solid) at both 2,316° and 2,149° C is found to be approximately 133 kcal mole^{-1}.

The estimated activation energy of 158 kcal mole^{-1} (± 20 per cent) for the β-silicon carbide to α-silicon carbide transformation as deduced from Baumann's data is not in disagreement with the value of 133 kcal mole^{-1} for the calculated heat of vaporization of gaseous species from silicon carbide. The latter value is reasonably close to the heat of formation of silicon gas since this material accounts for 86 mole per cent of the gaseous species over silicon carbide at 2,149° C, and 79 mole per cent at 2,316° C. It would appear, therefore, that it is not unrealistic to picture the conversion of β-silicon carbide to α-silicon carbide as a reconstructive transformation, proceeding through a vapour transport mechanism involving gaseous silicon, and various silicon carbide species.

The contentions of Scace and Slack[5], and of Kistler[6] appear to be very plausible, in light of the limited data which are available concerning the β→α transformation. In spite of this, however, some recent observations on the behaviour of silicon carbide at high temperatures are difficult to understand in light of Baumann's data. For example, Scace and Slack[11] have observed that cubic silicon carbide could be heated to approximately 2,830° C with no transformation taking place, and that both cubic

and hexagonal crystals of silicon carbide could be grown side-by-side from the vapour phase at 2,600° C.

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Syntheses of Poly-DL-Serine Derivatives containing Cytotoxic Groups

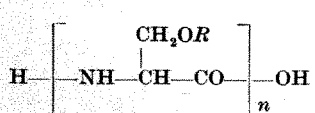
Nature has often used the trick of having a functional group carried by a larger structure of polypeptide character. This idea prompted me to use a polypeptide, namely polyserine, as carrier of groups with cytotoxicity and latent cytotoxicity.

Several serine derivatives have served as anti-tumour agents[1,2], but so far as I know polyserine has not yet been tried as a carrier moiety.

Poly-DL-serine was prepared from the *O*-benzyl-DL-serine-*N*-carboxylic acid anhydride by polymerization in boiling chloroform using triethylamine as initiator[3]. The benzyl groups were removed by suspending the polymer in dioxane saturated with hydrogen bromide and allowing the mixture to stand in the cold[1].

Poly-DL-serine, purified by dialysation followed by freeze-drying, was coupled with methanesulphonyl chloride, in pyridine. According to analytical data (required, S 17·5, N 7·7; found, S 16·4, N 7·7) and infra-red spectroscopic investigations nearly all OH-groups could be blocked with the well-known cytoactive methanesulphonyl-group (I).

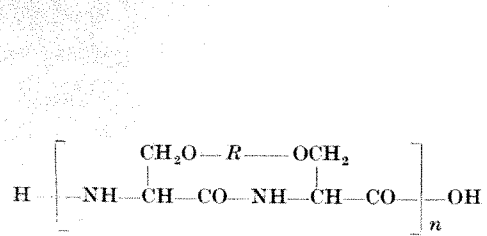
I succeeded in coupling polyserine in the same manner with chloromethanesulphonyl chloride[5] (required, S 14·7, Cl 16·3, N 6·4; found, S 13·8, Cl 15·5, N 6·7) (II).

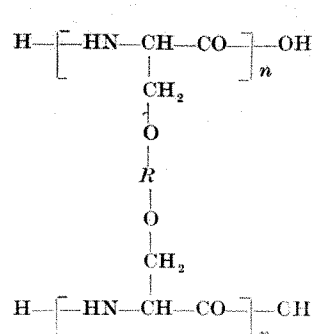


I $R=SO_2CH_3$

II $R=SO_2CH_2Cl$

I then examined the possibility of attaching latent mustard groups to polyserine. For this purpose polyserine was coupled with di-(2-chloroethyl)-phosphoramidic dichloride[6] in pyridine-dioxane. The resulting polypeptide derivative is insoluble in water, in alcohol and in most organic solvents. According to analytical data (required, C 33·2, H 4·5, N 11·6, P 8·6, Cl 19·6; found, C 33·1, H 4·4, N 11·3, P 8·2, Cl 19·1) and infra-red spectroscopic investigations, phosphoric acid esters are formed with the participation of two OH-groups. The two OH-groups may belong to two neighbouring serine units, forming cyclic esters, as formulated in III, but there is also a possibility of the formation of phosphoric acid ester linkages between two polyserine chains (IV).





III IV

III, IV $R=PON(CH_2CH_2Cl)_2$

It cannot be overlooked, naturally, that on each NH_2-terminated chain end there is a possibility for the formation of cyclic phosphamide esters with the participation of the OH- and NH_2-groups of the same, terminal standing serine-unit.

Further investigation and proof of the proposed structures are in progress.

The biological tests were carried out by Dr. B. Szende and Dr. K. Németh in the I. Institute of Pathological Anatomy and Experimental Cancer Research, Medical University of Budapest. Their results will be submitted for publication elsewhere.

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Use of Alkyltrimethylammonium Bromides for the Isolation of Ribo- and Deoxyribo-nucleic Acids

The use of cetyltrimethyl ammonium bromide for the isolation of nucleic acids and for the separation of RNA from DNA was first reported in 1951[1–3] and since has been used successfully for the isolation of DNA from bacteria[4]. The cetyltrimethylammonium bromide originally used was 'Cetavlon' (Imperial Chemical Industries Pharmaceuticals, Ltd.). A change in the nature of this material and modifications to the procedure used for the isolation of nucleic acids have necessitated a re-examination of the method.

Before May 1953 'Cetavlon' contained 85 per cent alkyltrimethylammonium bromides including a large proportion of the cetyl compound and some longer chain compounds. Since then a product of higher solubility, which contains a considerable proportion of the tetradecyl compound, has been issued. This change, which is advantageous from the pharmaceutical point of view, makes 'Cetavlon' less satisfactory for the separation of RNA from DNA, although it can still be used to separate nucleic acids from neutral compounds. In order to find a satisfactory alternative, a comparison has been made between 'Cetavlon', commercial cetyltrimethylammonium bromide (CTAB, British Drug Houses, Ltd.; found: C, 62·7, H, 11·9; N, 3·6; calc. for

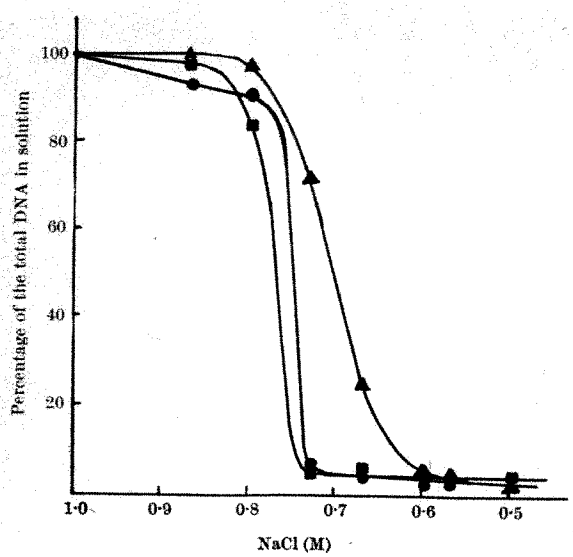


Fig. 1. Solubility of alkyltrimethylammonium salts of calf thymus DNA in sodium chloride solution at 20°. DNA concentration, 1·67 mg/ml. Concentration of alkyltrimethylammonium bromide, 6·67 mg/ml. ●, Pure synthetic cetyltrimethylammonium bromide; ■, commercial cetyltrimethylammonium bromide; ▲, 'Cetavlon'

$C_{19}H_{42}NBr$; C, 62·9; H, 11·6; N, 3·86 per cent) and a pure sample of cetyltrimethylammonium bromide (found: C, 62·9; H, 11·1; N, 3·84 per cent) which had been synthesized from cetyl alcohol, which had been found by gas phase chromatography to be 99 per cent pure (kindly supplied and analysed by Imperial Chemical Industries Dyestuffs Division, Manchester).

The solubilities of the deoxyribonucleates (from calf thymus DNA) of these alkyltrimethyl ammonium compounds are shown in Fig. 1. These show that the solubility of the deoxyribonucleate of the commercial CTAB decreased more rapidly with decreasing salt concentration than does that of the deoxyribonucleate of 'Cetavlon'. This indicates that the former should be more satisfactory for the purification of DNA. There did not appear to be any advantage in using the pure synthetic CTAB, and when this and the commercial CTAB were used to purify a sample of *Mycobacterium phlei* DNA which contained 17 per cent RNA (probably *s*RNA), in both cases samples of DNA were obtained which contained 1–2 per cent RNA.

The more recent methods for the isolation of nucleic acids, particularly the phenol method[5], give RNA of high molecular weight (microsomal RNA). This type of RNA was almost certainly lost in some previous methods, either by enzymatic degradation or by precipitation in 1 M sodium chloride and adsorption on the chloroform–protein gels formed during the deproteinization stages[6]. As this RNA is insoluble in 1 M sodium chloride its presence complicates the separation of RNA from DNA by the use of CTAB, but the microsomal RNA can be readily removed from the DNA by precipitation with 1 M sodium chloride before separating the DNA from the *s*RNA by means of CTAB. A typical procedure for the isolation of the nucleic acids from the aqueous layer of a phenol-*p*-aminosalicylate extraction[5] of a micro-organism is as follows:

The aqueous layer is poured into ethanol (3 vol.) and the precipitate removed and dissolved in water. The solution is made 1 molar with respect to sodium chloride and kept at 0° for 18 h. The precipitate of microsomal RNA is centrifuged off, dissolved in water and reprecipitated with M sodium chloride. It is finally dissolved in water, dialysed and freeze-dried. The supernatant liquid is brought to 20° and an aqueous solution of CTAB (3 per cent w/v) added until the sodium chloride concentration is reduced to 0·3 M. The resulting precipitate is centrifuged off and redissolved in 1 M sodium chloride so that the nucleic acid concentration is approximately 3 mg/ml. An aqueous solution of CTAB (2 per cent w/v) is then added (at 20°) until the sodium chloride concentration is 0·55–0·60 M (the exact concentration varies with the source of the DNA and the degree of polymerization; highly polymerized calf thymus DNA separates at a higher salt concentration) and the resulting precipitate of DNA (CTAB salt) centrifuged off. This can be purified by redissolving in 1 M sodium chloride and reprecipitating. The CTAB salt of DNA is converted to the sodium salt by dissolving the former in 1 M sodium chloride and precipitating the sodium salt with ethanol (3 vol.). Dialysis and freeze-drying give DNA, which contains 1–3 per cent RNA. The low molecular weight RNA (containing *s*RNA) is isolated from the supernatant obtained after precipitation of the DNA with ethanol. This precipitate is dissolved in water, dialysed and freeze-dried.

The foregoing method has been used to isolate DNA from *Aspergillus niger*, *Tetrahymena pyriformis*, *Polytomella papillata*[7], *Serratia marcescens*[8], and *Mycoplasma mycoides*, var. *capri*[9]. Precipitation with CTAB effectively separates the nucleic acids from neutral polysaccharides. Acidic polysaccharides are precipitated by CTAB, but most are redissolved in 0·3 M sodium chloride[2]. Some acidic polysaccharides and acidic cell wall material may contaminate the nucleic acids, however, and have to be separated by other means[8]. When the method is used in conjunction with phenol extraction, protein contamination is usually very slight, but any traces which remain can be removed by shaking the solution of nucleic acid in 1 M sodium chloride with chloroform and amyl alcohol[6].

The use of CTAB for the isolation of nucleic acids has several advantages. It is available cheaply in large quantities, the precipitates formed with nucleic acids are easy to separate, and the sodium nucleates can be easily recovered either by ethanol precipitation as described here, or by extraction of a solution of the CTAB salt in 1 M sodium chloride with chloroform. Also DNA can be readily separated from RNA without the use of enzymes. It has been shown[10] that long-chain quaternary ammonium compounds do not affect the biological activity of *s*RNA. A few batches of commercial CTAB have been found to be atypical. It is advisable, therefore, to test each batch with calf thymus DNA (Fig. 1).

For the biological testing of nucleic acids it is important that all the alkyltrimethylammonium compounds should have been removed. Their presence may be detected by the use of solochrome green *V*150 (Imperial Chemical Industries Dyestuffs Division, Manchester). Addition of an aqueous solution (0·05 per cent w/v) of this dye (pink, λ_{max} 500 mμ) to a solution of CTAB or 'Cetavlon' gives an intense purple colour (λ_{max} 550 mμ). An accurate estimate of the amount present could not be obtained because a standard curve of CTAB with the dye was not exactly reproducible and did not obey Beer's Law at concentrations of CTAB greater than 20 μg/ml. This appeared to be due to the formation of a precipitate of CTAB with the dye at certain concentrations. However, a semiquantitative estimate of the amount present could be made by adding 0·03 ml. of a 0·05 per cent aqueous solution of solochrome green *V*150 to 0·5 ml. of the solution containing CTAB. The presence of 10 μg/ml. of CTAB could be detected visually, the optical density at 550 mμ being 0·1. Normal constituents of aqueous extracts of biological materials, namely, proteins, polysaccharides and nucleic acids, did not interfere with the reaction, but high concentrations of organic solvents such as ethanol, acetone and dioxan also gave a purple colour. A 0·05 per cent aqueous solution of solochrome green *V*150 was also useful as a spray for the detection of CTAB and similar compounds on paper chromatograms.

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BIOCHEMISTRY

Isolation of Two Serologically Active Trisaccharides from Human Blood-group B Substance

A previous communication[1] reported the isolation of a B-active disaccharide, identified as 3-*O*-α-D-galactopyranosyl-D-galactose, from the products of partial acid-hydrolysis of a human blood-group B specific mucopolysaccharide. This sugar was the only disaccharide detected that showed B-activity, and its absence from the hydrolysis products of human blood-group A specific substance[2,3], together with other evidence, led us to infer that this disaccharide is the terminal non-reducing unit of the blood-group B determinant structure in the original mucopolysaccharide. However, the capacity of the disaccharide to inhibit the agglutination of B erythrocytes by a human anti-B serum, although much greater than that of any simple sugar previously examined, was nevertheless much lower than that of the intact B-active mucopolysaccharide in the same system, thus supporting the idea[1,4] that the complete B-determinant structure is larger than a disaccharide unit. To investigate this possibility, the trisaccharide components in the hydrolysis products were examined, and the isolation and identification of two B-active trisaccharides are now described.

A preparation of blood-group B-specific mucopolysaccharide (3·6 g) was partially hydrolysed, and the hydrolysate was adsorbed on a charcoal–'Celite' column, as described earlier[1]. After displacement of the monosaccharides and disaccharides from the column by successive elution with water, 1·5, 2·5 and 5 per cent (v/v) aqueous ethanol respectively, further elution with aqueous ethanol (7·5 per cent v/v, followed by 10 per cent v/v) displaced a mixture (about 120 mg) of trisaccharides. Fractionation of this mixture by preparative paper chromatography, using Whatman No. 3*MM* paper and ethyl acetate–pyridine–water (10 : 4 : 3 v/v) as the irrigant, afforded 35 mg of a fraction showing B-activity. This fraction proved to be a mixture of two components (I and II) having closely similar chromatographic mobilities ($R_{lactose} = 0{\cdot}87$ and 0·92 respectively). A solution of the mixture in anhydrous methanol deposited crystals of pure component I (about 20 mg), and component II (about 10 mg; 80 per cent pure) was recovered from the supernatant solution.

Chromatography of acid-hydrolysates of I and II indicated that galactose and glucosamine were present, and analysis by the phenol–sulphuric acid[5] and Elson–Morgan[6] methods, the latter after acid hydrolysis, showed that these monosaccharides occurred, respectively, in a molar ratio of approximately 2 : 1. However, when I and II were first reduced with sodium borohydride[7] and then analysed, it was found that the galactose content was essentially unchanged, whereas the hexosamine content was negligible.

Trisaccharide I had m.p. 235°–240° (decomp.) and $[\alpha]_D^{22} = +99°$ (equil.; $c = 1{\cdot}0$ in water). Under the conditions described by Aminoff, Morgan and Watkins[8], the substance gave a negative indirect Ehrlich reaction, indicating[9] that the reducing terminal *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) residue was substituted at position 4. On partial acid-hydrolysis (0·02 N polystyrenesulphonic acid[10] at 80° for 4 h), the trisaccharide yielded, in addition to D-galactose and *N*-acetyl-D-glucosamine, two disaccharides which were chromatographically indistinguishable from 3-*O*-α-D-galactopyranosyl-D-galactose[1,11] and 4-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine[12] respectively. However, when the glycitol of the trisaccharide, obtained by borohydride reduction, was partially hydrolysed in the same way, the only reducing sugars detectable by chromatography among the products were galactose and 3-*O*-α-D-galactopyranosyl-D-galactose.

Periodate oxidation of trisaccharide I (4·1 mg) in 0·01 M sodium metaperiodate (10 ml.) at 22° gave rise to an initial rapid uptake of 2·1 moles/mole of oxidant, with the liberation of 0·97 mole/mole of formic acid and 0·27 mole/mole of formaldehyde. Oxidation under the same conditions after borohydride reduction[13] led to the consumption of 2·9 moles/mole of periodate and the release of 1·0 mole/mole of formaldehyde; there was no over-oxidation. Trisaccharide I is therefore believed to be *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*N*-acetyl-D glucosamine.

A syrup containing at least 80 per cent of trisaccharide II had $[\alpha]_D^{22} = +92°$ ($c = 0{\cdot}5$ in water). It gave a strong indirect Ehrlich reaction[8], and the molar extinction was about 50 per cent higher than that given by *N*-acetyl-D-glucosamine, indicating[9] that the reducing *N*-acetyl-D-glucosamine residue was substituted at position 3. When partially hydrolysed with acid in the same way as trisaccharide I, it yielded, in addition to its constituent monosaccharides, two disaccharides which were chromatographically indistinguishable from 3-*O*-α-D-galactopyranosyl-D-galactose[1,11] and 3-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine[14] respectively. Partial acid hydrolysis of the derived glycitol yielded D-galactose and 3-*O*-α-D-galactopyranosyl-D-galactose as the only reducing sugars.

When heated in 0·1 N aqueous pyridine at 65° (ref. 15), trisaccharide II was rapidly degraded, to yield free chromogen and a disaccharide which was chromatographically indistinguishable from 3-*O*-α-D-galactopyranosyl-D-galactose. On periodate oxidation, the behaviour of trisaccharide II was very similar to that of trisaccharide I, but after borohydride reduction[13], it consumed 3·8 moles/mole of periodate, and liberated 1·04 moles/mole of formaldehyde. Trisaccharide II is therefore considered to be *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-*N*-acetyl-D-glucosamine.

Table 1. Oligosaccharides inhibiting the Agglutination of Human B Cells by Human Anti-B Serum

	Inhibiting substance*	Minimum amount giving complete inhibition: End-point†	: μmoles/ml.
1	Human B substance	512,000	$6{\cdot}5 \times 10^{-6}$‡
2	3-*O*-α-D-Galactosyl-D-galactose	800	4
3	3-*O*-β-D-Galactosyl-*N*-acetyl-D-glucosamine	< 50	> 100
4	4-*O*-β-D-Galactosyl-*N*-acetyl-D-glucosamine	< 50	> 100
5	3-*O*-β-D-Galactosyl-*N*-acetyl-D-galactosamine	< 50	> 100
6	3-*O*-β-*N*-Acetyl-D-glucosaminoyl-D-galactose	< 50	> 100
7	*O*-α-D-Galactosyl-(1 → 3)-*O*-β-D-galactosyl-(1 → 4)-*N*-acetyl-D-glucosamine	800	2·5
8	*O*-α-D-Galactosyl-(1 → 3)-*O*-β-D-galactosyl-(1 → 3)-*N*-acetyl-D-glucosamine	400	5
9	*O*-α-D-Galactosyl-(1 → 3)-*O*-α-D-galactosyl-(1 → 3)-D-galactose	400	5
10	6-*O*-α-D-Galactosyl-D-glucose (melibiose)	50	59
11	Methyl-α-D-galactoside	< 50	> 100
12	D-Galactose	< 50	> 111

* All non-reducing sugar residues are in the pyranose form.
† Expressed as the reciprocal of the dilution of inhibitor.
‡ Based on a molecular weight of 300,000.

The capacity of trisaccharides I and II (Nos. 7 and 8, Table 1) to inhibit the agglutination of group B cells by a human anti-B serum was compared with the activity of the five disaccharides isolated from human B substance[3] (Nos. 2–6, Table 1) and other galactose-containing compounds. The results showed that, on a molar basis, trisaccharide I (No. 7) was twice as active as an inhibitor as the active disaccharide 3-*O*-α-D-galactosyl-D-galactose[1] (No. 2), whereas trisaccharide II (No. 8) had approximately the same activity as the disaccharide and was not more active than the galactotriose (No. 9) isolated from λ-carrageenin[11]. The difference between the activities of the two trisaccharides from B substance is slight, and trisaccharide II has not yet been obtained in crystalline form, but the results may indicate that the *N*-acetyl-D-glucosamine residue makes a greater contribution to the total activity when joined through a 1→4 rather than through a 1→3 linkage. The small increase in activity between the active disaccharide and the most active trisaccharide suggests that the fourth sugar residue from the non-reducing end of the chain, and other residues still more remote from it, probably contribute little to the total activity. The degree of inhibition given by these oligosaccharide fragments is, however, very small compared with the inhibition given by the intact B substance and it is possible, as suggested earlier[1,4], that sugars attached as branching units to the fragments already isolated may make a significant contribution to the full specificity of the B determinant structure.

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Immunoelectrophoresis of Sweat Proteins

The presence of mucoproteins in human sweat has already been described by one of us (M. J.)[1]; mucoproteins were also found in the sweat of the horse, the chief protein of which is albumin (the sweat glands in the horse are of an apocrine type)[2,3]. We have now examined the immunochemical behaviour of proteins in human sweat, according to their specific antigenic properties.

Using a double-diffusion technique in gel of Ouchterlony[4], four precipitation lines were found when natural sweat was tested against a rabbit immune serum against sweat proteins (obtained by immunizing a rabbit with protein, which had been isolated from a greater quantity of collected thermal sweat, of a number of normal adults); when normal human serum was tested against the rabbit immune serum against sweat proteins, two precipitation lines were found. According to Ouchterlony's reaction types[4], two of these four protein components in sweat prove to be identical with those present in normal serum, whereas the other two do not.

Using an immunoelectrophoresis microtechnique[5], five precipitation lines were obtained when the immunoelectrophoretic pattern of sweat was tested with a corresponding rabbit immune serum against sweat proteins (Fig. 1).

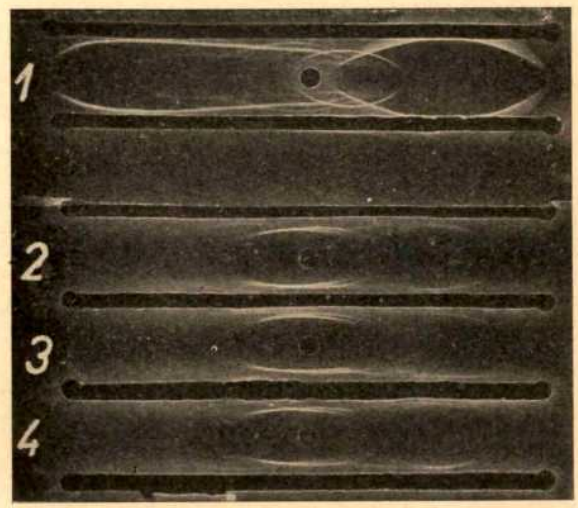


Fig. 1. Immunoelectrophoretic patterns of human sweat in comparison with human serum. 1, Normal human serum (in longitudinal basins a rabbit immune serum against human serum); 2, 3, 4, concentrated human sweat (in longitudinal basins a rabbit immune serum against sweat proteins)

At present we are trying to identify the protein components in human sweat and especially their nature as a specific product of the active secretive function of sweat gland cells.

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2,6,10,14-Tetramethylpentadecane (Pristane) from Wool Wax

The saturated polyisoprenoid hydrocarbon, 2,6,10,14-tetramethylpentadecane (pristane), has previously been shown to be a constituent of the liver oil of the basking shark (*Selache maxima* Gunnerus)[1,2] and a probable constituent of several other elasmobranch fish oils[3], sperm-whale liver[4] and ambergris[5]. It has more recently been identified in a distillate from petroleum crude oils[6].

We have now identified this compound as a constituent of wool wax. The hydrocarbon fraction was obtained, to the extent of 0·55 per cent, from a sample of the crude wool wax (the wool wax was generously provided by Mr. Clyde Rowntree, Wellman Combing Co., Johnsonville, S.C.) by alumina chromatography of the hexane-soluble materials. The normal and less highly branched components were removed by urea complex formation in *n*-propyl alcohol and the more highly branched components were

separated by gas-liquid chromatography utilizing a 'MicroTek 2500-*R*' instrument with dual columns and flame detection (MicroTek Instruments Inc., Oak Villa Boulevard, Baton Rouge, La., U.S.A.). Copper columns, 3/16 in. internal diameter by 6 ft. packed with 80–100 mesh 'Gas Chrom *P*' coated with 6 per cent silicone rubber *SE*–30 (Applied Science Laboratories, Inc., State College, Penn.), were used for the separation. The temperature of the columns was maintained at 130° C, and a helium flow rate of 70 ml. per min was utilized. Under these conditions pristane was eluted after 10·4 min.

Collection of the pristane eluate was accomplished with a differential temperature trap described by Stevens and Mold[7]. After rechromatographing under similar conditions, the column 'throw' was removed from the sample by chromatography in cyclopentane on alumina.

The infra-red spectrum for this purified sample was identical to that for an authentic sample of 2,6,10,14-tetramethylpentadecane and was in complete agreement with published spectra for this compound[8,6]. The gas-liquid chromatographic retention time for the isolated compound was also identical to that observed for the authentic pristane and approximated that for a normal hydrocarbon of two less carbon atoms.

Conclusive evidence for the molecular weight and structure was provided by a comparison of the mass spectrum for the compound isolated from wool wax with the spectrum for the authentic pristane. In addition to the molecular ion peak at m/e 268, major intensities were noted for ions of m/e 112, 113 and 183, in agreement with the mass spectrum published by Bendoraitis[6].

It is estimated that pristane constitutes about 1·1 per cent of the total paraffin fraction of crude wool wax. This is the first report of this compound from a non-marine source.

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A Crystalline Fragment from Human Gamma-globulin

PAPAIN hydrolysis of rabbit γ-globulin has yielded a crystallizable polypeptide fragment with a molecular weight of approximately 80,000 which has no antibody activity[1]. Although analogous fragments have been crystallized from purified human myeloma globulins[2], crystallization of a similar fragment from normal human γ-globulin thus far has not been reported. By performing the papain hydrolysis in relatively concentrated solutions of human γ-globulin, however, a crystalline polypeptide fragment has been obtained. Some work on this γ-globulin fragment is reported here.

7*S* γ_2-globulin (lot 337–1, Squibb) was dialysed as a 16 g per cent solution against distilled water. Part of the dialysed solution was lyophilized and then reconstituted to a concentration of 30 g per cent in 0·1 M potassium chloride containing 0·01 M cystein hydrochloride and 0·002 M sodium ethylenediamine tetraacetate. Twice-crystallized papain was added to either the 16 g per cent or 30 g per cent solutions of γ-globulin in an enzyme to protein weight ratio of 1: 1,000 and the γ-globulin hydrolysed for 30 min in the *p*H stat at *p*H 7·0 and 5 (ref. 3); at the end of this time, 4 μmoles of sodium hydroxide had been added per μmole of protein. The hydrolysate was then dialysed at 4° C for 48 h against several changes of distilled water at *p*H 5·5; during dialysis, crystals appeared.

The crystals were redissolved easily at a *p*H of 8·0 and crystallization was effectively achieved by dialysis at *p*H 5·5. Typical crystals are shown in Figs. 1 and 2. The crystals appeared to be monoclinic with the fast optical axis parallel to the long axis of the crystal.

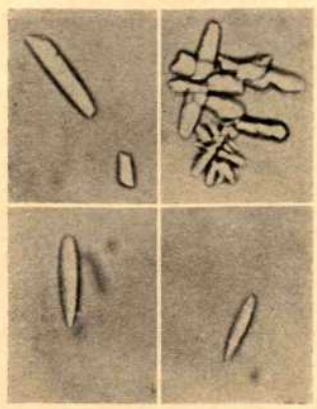


Fig. 1. Examples of crystals from first crystallization. (× *c.* 490)

Crystallized three times, the fragment had a sedimentation coefficient, $S_{w,20}$, of 3·4 *S* in a 0·6 g per cent solution, uncorrected for protein concentration and assuming a specific molal volume of 0·735. The average molecular weight was determined by sedimentation equilibrium[4] at 12,500 r.p.m. to be 63,000 ± 4,000. It was shown, however, that although thrice crystallized, this preparation contained two protein fragments, one component representing approximately 90 per cent of the total fraction: two-dimensional agar-gel diffusion studies showed two precipitin bands to be present. The two components were separated by filtration through 'Sephadex *G*-100' (Pharmacia, Uppsala, Sweden). The purified main fraction of the crystallizable protein had an average molecular weight of 49,000 by sedimentation equilibrium at 12,500 r.p.m.; in 0·2 M mercaptoethanol, the sedimentation velocity was not significantly altered. In 6 M urea, $S_{w,20}$ was 2·6 for this fraction and in 6 M urea containing 0·2 M mercaptoethanol, $S_{w,20}$ decreased to 1·7.

Immunoelectrophoresis revealed the main antigenic component of the crystallizable fragments to be the faster

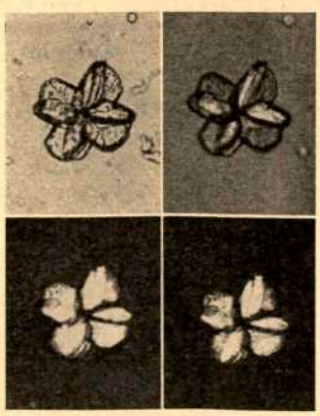


Fig. 2. In second and third crystallizations, crystals were flat and broad. Here, from third crystallization, 8 such crystals were joined and are viewed in various rotations of polarized light. (× *c.* 270)

of two antigenic determinants to be found in a papain digest of 7*S* γ_2-globulin. The 'slow' or *S* component of Edelman *et al.*[5] found in a papain digest of human 7*S* γ_2-globulin was separated from the 'fast' or *F* component in the digest[5] by chromatography on DEAE-cellulose in *p*H 7·6 phosphate buffer. The slow fraction was eluted directly from the column with phosphate buffer of ionic strength 0·01, and the *F* component was eluted by stepwise elution with buffer of ionic strength 0·4. Both fractions were further purified by gel-filtration, using 'Sephadex *G*-100'. In two-dimensional agar-gel diffusion, the *S* and *F* components gave a reaction of non-identity with each other, while the main component of the crystallizable fraction and the *F* component gave a reaction of identity. It would appear that the main crystallizable component represents the *F* fragment.

Tetanus antibody activity of the thrice-crystallized γ-globulin fragment and that of the *S* component were compared with that of unhydrolysed 7*S* γ_2-globulins. Total anti-tetanus antibody activity was determined by measurement of tetanus toxin neutralization in a mouse protection system (Division of Biologic Laboratories toxoid-coated red cells[6]. The neutralization test revealed that the immunochemically impure crystallized polypeptide had about 6 per cent of the activity of the original 7*S* γ_2-globulins while the *S* compound had an equal or slightly greater neutralizing activity than did the original 7*S* γ_2-globulin on a weight basis. In the hæmagglutination test, divalent activity of the crystallized fragment was 0·4 per cent that of the parent 7*S* γ_2-globulin, while the *S* component was devoid of divalent antibody activity. The antibody activity in the crystallizable fraction appeared to represent contamination with a univalent antibody component. The latter component was separated from the *F* component in the crystallized fraction by passage through 'Sephadex *G*-100'.

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Piloquinone: a New Phenanthrene-o-Quinone isolated from the Mycelium of *Streptomyces pilosus*

The mycelium of *Streptomyces pilosus* grown on a soy-bean meal containing medium produces several orange or red pigments. The principal one, which we propose to call 'piloquinone', crystallizes easily from a petrol ether or ether extract of the dried mycelium.

After recrystallization in chloroform-ether piloquinone is obtained as dark-red needles melting at 176°–178°. It is shown to be homogeneous by thin-layer chromatography on silicic acid.

The ultra-violet spectrum (in 95 per cent ethanol) has maxima at: λ_{max} 233 mμ ($\varepsilon = 32{\cdot}000$) and λ_{max} 286 mμ ($\varepsilon = 17{\cdot}000$) and a shoulder at 277 mμ ($\varepsilon = 15{\cdot}600$). The visible spectrum has maxima at 396 mμ ($\varepsilon = 4{,}075$) and 515 mμ ($\varepsilon = 5{,}560$). The infra-red spectrum (in 'Nujol') shows in the region of 1,500–1,700 cm^{-1} bands at: 1,570, 1,635 and 1,695 cm^{-1}.

Elementary analyses are in agreement with the formula $C_{21}H_{20}O_5$; the molecular weight 352 was confirmed by mass spectrometry. The pigment is easily bleached by sodium hydrosulphite and reoxidized by air; this indicates that it is a quinone. The easy formation of a quinoxaline $C_{27}H_{24}O_3N_2$ (m.p. 174°) after treatment with *o*-phenylenediamine suggests an *o*-quinone. A third oxygen atom is in a keto-group: piloquinone gives a mono-2,4-dinitrophenyl-hydrazone, $C_{27}H_{24}O_8N_4$, m.p. 254°; furthermore, reduction by $NaBH_4$ gives dihydropiloquinone, $C_{21}H_{22}O_5$, red brown needles, m.p. 230°, and Clemmensen reduction gives deoxy-dihydropiloquinone, $C_{21}H_{22}O_4$, red crystals, m.p. 175°; both these no longer show the CO band at 1,695 cm^{-1}.

Two oxygen atoms of piloquinone are present in strongly chelated phenolic hydroxyl groups (peaks at 734 and 762 c.p.s. in the nuclear magnetic resonance spectrum) (the nuclear magnetic resonance spectrum was

ive acetyla-

tion of piloquinone gives a colourless tetraacetate $C_{29}H_{30}O_9$, m.p. 182° (still showing the carbonyl absorption at 1,690 cm^{-1}).

The five oxygen atoms of piloquinone are thus accounted for: two in the quinonoid system, two phenolic OH groups and one carbonyl.

Zinc dust distillation in an atmosphere of hydrogen gave phenanthrene and 2-methyl-phenanthrene melting at about 55°; moreover, after reductive acetylation of deoxy-dihydropiloquinone, a tetraacetate $C_{29}H_{32}O_8$, m.p. 164°, was obtained, having an ultra-violet spectrum very similar to that of phenanthrene.

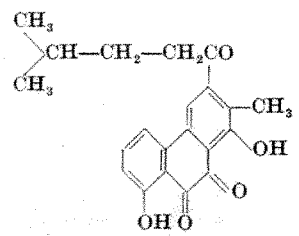
It thus seems established that piloquinone is a dihydroxy phenanthrene-*o*-quinone.

The presence of an isocaproyl side-chain was shown by chromic acid oxidation[1] of piloquinone: vapour phase chromatography of the methyl esters of the volatile acids thus obtained showed the presence of methyl isocaproate, isovalerate and lower fatty esters.

The nuclear magnetic resonance spectrum of piloquinone shows a doublet at 57 c.p.s. (6 H) corresponding to the two methyls of the isocaproyl side-chain and a singlet at 133 c.p.s. showing the presence of a methyl group on an aromatic ring.

The nuclear magnetic resonance spectrum of piloquinone and of several of its derivatives also suggests the presence of three hydrogen atoms adjacent to each other on one aromatic ring, a fourth being alone on a substituted benzene ring.

All these properties are in agreement with formula I, which is consistent with biogenesis from a polyacetate precursor[2].



I

A detailed paper giving further structural proofs and degradation to a diphenyl derivative will be published in *Bull. Soc. Chim. France*.

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Weever Venom

The venomous spines of the great weever (*Trachinus draco*) represent a great hazard especially to fishermen in areas where this fish is commercially important[1], since the wounds inflicted may cause severe and long-lasting pains[2].

Pharmacological[3] and serological[4] investigations have revealed little about the active principles. In the venom from the closely related lesser weever (*Trachinus vipera*), Carlisle[5] recently claimed to have found 5-hydroxytryptamine and a small molecular histamine releaser.

Our experiments have shown that the venom consists of three electrophoretically separable protein fractions. Evidence was obtained that noradrenaline, adrenaline, histamine and cholinesterase were present. Lecithinase and phosphodiesterase were not present.

When venom from the opercular spine of living weever was subjected to electrophoresis, the three spots shown in Fig. 1 were obtained. If the venom was diluted with a small volume of saline, coagulation occurred. Subsequent centrifuging and electrophoresis of the supernatant resulted in a single and faintly dyed spot at the application line indicating almost complete removal of the protein. The lability of the migrating components is also demonstrated by the tailing in Fig. 1.

The following experiments were all carried out with deep-frozen material. The opercular and dorsal spines were cut from living weevers (all about 30 cm long), immediately wrapped in aluminium foil, put on solid carbon dioxide and stored at $-20°$ C. For each experiment the venomous spines of two weevers were homogenized in Krebs–Ringer bicarbonate buffer using a small Waring blender.

Prior to adsorption on 'Amberlite *CG* 50' of the biological amines from an extract of venom, the protein was precipitated by the addition of an equal volume of ethanol. Elution of the amines and the subsequent paper chromatography were carried out according to Awapara *et al.*[6].

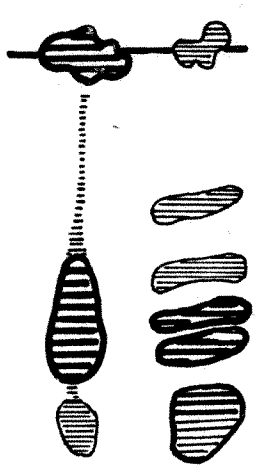


Fig. 1. Schematic drawing of an electropherogram run in 0·1 M veronal buffer *p*H 8·6, 200 V for 9 h. Dyed with amido black *B* 10. Left, venom; right, serum

The paper chromatogram revealed two spots, one of which was readily identified from its R_F value and colour reactions[7] as histamine. Similarly the other spot was identified as catecholamine[8]. As the paper chromatographic method used does not readily distinguish between the different catecholamines, further identification was carried out by a photofluorimetric method[9]. This revealed the presence of both adrenaline and noradrenaline in high concentrations.

Spraying of the chromatogram with 1-nitroso-2-naphthol reagent[10] did not reveal any 5-hydroxyindol derivative. In contrast to the lesser weever[5], therefore, 5-hydroxytryptamine does not seem to be present in significant concentrations in the great weever venom. The cholinesterase activity of homogenates was determined using standard manometric technique[11]. The high enzyme activity found in homogenized venomous spines (1,500 µl. carbon dioxide/100 mg wet wt.) and gill cover (3,000 µl. carbon dioxide/100 mg wet wt.) largely exceeded that obtained from fish muscle (300 µl. carbon dioxide/100 mg wet wt.). The homogenates were able to hydrolyse acetyl-, butyryl- and acetyl-β-methyl-choline, thus indicating the presence of both acetyl- and butyryl-cholinesterase. In a control experiment using gill cover from cod (*Gadus callarius*) no cholinesterase activity could be demonstrated.

The extracts of venom were examined for phosphodiesterase and lecithinase since these enzymes are common components of venoms from the animal kingdom. Using the methods of Boman and Kaletta[12] no activities could, however, be demonstrated. The reported hæmolytic properties of weever venom[4,13] can therefore scarcely be due to lecithinase.

Weever stings are known to produce ischæmia with subsequent hyperæmia, effects which may be explained by the amines present. These compounds will have a net effect according to their individual quantities and their rate of penetrating the surrounding tissue. In the initial ischæmic period the catecholamines, being vasoconstrictors, evidently are dominating. Since monoaminoxidase is present subcutaneously, the catecholamines are catabolized and the effect of histamine will prevail and produce hyperæmia. Admittedly this hyperæmia may at least partly be a reactive one.

The finding of a small molecular histamine releaser in the lesser weever venom was based on the weal and flare produced by the subcutaneous injection of a ninhydrin-positive substance eluted from a paper chromatogram[5]. Due to the close relationship between the two species, we suggest that this substance actually may be histamine itself.

The part played by cholinesterase in venoms from the animal kingdom has not been accounted for. We suggest that the high concentration of cholinesterase in the venomous spine and surrounding gill cover possibly reflects a cholinergic mechanism involved in the production of the venom.

The substances reported here are suggested to represent only a part of the toxic principles of the great weever venom, as the function of the protein fraction has been only partly accounted for. The coagulability of the proteins is evidently important, since the clot formed will greatly resist washing out. This view is in accordance with the prescriptions for the treatment of weever stings, which always state that good care should be taken to remove "traces of integumentary sheet" or "spongy white material" left in the wound. The clot will also promote a long-lasting effect of the inherent active principles as the diffusion into the surrounding tissue evidently will be retarded. Further, as it is well known that proteolytic enzymes are involved in coagulation processes, a significant part of the toxic principle may be related to the presence of such enzymes, as they may act further in the victim's tissue and split off toxic peptides. In this connexion, it is interesting to note that an enzyme promoting the coagulation of bovine plasma recently has been demonstrated and purified from Russell's viper venom[14].

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Ferulic Acid as a Component of a Glycoprotein from Wheat Flour

It has already been shown that the water-soluble wheat flour pentosans can be separated by DEAE-cellulose [illegible] into five fractions: a pure arabinoxylan

cipal glycoprotein fraction (No. 2) could be [illegible]. This fraction contains 80–90 per cent polysaccharide (components: xylose, arabinose and galactose) and 10–20 per cent proteins. The ultra-violet absorption spectra show in addition to the protein maximum a weak maximum at 320 mμ which disappears on oxidation[3]. It was suspected that polyphenolic compounds are responsible for this maximum.

Various degradation reactions were investigated, therefore, in order to obtain low-molecular weight fragments containing phenolic components. Degradation of the glycoprotein-2 by pure proteolytic enzymes released no phenolic compounds but showed that they were most probably linked to the xylan chain rather than to the protein part. Degradation by these enzymes produced two polysaccharide fragments of entirely different composition, indicating that in glycoprotein-2 the two polysaccharides are linked together by a polypeptide bridge[5].

Phenolic compounds could be split off from the glycoproteins (unfractionated flour pentosans), however, by treatment with aqueous 0·5 M potassium hydroxide at 60° C during 90 min under nitrogen. The hydrolysate was acidified (*p*H 3·5), extracted with ether followed by a bicarbonate extraction. The ether extract from the acidified bicarbonate solution was investigated by thin-layer chromatography. Three phenol positive spots were detected (solvent[6]: benzene–methanol–acetic acid 90 : 16 : 8, R_F values 0·46, 0·58 and 0·66). One of the spots (R_F 0·58) could be positively identified as ferulic acid (R_F values in various solvents, colour reactions, ultra-violet spectra of the extracted spot). When the flour pentosans were gelled by addition of hydrogen peroxide prior to hydrolysis with alkali, no ferulic acid could be detected. It is, therefore, very likely that ferulic acid and possibly other phenolic compounds in the soluble pentosans take part in the oxidative gelation of aqueous flour extracts and therefore constitute other types of compounds in wheat flour readily attacked by oxidizing agents.

It is tentatively assumed that ferulic acid is linked to the xylan chain via ester bonds. The deposition of toxic ferulic acid[7] and possibly other phenolic compounds in cell wall polysaccharides might be another way of detoxication of these compounds in certain plants, formation of simple glycosides being, of course, the most common way[8]. Aliphatic acids (formic and acetic acid) have long been known to be deposited as esters in certain plant cell wall polysaccharides[8].

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Sulphatases and Sulphated Polysaccharides in the Viscera of Marine Molluscs

Glycosulphatases are present in tropical[1] and British[2–4] molluscs. Molluscan preparations also contain chondrosulphatase[2,4], which may be an oligosaccharide sulphatase; but few examples of polysaccharide-sulphatases are known[5].

Recently the sulphatase activities of preparations from marine molluscs collected on the North Wales shore with respect to various sulphated carbohydrates of both high [illegible] weight have been studied. The

with the carbohydrate substrate [illegible] liberated sulphate was detected turbidimetrically[6]. Preparations from *Calliostoma zizyphinum* and *Patella vulgata* showed the widest range of activity, each liberating sulphate from D-glucose- and D-galactoso-6-sulphate, chondroitin sulphate *A* and fucoidin. Other polysaccharides, keratosulphate, carrageenin and mucilages from *Dilsea edulis* and *Porphyra umbilicalis*, were not desulphated. *Littorina littorea*[3] and *Mytilus edulis* preparations contained glycosulphatase, but were inactive against chondroitin sulphate *A* and the algal polysaccharide sulphates. *Buccinum undatum* and *Gibbula cineraris* were without action on the foregoing substrates.

It was of interest to consider the nature of the natural substrates for molluscan sulphatases and a further investigation had led to the isolation, from *Patella vulgata* viscera, of a sulphated polysaccharide(s), which is a substrate for the sulphatase preparation obtained from the same source. The polysaccharide material was obtained by extraction of *Patella* viscera with 5 per cent potassium chloride followed by trypsin digestion, Sevag deproteinization, dialysis and alcohol precipitation. It was further purified by precipitation with cetyl pyridinium chloride[7]. Sugars detected when the preparation was hydrolysed with acid were fucose, xylose and galactose. Fucose and xylose are not usually encountered in other than trace amounts in animal tissues and it is considered likely that the polysaccharides, in *Patella* viscera are derived, partially at least, from algal sources (where these sugars are common) which are ingested by the mollusc[8]. *Patella* sulphatases are thus regarded tentatively as digestive enzymes.

Patella viscera also contains a glucan, which is obtained by extraction with 10 per cent trichloroacetic acid, as well as enzymes, which degrade starch, maltose and cellobiose.

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PHYSIOLOGY

Role of Adenosine Triphosphate in Muscular Contraction

Loaded frog's stomach muscle at first relaxes and then contracts when treated with dinitrophenol (DNP)[1,2]. It was presumed that the relaxation and the subsequent contraction produced by DNP were due to depletion of stores of adenosine triphosphate. In the present research ATP, ADP and AMP have actually been estimated under the foregoing conditions.

These experiments were performed on circular strips of the stomach muscle of the frog *Rana tigrina*. Two sets of experiments were performed. In the first set two lightly loaded pieces of muscle from the same stomach were used; one of them, which served as a control, was suspended in Ringer solution and the other in Ringer solution containing 0·5 mM DNP. After 90 min both pieces were analysed for phosphate esters when the DNP-treated muscle had contracted after preliminary relaxation. In the second set of experiments, two pieces of muscle were similarly suspended in Ringer solution containing DNP, and one of them, which served as a control, analysed after 90 min, and the other was changed to Ringer solution and analysed after the next 120 min. To estimate the phosphate esters, the ether-extracted cold trichloroacetic acid extracts of tissue were chromatographed on paper using the solvent system of Krebs and Hems[3], and the esters were located by the acid molybdate spray of Burrows *et al.*[4]. The spots were cut out and wet washed[5] and analysed according to Bartlett's heating method[6] but read at 660 mμ (ref. 7).

DNP, after an initial stimulation, causes the loaded frog's stomach to relax and then contract again; on removal of DNP, the reverse happens, the muscle first relaxes and then contracts (Fig. 1). There are two variations from this usual result; the tonic contraction *B* may be little marked, suggesting the presence of some inhibitor in the muscle, and the relaxation *Y* may also be little marked, the contraction *B* passing into *C* without intervening relaxation. During the tonic contractions *A* and *C*, the muscle contracts when stimulated with electric current and acetylcholine, and shows spontaneous contractions; during *B* there was no response to stimulation and no spontaneous activity. *A* and *C* are relaxed by adrenaline and cyanide, but *B* is resistant to both.

The phosphate ester content in μmoles/g wet wt. of control muscles not treated with DNP was: ATP 1·512 ± 0·058, ADP 1·561 ± 0·053, AMP nil. That of 10 muscles treated with DNP for 1·5 h and in a contracted state was: ATP 0·114 ± 0·014, ADP 1·446 ± 0·085, AMP 1·650 ± 0·079, indicating a decrease of ATP by 92 per cent. In the second set of experiments, the phosphate ester content of 10 muscles treated with DNP was: ATP 0·104 ± 0·012, ADP 1·407 ± 0·079, AMP 1·634 ± 0·083. That of 10 muscles afterwards changed to Ringer and kept for 2 h therein was: ATP 0·809 ± 0·058, ADP 1·642 ± 0·065, AMP 0·539 ± 0·067, showing a recovery of about 50 per cent in the ATP content.

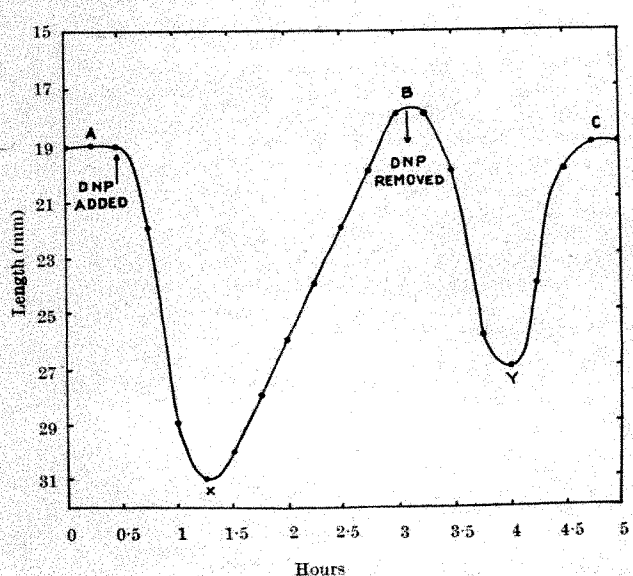


Fig. 1. Effect of 0·5 mM DNP on loaded frog's stomach muscle

These results suggest the two functions of ATP in unstriated muscle: (1) to provide energy for phasic contraction, active relaxation[1,2] and one kind of tone which is susceptible to asphyxia and metabolic inhibitors; (2) to keep the muscle relaxed. These probably correspond to two similar functions of ATP in the glycerol-extracted striated muscle[8].

In these estimations, an unidentified nucleotide phosphate ester band with R_F value 0·22 appeared and disappeared with the ATP band.

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Inhibition of Thyroid Function by a Dithiocarbamoylhydrazine

Paget, Walpole and Richardson[1] described some biological effects of 1-α-methylallylthiocarbamoyl-2-methylthiocarbamoyl-hydrazine ('ICI 33828') in rats, dogs and monkeys and postulated a selective and reversible inhibition of pituitary gonadotrophic function. Similar results have since been obtained in mice[2]. In view of these findings we decided to investigate the effect of the compound on pituitary thyrotrophic function.

The effect on the goitrogenic action of methylthiouracil was investigated in rats using the increase in thyroid weight as an index of thyrotrophin production by the pituitary. The mean thyroid weight in a group treated with methylthiouracil and 'ICI 33828' was significantly less than that in a group given methylthiouracil and saline; but the treated group also showed a significant fall in total body-weight. Since the precise relationship between changes in total body-weight and thyroid-weight was not known these results were inconclusive. Attempts to modify the experiment so as to reduce the loss in body-weight were unsuccessful.

We decided, therefore, to investigate the rate of discharge of thyroid hormone labelled with iodine-131 from the gland in rats and mice treated with 'ICI 33828'. An injection of carrier-free $Na^{131}I$ was given intraperitoneally and after 48 h potassium perchlorate was added to the drinking water to prevent recirculation of iodine and to discharge inorganic iodide from the gland. On the following day and for either two or three days thereafter half the animals were injected subcutaneously with a suspension of 'ICI 33828' and half with the suspending medium alone. After 24 h the animals were killed and the radio-iodine content of their thyroid glands assessed.

It can be assumed that, at the beginning of treatment, the mean iodine-131 content of the thyroid glands of the mice in the control and treated groups is not significantly different. After any given time the mean iodine-131

remaining in the gland will be diminished by the amount which has been discharged. The total radioactivity remaining in the thyroid gland was therefore used as a convenient measure of thyroid hormone secretion. In each of the six experiments detailed in Table 1, with the exception of those using adult male mice, the iodine-131 content of the thyroid gland was significantly higher in the treated group than in the control. This was interpreted as being the result of a decrease in the rate of discharge of thyroid hormone during treatment with 'ICI 33828'.

Table 1

Animal	Dose 'ICI 33828' mg/day	No. of doses	Mean thyroidal ^{131}I counts/100 sec	Significance of difference from control. P*
Mice, female immature	0	4	1,534	
	1	4	1,965	< 0·01
Mice, female adult	0	4	17,820	
	1	4	32,660	< 0·02
Mice, male adult	0	4	1,726	
	1	4	1,611	> 0·1
Mice, male adult	0	4	554	
	1	4	582	> 0·1
Rats, male immature	0	3	864	
	1	3	1,443	< 0·001
	3	3	1,161	< 0·02
Rats, male adult	0	3	2,175	
	3	3	3,869	< 0·02

* Derived from t-test.

The effect of 'ICI 33828' on the discharge of iodine from the thyroid gland suggests a decrease in the secretion of pituitary thyrotrophin or an inhibition of its action on the discharge of thyroid hormone. A reduction in secretion seems the more likely because we have been unable to show a significant effect of 'ICI 33828' on the discharge of thyroidal iodine in response to exogenous thyrotrophin.

Since 'ICI 33828' contains thiourylene groups it might be expected, in addition to its postulated action on the pituitary, to have a direct effect on the trapping and protein-binding of iodine by the thyroid gland. This action was demonstrated in another series of experiments in which, in both rats and mice, the injection of 'ICI 33828' produced a significant decrease in thyroid uptake of radio-iodine. This effect could not be overcome by exogenous thyrotrophin given 24 h prior to the measurements in intact mice the endogenous thyrotrophin of which had been suppressed by the administration of thyroxine.

It seems likely, then, that 'ICI 33828' acts on thyroid function both by a direct effect on the gland and by influencing pituitary thyrotrophic activity. This combination of effects probably explains why we have found that the injection of doses up to 12 mg daily for 10 days caused no significant change in the thyroid weight of adult rats. Doses much smaller than this cause a marked block of uptake of iodine by the gland and might be expected to be goitrogenic if the drug did not at the same time suppress pituitary thyrotrophic activity. The mechanism by which it produces the latter effect and the absence of this effect in adult male mice remain to be explained.

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Absorption of Vitamin B_{12} – Glutamic Acid Mixture

In their recently published papers[1,2], Heathcote and Mooney have expressed the belief that the concept of intrinsic factor is incorrect and that the absorption of vitamin B_{12} is dependent on gastric proteolysis. According to them, vitamin B_{12} is absorbed as either small peptide or amino-acid units. In addition, they reported excellent results of oral treatment of pernicious anæmia with a vitamin B_{12}–glutamic acid mixture[3].

For a study of the absorption of vitamin B_{12}, the Schilling test is routinely used. It is, in our opinion, also suitable for investigation of the supposed promoting effect of L-glutamic acid on the absorption of vitamin B_{12}. Accordingly, we performed a series of Schilling tests with this objective.

The series consisted of ten patients with Addisonian pernicious anæmia. The diagnosis was established by blood picture, bone marrow findings and a 'Histalog' test meal. The following tests were made on each patient: (1) ordinary Schilling test with 0·5 μg of vitamin B_{12} labelled with cobalt-60; (2) Schilling test with the same B_{12} dose together with 10 μg of L-glutamic acid; (3) Schilling test with the same B_{12} dose and 30 mg of purified hog intrinsic factor (Abbott Lab., Inc.).

The results are presented in Fig. 1. In seven cases the values of the Schilling test with L-glutamic acid were not significantly different from the ordinary Schilling test values. In three cases the Schilling-L-glutamic factor values rose moderately, but even in these cases the increase was not of the same order as the increase in all cases of Schilling-intrinsic factor tests. The mean value of the ordinary Schilling tests was 1·0 per cent, that of the Schilling-L-glutamic acid tests 2·9 per cent and that of the Schilling-intrinsic factor tests 13·1 per cent.

In their therapeutic trial, Heathcote and Mooney[3] have used control periods of 10 and 13 days with crystalline vitamin B_{12} before starting therapy with the B_{12}–L-glutamic acid mixture. In both their cases the rise in hæmoglobin

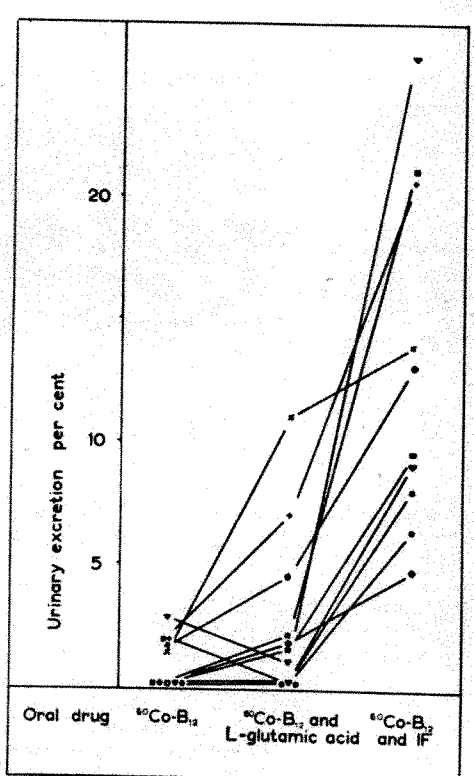


Fig. 1. Results obtained in the Schilling tests with vitamin B_{12} alone, with vitamin B_{12} and L-glutamic acid, and with vitamin B_{12} and intrinsic factor

and packed cell volume began on the first day of B_{12}–L-glutamic acid treatment. In our opinion, this rise occurred too early to have been an actual response to B_{12}–L-glutamic acid treatment. It appears more likely to have been an effect of the crystalline vitamin B_{12} therapy given during the preceding control period. On the other hand, we admit that the second reticulocyte response following B_{12}–L-glutamic acid treatment indicated that L-glutamic acid promotes the absorption of vitamin B_{12} to some extent at least.

Our results with Schilling tests, however, indicate that L-glutamic acid does not in general increase the absorption of vitamin B_{12}, or at least not to anywhere near the same extent as the intrinsic factor preparation. Accordingly, we do not find ourselves in agreement with Heathcote and Mooney and we cannot deny the existence of an "intrinsic factor".

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The authors of the g communication state that "According to them [H te and Mooney], vitamin B_{12} is absorbed as either oeptide or amino-acid units". We trust that this mis retation of our actual beliefs is due to lingual difficulty does not imply that we believe in the peptide or amino- nature of vitamin B_{12} itself.

Dr. Heinivaara and Dr. Palva state her that the Schilling test is "suitable for investigation he supposed promoting effect of L-glutamic acid on the absorption of vitamin B_{12}". It is unfortunate that they do not give their reasons for this statement because the significance of their own work depends entirely on the validity of this assumption. We should particularly like to know why they think that this test—which actually measures the urinary excretion of radioactive vitamin B_{12}—necessarily gives a true measure of the amount absorbed? Can they demonstrate—as the Schilling test presumes—that all forms of vitamin B_{12} when absorbed into the blood stream are incorporated into the stores and blood-forming tissues at an equal rate? Furthermore, can the "flushing dose"—assuming that it dislodges some radioactive B_{12} from the serum—also release that which has been actively incorporated into the liver and blood-forming tissues? Unless they can produce such evidence, their own results can be equally well interpreted simply as demonstrating an improved retention or an increased "resistance to flushing" of radioactive B_{12} in the presence of glutamic acid. Do they also believe that the Schilling test is a better index of therapeutic efficiency than the type of test which we have used, whereby hæmatological progress is assessed? Has it in fact any physiological merit at all?

With reference to our work, Dr. Heinivaara and Dr. Palva "admit that the second reticulocyte response following B_{12}–L-glutamic acid treatment indicated that L-glutamic acid promotes the absorption of vitamin B_{12} to some extent at least". They go on to state that "In both their cases the rise in hæmoglobin and packed cell volume began on the first day of B_{12}–L-glutamic acid treatment. In our opinion, this rise occurred too early to have been an actual response". Examination of the figures in our paper will show, however, that in case 49 (Fig. 1) neither the hæmoglobin nor the packed cell volume was estimated during the first four days of treatment with B_{12}–L-glutamic acid mixture, and that in case 52 (Fig. 2) a fall in both hæmoglobin and packed cell volume was recorded in the first estimation performed after the commencement of B_{12}–L-glutamic acid treatment.

Finally, it may be of some interest to mention, briefly, the present state of health of the two patients who formed the basis of our communication. Both are still on the same form of therapy, are well, and have no neurological symptoms. Case 49 has now been maintained for 973 days on an average daily dose of 28 μg of the mixture (expressed as B_{12} content). At present his hæmoglobin is 91 per cent and packed cell volume 40 per cent. Case 52 has been maintained for 885 days on an average daily dose of 24 μg of B_{12}–glutamic acid mixture. His hæmoglobin is 107 per cent and packed cell volume 44 per cent. A third patient (case 62) has also been treated with B_{12}–glutamic acid, since our earlier publication. He is a man aged seventy-five who had, on admission, a hæmoglobin of 52 per cent and packed cell volume of 25 per cent. He has been treated for 639 days with an average daily dose of 16 μg of the mixture. At present his hæmoglobin is 95 per cent and packed cell volume 40 per cent.

In conclusion, although we have no particular desire to undermine any faith which they may hold in "intrinsic factor", might we suggest to Dr. Heinivaara and Dr. Palva that a critical re-appraisal of the value of the Schilling test, as a basis for their belief, might not be entirely without profit?

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Increase of Trypsin Inhibitor in Serum during Pregnancy

In normal pregnancy increased quantities of trypsin inhibitor are excreted in the urine, especially during the second and third trimester, followed by a rapid decrease to normal after parturition[1].

Excretion of trypsin inhibitor seems a response to stress, since it is increased after various forms of stress and after administration of ACTH or glucocorticoid hormones[2,3]. The amount excreted appears to be regulated by the concentration of glucocorticoids (endogenous or exogenous) in the body[3,4]. Hence, it is also believed that the increased trypsin inhibitor excretion observed in pregnancy is produced by an increase in glucocorticoid production.

The physiological mechanism of the excretion of trypsin inhibitor is unknown, but we have found that in certain forms of stress (surgical) the increase in trypsin inhibitor excretion parallels an increase in trypsin inhibitor concentration in blood serum, suggesting that variations in trypsin inhibitor excretions reflect variations in serum concentration of the same inhibitor[5]. In order to substantiate this concept we wish to report an increase of trypsin inhibitor concentration in serum during pregnancy, corresponding in pattern to the variations in urinary trypsin inhibitor excretion[1] and in serum concentration of glucocorticoid hormones[6].

The estimation of trypsin inhibitor in serum was performed as before[5]. One trypsin inhibitor unit represents the amount which inhibits 1 μg of a preparation of crystalline, pure trypsin containing 24·4 Anson units per g (kindly supplied by the NOVO Laboratories, Copenhagen).

Blood samples were obtained from 11 normal women and from 47 women with normal pregnancies of different durations. Blood was centrifuged immediately after spontaneous coagulation, and serum was stored at −8° C until analysed.

The results (Table 1) show great individual variations in the serum concentration of trypsin inhibitor. This was also encountered in the amount excreted in the urine during 24 h (ref. 1). Thus, in the non-pregnant group the serum concentration ranged from 300 to 1,965 trypsin inhibitor units per ml. with an average value of 1,140 units per ml. (*S.E.*, 134). In the pregnant groups the deviations tended to be slightly less. The difference between the average concentration in the first trimester

Table 1. Concentration of Trypsin Inhibitor in Units per ml. Serum in Pregnant Women

Non-pregnant	1st trimester	2nd trimester	3rd trimester
300	1,060	1,510	525
765	1,230	1,590	1,380
810	1,275	1,760	1,380
870	1,410	1,770	1,620
1,140	1,545	1,860	1,860
1,245	1,680	1,860	1,905
1,260	1,770	1,950	1,965
1,320	1,820	1,950	2,030
1,350	1,830	2,055	2,040
1,530	2,010	2,055	2,100
1,965		2,070	2,100
		2,100	2,115
		2,130	2,145
		2,145	2,220
		2,220	2,325
		2,340	2,460
		2,340	2,475
		2,370	2,520
			2,520
Average: 1,140 ± 134 (*S.E.*)	Average: 1,565 ± 98 (*S.E.*)	Average: 2,005 ± 58 (*S.E.*)	Average: 1,985 ± 112 (*S.E.*)

(1,565 units per ml. with the *S.E.* 98) and in non-pregnant women is not statistically significant.

The average concentrations in the 2nd and 3rd trimesters are both statistically different from the non-pregnant group ($P < 0.001$) and the 1st trimester ($P < 0.01$ and $P < 0.02$, respectively). The small difference between the average values in the 2nd and 3rd trimesters is caused by one single person, who deviated considerably from the remaining individuals in the group.

The increase in trypsin inhibitor in serum during pregnancy parallels in time the increased urinary excretion observed before[1]. These results are comparable with those obtained in surgical stress as mentioned above. Hence, it is suggested that the increase in trypsin inhibitor excretion in urine is caused by the increased serum concentration of trypsin inhibitor and not by a possible alteration of the kidney function, respective to the treatment of the trypsin inhibitor. It is further suggested that not only the increase in urinary excretion of trypsin inhibitor but also the increase in serum trypsin inhibitor concentration in pregnancy are caused by an increase in glucocorticoid production.

The trypsin inhibitor responsible for the increase in trypsin inhibitor concentration in serum and trypsin inhibitor excretion in urine during stress and pregnancy could be called the stress-regulated or the glucocorticoid-regulated trypsin inhibitor[5]. Also the hyaluronidase inhibitor in serum increases during pregnancy[7]. This observation is of interest because the trypsin inhibitor in urine is a hyaluronidase inhibitor[8], suggesting that the substances, which produce increase in hyaluronidase-inhibiting activity and in trypsin-inhibiting activity of blood are identical.

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IMMUNOLOGY

Delayed Hypersensitivity to a Conjugate of Polytyrosine and its Inhibition by Haptens

Recent investigations concerning the nature of the antigenic determinants involved in delayed hypersensitivity to hapten–protein conjugates have pointed to the important part played by the protein carrier[1,2]. In addition, some aspects of the heterogeneity of the response have been clarified[3]. In recent experiments reported by us[4], guinea pigs were immunized with arsanilic acid (ars) coupled to guinea pig serum albumin. They reacted with the same hapten coupled to many unrelated proteins, suggesting a high degree of hapten-specificity. Positive reactions elicited by heterologous haptens coupled to guinea pig serum albumin were also noted and considered to be a response to antigenic determinants produced or revealed by the chemical process of conjugation with diazonium salts.

In order to limit to some extent the heterogeneity of response attributed to antigenic determinants arising from the non-repeating amino-acid sequences present in proteins as well as from the many different sites available for hapten conjugation, attempts were made to induce delayed hypersensitivity to haptens conjugated to a polymer of a single amino-acid.

This communication is concerned with investigations of delayed hypersensitivity produced to polytyrosine (obtained from Yeda Research and Development Co., Rehovoth, Israel) conjugated with diazotized arsanilic acid in a ratio of 5 moles arsanilate per mole of polytyrosine. A total of 100 μg polytyrosine–azobenzenearsonate (ars–polytyr) emulsified in complete Freund adjuvant containing killed mycobacteria was injected in a total volume of 0·1 ml. in the four foot pads of 400 g white guinea pigs. Two weeks later, skin tests were performed with 1 μg nitrogen amounts of conjugates prepared from a variety of proteins and arsanilic acid. Some typical results seen in Table 1 illustrate what appears to be a large measure of hapten specificity. Thus, arsanilic acid conjugated to proteins as diverse as insulin and ribonuclease produced delayed reactions which were, if anything, larger than the reactions produced by the immunizing antigen itself. These results may be compared with those reported[4] previously for immunization with a conjugate prepared from arsanilic acid and guinea pig serum albumin, in which a large degree of hapten specificity was also noted. In this latter case, however, conjugates prepared from heterologous protein carriers usually produced smaller reactions than did the immunizing antigen. Both these results stand in striking contrast to the narrow range of carrier specificity observed with other haptens, such as *p*-aminobenzoic acid and picryl chloride[1], in which conjugates prepared from heterologous protein carriers were unreactive.

Table 1. Skin Reactions to Azobenzenearsonate-conjugated Proteins in Guinea Pigs immunized with 100 μg Ars–Polytyrosine

No. of animals	Delayed reactions* to skin test antigens: ars–polytyr (50 μg)	ars–gelatin (1 μg N)	ars–GSA (1 μg N)	ars–ribonuclease (1 μg N)	ars–insulin (1 μg N)	ars–elastoidin (1 μg N)
6	15	—	16	18	—	—
5	13	14	—	—	18	—
5	13	—	—	—	—	13

* Average induration and erythema (mm).

It seemed evident from these findings that a large portion of the reaction spectrum produced by immunization with ars–polytyr might be directed towards the azobenzenearsonate group. To provide further evidence for this, attempts were made to suppress the delayed reaction to this moiety by the administration of simple haptens. The most obvious hapten, other than arsanilic acid itself, was considered to be its conjugate with tyrosine. Accordingly, equimolecular amounts of diazotized arsanilic acid and *N*-acetyltyrosine were allowed to react in cold alkaline solution and the resulting dye

precipitated by acidification. Similar dyes were prepared by conjugation with *N*-benzoyltyrosine as well as the other aromatic amino-acids *N*-acetyltryptophan and *N*-acetylhistidine.

In Table 2 are seen the results of skin tests with ars–guinea pig serum albumin before and after hapten administration in animals immunized with ars–polytyr. With the dose-levels used, a striking suppression of reaction was observed only with the ars–*N*-acetyltyrosine, so that 24-h reactions to skin tests performed immediately after intracardiac injection of this hapten consisted only of occasional erythema alone. It is of interest that arsanilic acid itself in doses four times as large was not inhibitory. Also inactive was the conjugate prepared from the *N*-benzoyl derivative of tyrosine. Because of the unknown effects of differences in hapten solubility and rates of excretion, it cannot be stated with certainty that these results are due to steric interference of the benzoyl group with the binding by the reactive component in delayed hypersensitivity and the tyrosine–azobenzenearsonate group. Similarly, conclusions concerning the inability of haptens prepared from histidine and tryptophan to suppress delayed hypersensitivity must also await determinations of their *in vivo* solubility and persistence. Nevertheless, these results provide strong confirmatory evidence for the conclusion that the size of the antigenic determinant responsible for the reactions investigated here lies between arsanilic acid itself and tyrosine–azobenzenearsonate.

Table 2. Skin Reactions to Ars–Guinea Pig Serum Albumin (GSA) before and after Administration of Haptens in Guinea Pigs immunized with Ars–Polytyrosine

No. of animals	Hapten given (1×10^{-5} moles)	Delayed reactions* to skin tests with 1 μg N ars–GSA: Pre-hapten	Immediately post-hapten	24 h post-hapten
5	ars–*N*-acetyltyrosine	15	0†	11
3	ars–*N*-benzoyltyrosine	14	13	12
4	ars–*N*-acetyltryptophan	12	9	8
4	ars–*N*-acetylhistidine	12	11	10
4	arsanilic acid plus *N*-acetyltyrosine (4×10^{-5} moles each)	13	12	—

* Average induration and erythema (mm).
† No induration, 8 mm erythema.

The delayed response to conjugates prepared with proteins undoubtedly represents a more complex reaction pattern[3,4], since some degree of reactivity is probably directed towards antigenic determinants of the protein carrier and some towards determinants consisting of hapten plus the amino-acids at its site of attachment. If this analysis were correct, it would follow that hapten inhibition of delayed reaction would be observable only in the event that the major contribution to the reaction was made by the antigenic determinant to which the hapten was related. To test such a hypothesis, inhibition investigations were conducted on guinea pigs immunized with ars–guinea pig serum albumin and tested with this antigen before and after intracardiac injection of ars–*N*-acetyltyrosine. The results in Table 3 show that in 3 of the animals a slight decrease was noted. However, in no case was there so marked a suppression of reaction as that seen in the animals immunized with ars–polytyr. An eight-fold larger dose of arsanilic acid was completely non-inhibitory.

Table 3. Skin Reactions to Ars–Guinea Pig Serum Albumin (GSA) before and after Administration of Haptens in Guinea Pigs immunized with Ars–GSA

Guinea pig	Hapten given	Delayed reactions* to skin tests with 1 μg N ars–GSA: pre-hapten	post-hapten
1	ars–*N*-acetyltyrosine (1×10^{-5} moles)	17	9
2	,,	19	11
3	,,	19	9
4	,,	10	12
5	,,	19	20
6	,,	16	20
7	arsanilic acid (8×10^{-5} moles)	11	7
8	,,	13	15
9	,,	16	20

* Average induration and erythema in mm.

Since the degree of sensitivity achieved by immunization with ars–polytyr and ars–guinea pig serum albumin cannot be quantitatively ascertained, comparisons of the suppressive effect produced by haptens in the two cases is attended by some uncertainty. Nevertheless, the results reported here provide suggestive evidence of the narrowed range of antigenic determinants resulting from immunization with conjugates prepared from a polymer of a single amino-acid. Secondly, and of considerable importance, the demonstration of suppression of delayed hypersensitivity by simple haptens in these animals introduces a powerful tool for the elucidation of the nature of the interactive forces involved in delayed hypersensitivity.

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PATHOLOGY

Influence of Urethane on Susceptibility to Leukæmia Induction by Graffi Virus in Adult Mice

The occurrence of leukæmia in mice of low-leukæmic strains, injected with urethane (ethyl carbamate) at birth or soon after, has recently been reported by several investigators[1]. Adult animals, on the contrary, do not seem to be very susceptible to the leukæmogenic activity of urethane, though there is some contradictory evidence[2]. However, urethane has been shown to be highly effective in increasing the incidence of leukæmia induced in adult mice by other agents, such as X-ray, œstrogenic hormones, or methylcholanthrene[3].

The enhancing effect of urethane on radiation leukæmogenesis was well demonstrated by Berenblum and Trainin[4], who observed a remarkable increase in leukæmia in *C57BL* mice receiving repeated doses of urethane after total-body irradiation. Since no comparable results were obtained when the sequence was reversed (that is, urethane prior to X-ray), these authors suggest, in line with the two-stage theory of carcinogenesis, that, in leukæmia induction, urethane acts as a 'promoting' agent. In further experiments[5] the same investigators observed leukæmogenic activity of urethane also when administered to non-irradiated recipients previously implanted with various tissues from irradiated isogenic donors. It was therefore suggested that urethane operates as a co-leukæmogenic agent, possibly by converting into an active leukæmogenic virus a 'transmissible factor' appearing in a precursor stage under the 'initiating' influence of X-radiation.

On this basis, experiments were undertaken to test in a simple experimental system the possible influence of urethane on virus leukæmogenesis. Since in preliminary investigations we obtained evidence that mice of certain low-leukæmic strains did not develop leukæmia after inoculation at adult age with cell-free extracts containing Graffi virus, it was decided to investigate whether the susceptibility to induction of leukæmia by this virus could be modified by supplementary treatment with urethane.

Male and female *C57BL* and *C3Hf/Gs* mice, three months old, were divided in four groups and treated as follows: One group received intraperitoneally four doses of 1 mg/g of urethane at intervals of 3–4 days; 24 h after the last injection, the animals were inoculated intraperitoneally with 0·10 ml. of cell-free extract obtained from leukæmic tissues of mice bearing leukæmia induced by Graffi virus. In another group the sequence of the

treatment was reversed, the interval between the inoculation of the extract and the first dose of urethane being 4 days. The remaining two groups, which served as controls, were given injections of cell-free extract alone, and of urethane alone, respectively. The Graffi virus was originally obtained from a leukæmic *C57BL* mouse received in August 1961 from Prof. Graffi's laboratory and carried since then in this laboratory by inoculation of cell-free extracts in new-born mice of the same strain. The extracts were prepared from homogenates of thymus, lymph nodes, and spleen, as a 20 per cent suspension in saline, centrifuged at 1,400*g* and then at 7,000*g*. The preparations used in this experiment yielded about 90 per cent leukæmia when injected in new-born animals of several strains, including mice of *C57BL* and *C3Hf/Gs* strain.

Table 1. INFLUENCE OF URETHANE ON LEUKÆMOGENESIS BY GRAFFI VIRUS IN *C57BL** AND *C3Hf/Gs** ADULT MICE

Treatment	*C57BL*			*C3Hf/Gs*		
	Total No. of mice	No. of mice with leukæmia	Leukæmia incidence (%)	Total No. of mice	No. of mice with leukæmia	Leukæmia incidence (%)
Urethane	24	2	8	20	1	5
Virus	38	2	5	27	0	0
Urethane followed by virus	19	3	16	28	2	7
Virus followed by urethane	18	10	55·5	20	0	0

* Incidence of spontaneous leukæmia less than 1 per cent.

The results, which refer to a 10-months' period of observation from the time of treatment, are shown in Table 1. The mean latent period in all the four groups was about 7 months after treatment. A slight prevalence of leukæmia was observed in female mice. Histologically, leukæmia was of the lymphoid type in all animals except in three *C57BL* mice of the group receiving virus prior to urethane in which a myeloid leukæmia developed. In one of them the neoplastic tissue exhibited grossly the greenish appearance characteristic of chloroleukæmia. The occurrence of myeloid leukæmia in mice of this group strongly suggests that the leukæmic disease is of viral origin, since in our laboratory this type of leukæmia has never been observed in *C57BL* strain except after inoculation of Graffi virus. On the other hand, the prevalence of lymphoid leukæmia in our experiments is not surprising, since it has been found that the type of leukæmia resulting from injection of Graffi virus in new-born mice of several different strains is mainly conditioned by the host environment (unpublished results).

The remarkable increase of the incidence of leukæmia obtained in *C57BL* mice only when urethane was administered after the virus seems almost exactly like that observed by Berenblum and Trainin[4] using X-radiation and urethane, thus confirming under different experimental conditions a 'promoting' action of this chemical. That both exposure to X-radiation and inoculation of a cell-free extract containing an active virus have the same effect with regard to the capability of urethane to influence leukæmogenesis in adult *C57BL* mice may add support to the hypothesis that a virus is involved in X-ray leukæmogenesis[6]. However, since in our experiments there is no doubt that the virus concerned is an active, complete virus, the 'promoting' effect of urethane could be explained either as a direct influence of the drug on infected cells or, more generally, in terms of modification of the degree of susceptibility of the host to the leukæmogenic action of the virus. The possibility that in animals receiving combined treatment leukæmia would result from activation by urethane of a different, pre-existing virus harboured in normal *C57BL* mice can readily be ruled out in view of the negligible leukæmogenic activity exhibited by urethane when administered alone in the same strain of mice.

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Mast Cell Reaction and 5-Hydroxytryptamine Content in the Skin of Syrian Golden Hamsters painted with 9 : 10-Dimethyl-1 : 2-benzanthracene

IN the skin of mice painted with chemical carcinogens a remarkable accumulation of mast cells is usually observed in the altered dermis. Cramer and Simpson[1] first observed that the accumulating mast cells acquire a strong primary golden-yellow fluorescence in ultra-violet light, a property which is not possessed by normal skin mast cells of mouse.

In experiments previously reported[2], designed to investigate the development of mast cell reaction during subsequent stages of skin chemical carcinogenesis in Swiss mice, we found that the increase of mast cell number closely paralleled the degree of intensity of mast cell fluorescence and the rise of skin-level of 5-hydroxytryptamine (5-HT). In addition, prolonged treatment with acetone (the usual solvent of 5-HT) prior to formol fixation was found to abolish completely the fluorescence of mast cells. These findings gave support to the hypothesis, first advanced by Riley[3], that the fluorescence acquired by dermal mast cells under such experimental conditions was due to a remarkable increase of the amount of 5-HT contained in the mast cells. Actually, a similar strong golden-yellow fluorescence is a characteristic feature of enterochromaffin cells, that is, the main physiological cellular source of this amine, as well as of 5-HT examined *in vitro*. However, the fluorescing mast cells fail to give positive results when tested with the argentaffin reaction of Masson–Hamperl, which is considered a sensitive method, although not entirely specific, for evidencing histochemically 5-HT in enterochromaffin cells (unpublished observations). Such results leave some doubt on the identity of the fluorescing material with 5-HT.

Since 5-HT has been found only in mast cells of mice and rats and not in those of other animal species[4], it was considered of interest to test whether in hamsters, which are also susceptible to skin carcinogenesis, the mast cell reaction would occur with morphological and biochemical characteristics comparable with those observed in mice.

In experiments reported here, Syrian golden hamsters, originally obtained from ARSAL Co., Rome, and bred at random in this laboratory, were used. Twenty animals of both sexes, aged 8 months, received twice a week on the

clipped skin of the back 0·10 ml. of a 1 per cent solution of 9:10-dimethyl-1:2-benzanthracene (DMBA) in paraffin oil. The animals were killed 7 weeks after the beginning of the treatment, when they had received 14 applications of DMBA. As controls, 20 untreated animals of both sexes were killed at the same age of the experimental group. Bioassay of the 5-HT content of the skin and histological or ultra-violet examination of mast cells were performed according to the methods previously described[2].

In comparison with controls, a remarkable accumulation of mast cells was detected in the dermis of the painted area in all the treated animals. The mast cell reaction was practically comparable with that observed in previous investigations in mice, although less pronounced. The accumulating mast cells appeared smaller and less granulated than normal hamster mast cells and located mostly beneath the hyperplastic epidermis. However, when the sections were examined in ultra-violet light both the normal and reacting mast cells failed to exhibit any fluorescing property. Moreover, the determination of 5-HT skin level did not reveal any difference between control and treated animals, the values obtained being less than μgm/gm 0·08 in both groups.

These findings indicate that no production of 5-HT occurs in normal skin mast cells of hamsters as well as in mast cells appearing in the dermis during carcinogenic treatment in these animals. In skin carcinogenesis of mice, as already stated, the close parallelism between golden-yellow fluorescence of mast cells and high content of 5-HT in the skin suggested that the increased level of this compound was the factor responsible for the fluorescing properties acquired by the reacting mast cells. The results of the experiments recorded here give further support to this hypothesis, since in hamsters painted with DMBA neither increase of 5-HT nor appearance of fluorescence in the accumulating mast cells was observed in the skin. On the other hand, the lack of appreciable amounts of 5-HT in mast cells of DMBA-treated hamsters seems to indicate that this amine plays no significant part in the mast cell reaction developing in the course of chemical skin carcinogenesis.

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RADIOBIOLOGY

Cæsium-137 Content of Lambs

The cæsium-137 γ-activity of 10 lambs, which had been used previously to investigate some relationships between potassium-40 content and composition[1], was measured concurrently with potassium-40 γ-activity on the Los Alamos human counter[2]. The lambs averaged 88 lb. in live-weight and were purchased from a feed-lot at La Jara, in southern Colorado, in March 1960. The counting conditions have been described earlier[1]. The γ-counts from the upper and lower channel settings were entered on I.B.M. cards, and a computer tabulated and printed the cæsium results as μμc./*g* potassium[3]. Calculations were based on previously determined calibration curves for the counter.

The cæsium content of the 10 live lambs and their components are presented in Table 1. The increase in the cæsium/potassium ratio which was recorded after the lambs had been washed resulted from the removal of a large amount of potassium from the surface of the lambs by the washing process[1]. The results (Table 1) show that, when the cæsium-levels were converted to a per kilogram of tissue basis, little cæsium was removed by washing. Results indicate that the cæsium/potassium ratio in the washed lambs and their components was fairly constant, which would be expected as cæsium is metabolically similar to potassium[4]. This resulted in a higher concentration of cæsium per unit of muscular tissue than in the live lambs, as the lean tissue also has a higher concentration of potassium[1].

Table 1. Cæsium-137 Content of Ten Live Lambs and their Components

Item	Mean	*S.D.*	Range
Live unwashed (μμc. cæsium-137/g K)	58·0	10·6	41·8–74·5
Live washed (μμc. cæsium-137/g K)	84·8	17·9	60·2–121·0
Dressed carcass (μμc. cæsium-137/g K)	81·5	23·0	43·0–122·4
Non-carcass components (μμc. cæsium-137/g K)	96·8(9)*	59·4	25·9–189·2
Separable lean (μμc. cæsium-137/kg K)	79·5	18·1	59·1–107·7
Live unwashed (μμc. cæsium-137/kg tissue)	155·4	24·1	126·3–182·8
Live washed (μμc. cæsium-137/kg tissue)	148·7	21·4	118·8–183·3
Separable lean (μμc. cæsium-137/kg tissue)	236·6	54·3	167·8–323·1

Counts for separable fat and bone on the lower channel were so close to background that several of the samples were estimated to have a negative cæsium content and all results on these tissues were obviously inaccurate. For this reason they have not been reported.
* Mean is based on 9 observations as one negative value was discarded. If a value of zero is used for the negative observation, the mean becomes 87.

The levels of cæsium reported are similar to those found in cattle tissue in 1957–59 in the United States[5], in pigs and calves prior to 1962 in Canada[6,7] and in human beings prior to 1961, largely from the United States[4]. However, the values are very much lower than those observed in Norway and Sweden for beef[8,9], horse[9], mutton[9], pork[9], reindeer[9,10], and, to a lesser extent, man[10,11]. As all these observations were made prior to the recent nuclear weapons test series of the U.S.S.R. and the United States beginning in August of 1961, it would appear that these levels resulted from earlier weapon tests. Differences may be due to different world fall-out patterns, time interval from previous atomic tests and different eating habits.

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Lead-210 and Polonium-210 in Human Tissues

It has recently been shown[1] that appreciable amounts of lead-210 and polonium-210 may occur in certain human foodstuffs as a result of deposition from the atmosphere of long-lived radon decay-products (Table 1). Absorption of these products may therefore occur by both inhalation and ingestion, but, although measurements of the activity of lead-210 in many human bone samples have been reported[3–6], the ratio of polonium-210 to lead-210 in bone and the activities of these nuclides in human soft tissues have been reported in only a few samples[1,3,7,8]. This communication describes the results of preliminary radiochemical estimations of polonium-210 and lead-210 in human bone and soft tissue samples.

Table 1. Radon Decay Products

	Nuclide	Half-life (ref. 2)	Decay
Radon	Rn-222 (Rn)	3·823 d	α
Short-lived daughters	Po-218 (RaA)	3·05 m	α
	Pb-214 (RaB)	26.8 m	β^-
	Bi-214 (RaC)	19·7 m	β^-
	Po-214 (RaC′)	160 μs	α
Long-lived daughters	Pb-210 (RaD)	20 y	β^-
	Bi-210 (RaE)	5·0 d	β^-
	Po-210 (RaF)	138·4 d	α
Stable lead	Pb-206	—	—

The method used was similar to that of Black[7]. Up to 80 g of wet tissue were digested with nitric and perchloric acids, care being taken to keep the temperature below 200° C because of the volatility of polonium. From a final solution, 0·5 N in hydrochloric acid, polonium was electrochemically deposited on silver foil and the α-activity was measured in a low background scintillation counter[9]. The specificity of the separation for polonium-210 was checked by α-spectroscopy of several of the foils, using the α-spectrometer which is described elsewhere[10]. By low-level β-counting for bismuth-210 in an aged deposit on silver from an equilibrated lead-210 solution, it was shown that less than 0·01 per cent of lead-210 was deposited under the plating conditions used in the tissue analyses. Hence, lead-210 was estimated in some of the tissue samples by deposition of the polonium-210 from the tissue solutions several months after the first deposition. Reproducibility was checked by several estimations of polonium-210 and lead-210 in samples from the same tissue. The overall yield was estimated by spiking one member of each of ten pairs of samples with an equilibrated lead-210/bismuth-210/polonium-210 solution and the measured overall yields for the first and second depositions were 90 ± 5 per cent and 90 ± 9 per cent respectively. The average activities of the tissues determined in this manner are as shown in Table 2.

Table 2. Po-210 and Pb-210 in Human Tissues

Tissue	Po-210 pc./100 g wet.		Po-210/Pb-210
Vertebra	1·7	(9)	0·82 0·43
Liver	1·0	(4)	3·1 1·8
Kidney	0·71	(2)	8·9 4·1
Spleen	0·32	(3)	1·8 0·4
Lung	0·30	(2)	—
Skeletal muscle	0·11	(5)	—
Testis	0·33	(4)	—

The figures in parentheses (Table 2) indicate the number of specimens of each type of tissue analysed. The ratios in column 3 are from individual determinations. Taking account of the uncertainties in the yields and the counting statistics, the probable errors of the individual determinations were less than 10 per cent for polonium-210 and 20 per cent for lead-210. The extreme values from separate determinations for a given type of tissue were within a factor of 2·5 of the mean value tabulated.

A few observations may be made in the light of these results. The average polonium-210 concentration in the bone samples is comparable with previously reported values and there are indications that the lead-210–polonium-210 series may not be in radioactive equilibrium in vertebræ. This is in keeping with the findings of Black[7] and Holtzman[3]. The latter author has suggested that the lead-210 concentration (per gram ash) in vertebræ is close to the average for the whole skeleton. On this basis, assuming an ash content in vertebræ of 21 per cent, a total skeletal ash of 2,800 g and, from the foregoing, a polonium-210/lead-210 ratio in vertebræ of 0·6, the skeletel burden of lead-210 is approximately 400 pc.

The results of tracer studies with animals and a few human beings, summarized in the I.C.R.P.[11] tables, have indicated that polonium-210 may be concentrated in the soft tissues, particularly liver and kidney, and the foregoing measurements suggest that this is also the case at lower levels, the polonium-210 being measurably in excess of lead-210. α-spectroscopic measurements on other tissue samples[1] have demonstrated that polonium-210 is in excess of other α-emitters. The measurements reported here give average values for polonium-210 activity in the major soft tissues higher than those spectroscopic determinations, probably because of volatilization of polonium in the dry-ashing procedure required for the spectroscopy.

If the concentration in those types of soft tissue not measured here is comparable with that measured in skeletal muscle, the foregoing results indicate an average body burden of polonium-210 in the range 330–450 pc. with at least 20 per cent of the activity in the soft tissues. The upper and lower limits correspond to the assumptions that the more highly mineralized cortical portion of the skeleton has a polonium-210/lead-210 ratio equal (*a*) to that in the trabecular region, taken here as 0·6, or (*b*) to unity, respectively.

The radiation dose rate from the concentrations of lead-210 (β-energy, 17 keV maximum) and polonium-210 (α-energy, 5·30 MeV) in tissues, reported here, is due predominantly to the polonium-210 α-particle. The *in vivo* activity of bismuth-210 (β-energy 1·16 MeV maximum) is difficult to measure because of its rather short half-life. However, since bismuth does not appear to be concentrated very markedly in any of the larger organs[11], it seems unlikely that redistribution of bismuth-210 following the decay of lead-210 would affect the validity of the foregoing statement. A polonium-210 concentration of 0·1 pc./100 g wet. tissue gives a dose rate of approximately 1 mrem/y, so estimates of the dose rate to the various soft tissues from the measured activities may readily be obtained from Table 2. Comparison of these dose rates with those from radium-226 plus short-lived daughters and the radium-228 series in human bone and soft tissues[12] indicates that the rem dose rate from polonium-210 in bone may be comparable with the rem dose rate from those nuclides and, in soft tissues, the rem dose rate from polonium-210 may be more than an order of magnitude greater than that from radium-226 plus short-lived daughters and the radium-228 series.

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BIOLOGY

Culture of Single Mammalian Cells

The single-cell culture method of mammalian cells was established by Puck *et al.*[1], and is now applied in cell physiology and genetics. Edlinger[2] has simplified this method, using a rubber-stoppered bottle without 5 per cent carbon dioxide incubator. However, this was used only for the nutritionally non-exact *L* strain cell: the method has also been used for the nutritionally more exact HeLa strain *S*3 clone[3] as well as the other two strains, and has provided results applicable to the cell-cloning technique. This communication is concerned with the technical details of this method, and presents a nutritional investigation of the single-cell culture medium for bottle technique.

All the cell strains used were those maintained in lactalbumin hydrolysate Hanks's solution medium containing bovine serum. The basal culture medium through the single cell culture test was new Eagle's medium[4] and the serum added to the foregoing medium was the bovine serum inactivated at 56° C for 30 min. The whole medium component was adjusted to *p*H 6·8 with 5 per cent carbon dioxide in air to avoid possibly the alkaline shifting of medium *p*H during the incubation period, and it was sterilized through a porcelain filter. For the test 36 cm² virus-plaque bottles were used. Four ml. of the culture medium were put in each bottle and 300 trypsin-dispersed cells were inoculated to the culture medium. The culture was incubated at 37° C under rubber-stoppered conditions for 14 days. The experimental results are expressed as plating efficiency, which is the percentage ratio of the performed colony number to the inoculated cell number. The colony count was carried out after the 14th culture day, the colonies being stained with Giemsa (Fig. 1).

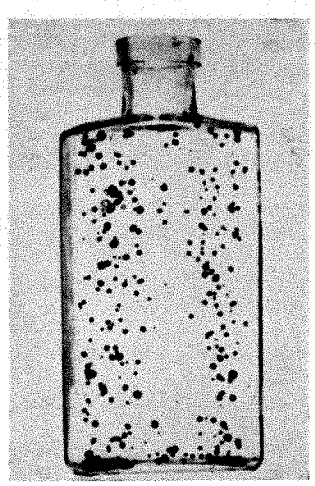


Fig. 1. The colonies of HeLa–*S*3 cell developed in a square bottle after 14 days of culture

Before considering the experimental results, it should be noted that the serum used in this test had a critical controlling effect. Accordingly, all the sera used were checked roughly for the plating efficiency beforehand and any toxic serum was omitted from the test. At the outset of this work the effect of the serum concentration to the plating efficiency was tested. The medium containing 20 per cent serum showed the best results through all the cell strains examined over the range tested. In the past, tests of plating efficiency of HeLa strain with 20 per cent serum medium was 67–88 per cent. The results of *DK* strain (established dog kidney strain) and *FL* strain (established human amnion strain) were similar to those for HeLa strain, while those of *L* mouse strain usually showed 100 per cent with 20 per cent serum medium.

Table 1. Effect of Nutritive Supplements on the Plating Efficiency of HeLa-*S*3 Cell in Eagle's Medium supplemented with 5 per cent Whole Serum or 15 per cent Dialysed Serum

Serum	Supplement*	Plating efficiency	Serum	Supplement*	Plating efficiency
5% Whole serum	Nothing	0	15% Dialysed serum	Nothing	8·5
	Vitamin B_{12} (1)	4·0		Cholesterol (5)	6·8
	Thymidine (2)	0		L-Serine (6)	5·7
	Folinic acid (3)	24·0		(4)	4·8
	Lactalbumin hydrolysate (4)	25·0		(5)+(6)	11·8
	(1)+(2)	8·0		(4)+(5)+(6)	22·5
	(1)+(3)	4·5		(1)+(2)+(3)+(4)+(5)+(6)+Protamine insulin	40·0
	(2)+(3)	3·5			
	(1)+(2)+(3)	45·0			
	(1)+(2)+(3)+(4)	25·0			

* Concentration of each supplement is as follows (mg/l.): Vitamin B_{12}, 0·02; folinic acid, 0·1; thymidine, 2·4; lactalbumin hydrolysate (Nutritional Biochemicals Co.), 300·0; cholesterol, 0·8; L-serine, 10·0; protamine insulin, 200 unit/l.

When the serum concentration was decreased to 5 per cent and its plating efficiency dropped to zero, the addition of lactalbumin hydrolysate (Nutritional Biochemicals Co.) and the combination of thymidine, folinic acid and vitamin B_{12} to the medium could increase the plating efficiency considerably (Table 1). Also when the serum was dialysed for 24 h against Earle's balanced salt solution, following Puck's method[5], the plating efficiency of 15 per cent dialysed serum medium was quite low. But the addition of lactalbumin hydrolysate, folinic acid, vitamin B_{12}, thymidine and protamine insulin to the medium in addition to cholesterol[5] and serine[4] increased the plating efficiency to each additive, while a combination of them together could raise the plating efficiency still further (Table 1). These findings show that under a closed system the nutritional requirement for colony formation is somewhat different from the results reported by Eagle[6] and Puck[1].

The plating efficiency of the present method is not complete. But using the foregoing technique, a special 5 per cent carbon dioxide incubator was not needed; an ordinary incubator was good enough. The cell-cloning technique was simply performed with a platinum loop and was successfully applied for cloning the antibiotic-resistant HeLa strain cell clone and the HeLa clone more sensitive to adenovirus[7].

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Skin Biopsy for Cell Culture

One of the most useful tissues available to the human geneticist for cell culture is skin. It is plentiful, easy to grow, remains diploid for several months, and can be frozen, stored for probably years, then thawed and re-cultured[1]. What is needed for large-scale family or population studies is a simple, rapid, relatively painless procedure for obtaining suitable skin biopsies. Just such a procedure, using an electric rotary punch, is described in the literature by Urbach and Shelley[2]; but few investigators outside the field of dermatology seem to be aware of the technique. We have had such good results with an adaptation of the punch drill that we call attention to it in this communication and present our results to date. The procedure is as rapid and simple as a venipuncture, and certainly no more painful. No anæsthesia or suture is required, and the biopsy can be performed in the clinic, the office, or the patient's home.

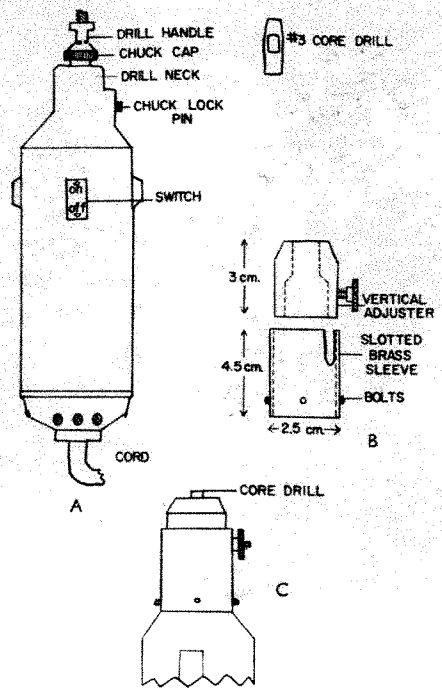


Fig. 1

The drill and component parts are illustrated in the diagrams. Fig. 1*A* depicts the electric drill with the drill handle in place in the chuck. (Moto-Tool model No. 2. 110 V a.c.—d.c. 65 amp. Manufactured by Dremel Manufacturing Co., Racine, Wisconsin: obtainable from the Scientific Glass Apparatus Co., Inc., 735 Broad Street, Bloomfield, New Jersey.) The drill handle and the core drills are part of a punch biopsy set (Martin *M–H* Biopsy Set, a United Product, Port Chester, New York). The 5-5 in. handle from the set must be cut to about 2 cm in length and tooled down to fit the chuck. Fig. 1*B* shows the slotted brass sleeve and the hollow brass vertical adjuster made for us by a machinist. Fig. 1*C* shows the assembled apparatus ready to operate. The brass sleeve is bolted on to the neck of the drill and the vertical adjuster is telescoped inside it to set the depth of the incision. A core drill is autoclaved along with a pair of forceps and a pair of sharp scissors.

The biopsy is performed as follows: the skin is swabbed with alcohol or zephiran, rinsed with sterile saline, and dried with sterile gauze. Almost any area of the body can be used. We have biopsied the buttocks of babies, the back medial to the upper border of the scapula, the flexor surface of the forearm[3] and the deltoid region. The core drill is grasped with the sterile forceps and screwed on to the drill handle. The chuck lock pin will prevent the chuck from rotating as the drill is tightened. Then the vertical adjuster is telescoped into the brass sleeve, taking care not to contaminate the sterile core drill. The desired depth is maintained by the nut on the vertical adjuster. We have found that a depth of 2–3 mm is adequate for cell culture. The rotating drill is momentarily touched to the skin with a 'flick of the wrist' motion. The small core of skin is then lifted gently with the sterile forceps and snipped off quickly with the scissors.

Our experience is as follows: using a No. 3 core drill (3 mm diam.) and a depth of about 2 mm, we have obtained 21 biopsies, all of which were successfully cultured in modified Eagle's medium with 20 per cent human serum and 5 per cent bovine embryo extract ultra-filtrate. Each specimen was cut into as many small pieces as possible and incubated at 37° C in an atmosphere of 5 per cent carbon dioxide in air. Seventeen of the skin specimens were collected by one of us in Sardinia and sent by air to Baltimore in medium, unrefrigerated. The trip took 8 days, and handling of the packages was far from ideal. One specimen arrived stuck on the side of the glass tube out of the medium and quite dried out. Although slow in starting, it has since grown well. We have, however, lost one specimen by contamination before any investigations were made, and one has never grown well and will probably be lost.

In each case we enquired about pain. The electric drill itself is virtually painless. The snipping of the pedicle usually produced some slight discomfort, but only for an instant. One sleeping infant did not even waken during the entire procedure. With the No. 3 drill, bleeding was either absent or slight, and easily stopped by a minute or two of pressure. The site was covered for 2 days by a simple band-aid, and the resultant scar is barely noticeable in most cases. One of us is using the apparatus for sweat gland biopsy[2], and uses a No. 5 core drill (5 mm diam.) with depths up to 1 cm. Even in these cases pain was minimal, and bleeding was arrested by a simple pressure dressing.

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Cultivation of Mouse Embryos *in vitro*

EXPERIMENTAL embryology of mammals is greatly restricted by the difficulties of growing mammalian embryos *in vitro*. Despite recent successes with the cultivation of mouse and rabbit eggs[1], techniques for the cultivation of post-implantation embryos have not hitherto advanced beyond those devised by Jolly and Lieure[2], and Nicholas and Rudnick[3]. Jolly and Lieure obtained development of rat and guinea pig embryos explanted into homologous serum at stages between primitive streak and a few somites. They report that of their explanted rat embryos 37 per cent developed an embryonic axis with a rhythmically beating heart, but only 9 per cent a functioning circulation. None formed limb buds or a functioning allantoic circulation. Nicholas and Rudnick[3] appear to have had a similar degree of success with rat embryos explanted into heparinized rat plasma and embryo extract.

We have obtained much better results by growing mouse embryos on chick plasma clots in an atmosphere consisting of 5 per cent carbon dioxide in air. For a few of the cultures the oxygen was also increased (to about 60 per cent) and this improved the results. The clots were made by adding one part of embryo extract to three parts of chick plasma. The embryo extract was obtained from either 18-day mouse or 13-day chick embryos; both gave equally good results. The embryos to be cultured were obtained from *C3H*, hybrid and other genetic strains of mice mated 8–9 days previously, and ranged from primitive streak stage to about six somites. They were dissected free of the decidua and of Reichert's membrane but were not separated from the yolk sac or ectoplacental cone. The whole explant therefore consisted of a small

Table 1. Number and Percentage of Embryos surviving to Different Stages in Culture

			Percentage of No. explanted
Stage attained in culture	No. explanted	126	
	8 or more somites; beating heart	107	85
	Yolk sac circulation	98	78
	16 or more somites	58	46
	Allantoic circulation	30	24
	Limb buds	34	27

sphere of yolk sac with the embryo at one pole and the ectoplacental cone at the other (Fig. 1).

Each explant was placed on a clot in a drop of embryo extract diluted with an equal volume of Tyrode solution containing an extra 1 per cent glucose. During incubation the fluid within the yolk sac rapidly increased in volume so that the diameter of the yolk sac sphere, which at the time of explantation was about 1 mm, increased after 48 h to 3–4 mm. As it grew it digested a cavity in the clot and sank into it. Despite the close contact between the yolk sac and the clot no firm adhesion was formed between the two. Cells of the ectoplacental cone, however, attached firmly to the clot and migrated for a short distance into and over it.

We have cultured 126 embryos. They were usually examined 24 h after explantation and again after 48 h. By 24 h most of the embryos had developed a complete embryonic axis with more than 8 pairs of somites, a beating heart and a functioning embryonic and yolk sac circulation. The allantois had fused with the ectoplacental cone forming the allantoic placenta, and in some of the cultures blood could be seen circulating in the allantois. After 48 h many of the embryos still had a functioning blood circulation and a few had developed limb buds and 20 or more pairs of somites (Figs. 2 and 3). Histological examination showed them to have well-differentiated internal structures (Fig. 4). Circulation of the blood often continued for a further few hours and a rhythmical beating of the heart for considerably longer, but no further development occurred. Table 1 shows the number of embryos developing to different stages.

The proportion of explanted mouse embryos that develop in our cultures, and the amount of growth and differentiation that occurs, is similar to that obtained with chick embryos of equivalent age when cultured by the standard methods. It should now be possible, therefore, to apply to mammalian embryos many of the embryological techniques that have been used so successfully on

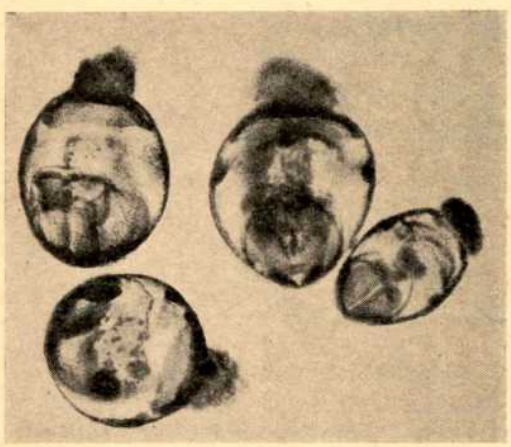


Fig. 1. Embryos as explanted. Amnion, yolk sac and most of ectoplacental cone left intact, but Reichert's membrane removed (× 24)

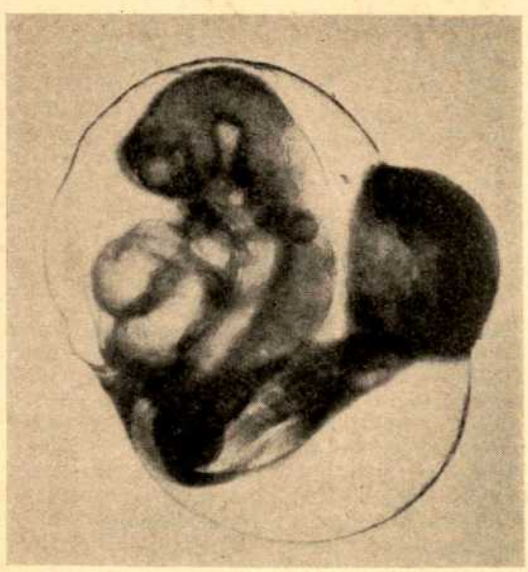


Fig. 2. Embryo explanted at the 5-somite stage and grown in culture for 48 h. More than 20 somites, optic and otic vesicles have developed, together with functioning yolk sac and allantoic blood circulations (× 24)

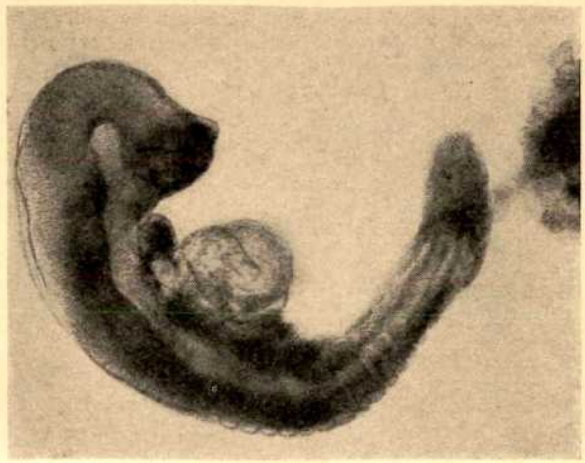


Fig. 3. Embryo explanted at the 6-somite stage and grown in culture for 48 h. Membranes removed before fixation (× 24)

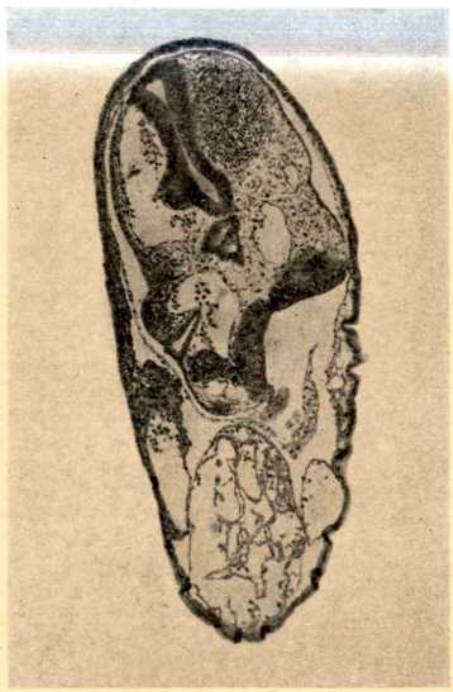


Fig. 4. Embryo explanted at the head fold stage and grown in culture until 18 somites developed. Section through brain, pharyngeal pouch and allantois (× 60)

the chick. We are at present studying the physiological requirements of mouse embryos *in vitro* and the possibility of grafting tissues from one embryo to another.

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Thyroid Gland and Temperature Tolerance Relationships in Cold-blooded Vertebrates

Although the thyroid gland is of extreme importance in the metamorphosis of amphibians, its function in cold-blooded vertebrates in general is still obscure[1]. Several workers have suggested that thyroid hormones enable poikilotherms to withstand changes in environmental temperature. For example, Evropeitzeva[2] reported that larvæ of *Coregonus lavaretus ludoga* withstood exposure to 29° C for 5 min after treatment with 0·033 per cent thiourea, whereas untreated animals died. Suhrmann[3] found that immersion in a solution of thiourea increases the upper lethal temperature in the goldfish, *Carassius auratus*. Dent and Lynn[4] also indicated that the thyroid may play a part in tolerance to changes in temperature. But the most striking report in this field comes from the work of Fortune[5,6], who has reported that in the minnow, *Phoxinus phoxinus* L., the thermal death point is raised some 10° C by treatment with thiourea. She states: "*Phoxinus* already treated with thiourea for 3 days, were subjected to a rise in temperature of 10° C (that is, to 33° C) over 2 days. They survived indefinitely at this temperature and appeared normal, the controls dying under such conditions between 23° C and 24° C". On the other hand, La Roche and Leblond[7] state that the ability of salmon parr to withstand rising temperatures is impaired by radiological thyroidectomy and is restored by the administration of thyroid powder.

As a preliminary to further work on these aspects of thyroid physiology, and in an attempt to confirm and extend the work of Fortune, one of us (J. M. D.) set out to repeat the work on minnows already described. Fish were obtained from Lake Windermere in September and October and a series of experiments was started. A repetition of Fortune's protocol gave thermal death points of 29°–31° C in both thiourea-treated and control fish. The preliminary treatment with thiourea was then extended, first to 10 days at 23° C and afterwards to 27 days at 15° C without effect on the thermal death point. In the latter experiment all fish died at about 30° C whether the rise was sudden (15°–30° C a day) or more gradual (5° C a day). In conjunction with these studies, tests of the temperature tolerance of a fish with an extensive, naturally occurring goitre and a group of fish that had been treated with sodium thyroxine at a concentration of 1 in 10^7 at 23° C for 3 days were carried out. All died at about 30° C, indicating that neither depression nor elevation of thyroid function affects temperature tolerance in this type of experiment. This work was extended to include tadpoles of *Xenopus laevis*, and it was found that neither thiouracil treatment nor surgical thyroidectomy enabled the tadpoles to withstand temperatures higher than 34°–36° C, at which control specimens also died.

We have more recently repeated part of this work and have once again found no elevation of the thermal death-point. Minnows, received from Lake Windermere in mid-September, were kept at 15° ± 1° C for about a month and were fed on 'Bemax' fish food. On October 17, 20 fish, each about 5 cm in length, were selected and divided into two groups of 10. Each group was put in 4 l. tap water in a 5-l. beaker and the beakers were submerged to the level of their contents in a constant-temperature bath at 15° C. The temperature of the bath was gradually raised from 15° C to 19° C on the first day and from 19° C to 22° C on the second. By the end of the second day one fish in each beaker was dead. On the third day the water in one beaker was replaced with fresh tap water and the other with tap water containing 0·05 per cent thiourea, both at 22° C, and the temperature was raised to 23° C, at which level it was maintained for 3 days. This concluded the conditioning period. The gradual elevation of the temperature was then continued. After 22·5 h the temperature reached 27·6° C and the first fish died. Between 22·5 and 31 h the temperature was raised to 29·7° C and the progressive mortality of the treated and control groups was virtually identical. At 31 h and a temperature of 29·7° C one control and one experimental fish remained alive. By 45 h and without raising the temperature further all the fish were dead.

These findings are not sufficiently extensive to serve as a firm basis for sweeping conclusions, particularly in the face of some contradictory evidence; but clearly they give support to the view that hypofunction of the thyroid gland of the minnow does not enhance the ability of the fish to withstand exposure to lethal and sub-lethal temperatures under the conditions described here. However, they do not necessarily run counter to Evropeitzeva's findings since her fish were exposed to near-lethal temperatures for only 5 min.

This work, which was supported in part by U.S. Atomic Energy Commission contract *AT* (40–1)–2000, was carried out at the Gatty Marine Laboratory of the University of St. Andrews.

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Rapid Detection of the Pathogenicity of Phytopathogenic Pseudomonads

Most bacterial plant diseases are caused by bacteria belonging to the genus *Pseudomonas*. A number of saprophytic pseudomonads occur in large amounts on the surface of the plant (*Pseudomonas fluorescens*, *Ps. denitrificans*, etc.). Therefore, isolation of phytopathogenic pseudomonads from the diseased plant is difficult. Generally the morphology of the colonies, the biochemical properties[1], the antigenic structure[1] and the phage sensitivity[2] of the pathogenic and saprophytic pseudomonads are so similar that they can scarcely be distinguished on the basis of the foregoing characteristics. In practice the pathogenicity test is the sole reliable method which can be used for identification. To carry out pathogenicity tests one must have suitable hosts, but in this case the entire procedure of artificial infection in the greenhouse is tedious. Testing of woody plants is particularly difficult.

An 'injection-infiltration' method has been developed as a rapid test of pathogenicity. Potted tobacco plants instead of the normal hosts of the pathogens are used



Fig. 1. Infiltration by injection of an intact tobacco leaf

for the tests, which can easily be carried out even in winter time. *Nicotiana tabacum* var. White Burley plants are particularly suited for the tests but other tobacco varieties will do as well. Infection is carried out as follows: colonies of the *Pseudomonas* type which have been obtained by plating from diseased plants are suspended one by one in 3-ml. sterile tap-water. The suspension should contain about 10^7 bacterial cells/ml. Adjustment of the number of cells can be carried out by the use of a densitometer or, after some practice, also by subjective judgment. These suspensions are then injected by fine hypodermic needle into the intercellular spaces of the tobacco leaf (Fig. 1). Older leaves are more suitable for injection than the young ones. The injection can be made more easily near the lateral veins of the leaf (lower surface), where the tissues are thicker. The carefully injected suspensions will spread over a larger surface usually surrounded by the bigger veins. When several bacterium strains are under investigation these can be injected one by one into the various inter-veinal areas of the leaf (Fig. 1). Thus 10–12 different bacterial strains can be injected into a single leaf. After infiltration, the infiltrated water is evaporated from the intercellular spaces and the leaf regains its original appearance.

24–48 h after injection with phytopathogenic bacteria the injected leaf areas die off and in 3–4 days the treated tissues become entirely dry and white. Saprophytic bacteria do not induce necrosis in the leaf. After proof of pathogenicity, knowing the host plant from which the isolation has been made, the bacterium can be identified.

The method has the advantage that the investigations can be carried out on intact plants. Until now infiltration of intact plants was difficult. Complicated procedures were needed for the infiltration of potted plants. Much material to be infiltrated was needed for the infiltration either of whole plants or of detached leaves. The injection infiltration method makes it possible to feed the leaf with substances in small amounts and even dilution series can be injected into various parts of the same leaf. In view of this the method is particularly suitable for plant physiological experiments.

Table 1. Reactions induced by *Pseudomonas strains* in the Tobacco Leaf Three Days after Injection

Bacteria	Cell No. of the inoculum/ml. 10^8	10^7	10^6	10^5
Ps. aptata	++	++	++	−
Ps. atrofaciens	++	++	+	−
Ps. cannabina	++	++	+	−
Ps. caryophylli	++	++	+	−
Ps. lachrymans	++	++	+	−
Ps. mori	++	++	+	−
Ps. mori var. *huszi*	++	++	+	−
Ps. mors-prunorum	++	++	+	−
Ps. phaseolicola	++	++	+	−
Ps. pisi	++	++	+	−
Ps. oryzicola	++	++	+	−
Ps. syringae	++	++	++	−
Ps. syringae var. *capsici*	++	++	+	−
Ps. syringae f.sp. *populea*	++	++	+	−
Ps. tabaci	++	+	+	+−
Ps. tomato	++	++	++	−
Ps. aeruginosa	−*	−	−	−
Ps. denitrificans (4 strains)	−*	−	−	−
Ps. fluorescens (6 strains)	−*	−	−	−
Ps. sp. (2 strains)	−*	−	−	−

++, Total necrosis; +, partial necrosis; +−, weak necrosis; −, no necrosis; *, weak yellowing.

By the use of the method described here a number of pathogenic and saprophytic *Pseudomonas* strains derived from different localities were investigated. The bacteria were injected into tobacco leaves in different cell-concentrations. The results are shown in Table 1.

It may be seen that all phytopathogenic bacteria-induced deaths of cells occur at a concentration of 10^6 cells/ml. With inocula of a lower cell number macroscopic symptoms were not obtained. Infiltration of less-concentrated suspensions has led to the development of small local lesions extending to a few cells which could be seen by 10- to 12-fold magnification. The number of these small lesions was proportional with the number of bacteria infiltrated.

Pseudomonas tabaci, the causal agent of the 'wildfire' disease of tobacco, evokes the development of symptoms different from those induced by other pathogenic bacteria. On one hand the dead parts of the leaves do not bleach; on the other, the cells die more slowly. The lower the cell number of the inoculum, the slower the death of the tissues in the case of injection with *Ps. tabaci*.

Saprophytic bacteria do not cause death of cells. Infiltration with cell numbers as high as 10^8–10^9 cells/ml. results later on in a weak yellowing of the treated areas.

The detection of pathogenicity with the foregoing method is based on the hypersensitive reaction of the tobacco leaf. A hypersensitive reaction of a similar nature has already been described for bean pods infected with bacteria[3,4].

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MICROBIOLOGY

Sucrose-fermenting Strains of *Clostridium septicum*

Several authors[1–5] have described *Clostridium septicum* as an organism which does not ferment sucrose. Smith[6], describing the fermentation reactions of strains of *Clostridium septicum*, mentions that some strains do ferment sucrose but that they need several days for sufficient acid to be produced to be detectable by the usual indicators.

During the investigation reported here, strains of *Clostridium septicum* were isolated from various samples of soils, and a collection of 52 strains formed the basis of the work. The strains were tested for fermentation of sugars. The sugars used were glucose, fructose, galactose, maltose, lactose, sucrose, inulin, glycerol, mannitol and salicin.

Sugars were added to peptone water as Seitz filtrates to give 1 per cent (w/v) solutions. Andrade's indicator was incorporated to detect change in acid. The sugar media were inoculated with 4 drops of an overnight culture of *Clostridium septicum* grown in sugar-free media. Two sets of sugars were tested with each strain of *Clostridium septicum*. One set was incubated under anaerobic conditions in a McIntosh and Fildes jar. In the other set anaerobiosis was brought about by the addition of a sterile iron nail about 0·5 in. long to each tube. Both conditions were found to be equally effective for testing the ability of *Clostridium septicum* to ferment sugars. The tubes were examined for acid and gas production in 24 h and 48 h.

Fifty of the strains produced acid and gas from glucose, fructose, galactose, maltose, lactose and salicin; sucrose, inulin, mannitol and glycerol were not fermented by them. Two of the strains produced acid and gas from sucrose in addition, which was detectable in 24 h. Both these strains were pathogenic to guinea pigs, and cultures obtained from the heart blood of the dead guinea pigs retained the ability to ferment sucrose. The fermentation of sucrose and glucose was very similar: this is a significant finding as the fermentation of sucrose by *Clostridium chauvoei* and the inability of *Clostridium septicum* to ferment sucrose are used as one of the distinguishing tests between the two, which are very similar in other reactions and also possess a common antigen.

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Membrane Stability and Salt Tolerance in Gram-negative Bacteria

THE ability of a bacterium to tolerate any particular set of ionic conditions is likely to be determined in part by the outermost permeability barrier of the organism. This barrier, which is usually a lipoprotein membrane, is in contact with the extracellular environment and must remain functional under all conditions which permit growth of the organism. It has already been shown that the cell envelopes (or membranes) of two Gram-negative bacteria, one a marine organism (N.C.M.B. 845) requiring moderate concentrations of salt and the other a halophil (*Halobacterium halobium*) requiring high concentrations of salt, are unstable at low ionic strengths. The structural integrity of these two types of envelope depends, to a different degree in each case, on ionic strength or the concentration of bivalent cations[1,2]. The work recorded here was undertaken to determine if cation-sensitive instability of bacterial membranes is a general phenomenon and if there is evidence of a correlation between membrane properties and salt tolerance of the growing organism.

Gram-negative bacteria isolated from marine and other sources were used. For determination of salt tolerance they were incubated at 30° in stagnant media containing peptone (1 per cent w/v) and sodium chloride (0, 1, 2, 4 and 8 per cent w/v). Growth was assessed by inspecting the tubes after 1, 2, 3 and 6 days. The organisms and their salt tolerances are listed in Table 1, which shows that all the non-marine bacteria grew without added salt and one of them (*Proteus* sp.) tolerated 8 per cent sodium chloride; on the other hand, none of the marine bacteria grew with less than 1 per cent sodium chloride. Minimal salt requirement was the only conspicuous and constant difference between the two groups of organisms. For the preparation of cell envelopes, bacteria were grown for 22–24 h with aeration in a medium (500 ml.) containing peptone (1 per cent w/v). The medium was prepared in sea-water for the marine organisms and distilled water for the others. Two organisms, the *Pseudomonas* sp. and *Escherichia coli*, were grown also in half-strength and full-strength sea-water respectively. Envelopes were prepared in cold distilled water as previously described[3]. Envelope breakdown was measured turbidimetrically at 700 mμ in a solution containing *tris* (hydroxymethyl) amino methane buffer (*p*H 8·0, 0·0125 M) and sodium

Table 1. LIMITS OF SODIUM CHLORIDE CONCENTRATION (%) FOR GROWTH OF GRAM-NEGATIVE BACTERIA

Organism	Minimal	Maximal	Optimal*
Marine			
11 (N.C.M.B. 845)	1	8	4
12	1	8	2–4
13	1	8	4–8
14	1	8	2–4
26	1	8	8
56	1	8	1–4
60	1	4	4
67	2	4	2–4
68	4	4	4
72	1	8	2–4
73	1	8	2–4
74	4	8	4–8
Non-marine			
Pseudomonas sp.	0	4	1
Escherichia coli	0	4	1–2
Achromobacter sp.	0	4	1–2
Proteus sp.	0	8	1–2
Serratia sp.	0	4	0
Spirillum undula	0	1	0–1
Spirillum serpens	0	1	0

Marine bacteria are listed by their catalogue number in the culture collection of C.S.I.R.O., Division of Fisheries and Oceanography, Cronulla, Sydney. They were mostly motile rods, probably related taxonomically to N.C.M.B. 845. Exceptions were an agar-digesting species of *Cytophaga* (No. 68), an organism with a low incidence of branching (No. 72) and a very pleomorphic organism of average length about 2μ (No. 74). Halophilic bacteria were omitted from the survey because it has been shown that breakdown of the membrane *Halobacterium halobium* is not autolytic as in N.C.M.B. 845 but instead consists of non-enzymatic dissociation into subunits (ref. 2).
* Optimal salt concentration was assessed from rate of development of turbidity and final maximal turbidity reached by the cultures.

chloride (0·01 M) at 35°. The amount of envelope breakdown was expressed as the percentage reduction in turbidity after 20 min at 35°. Protein was estimated by the method of Lowry *et al.*[4].

No direct correlation was demonstrated between envelope lysis and salt tolerance but, by analogy with N.C.M.B. 845[3], it was thought that lysis would be affected by the amount of protein in the cell envelopes. Envelope protein lay between about 21 per cent (*S. serpens*) and about 85 per cent (*E. coli* grown in sea-water). There was no statistically significant correlation between envelope protein and the extent of lysis. There was, however, a significant relation between 'specific lysis' (lysis (per cent)/protein (per cent)) and salt tolerance. For maximal salt concentration tolerated during growth the mean values of specific lysis were: 1 per cent sodium chloride, 0; 4 per cent sodium chloride, 0·35; 8 per cent sodium chloride, 0·54. The differences were significant at the 1 per cent level. A similar relation was demonstrated between specific lysis and minimal salt tolerance. There was no effect between optimal salt concentration and specific lysis.

Envelope lysis was inhibited by calcium chloride (0·001 M) and the effect was related significantly to maximal salt tolerance (Fig. 1). No significant relation was demonstrated between inhibition by calcium chloride and either optimal or minimal salt concentration. Sodium chloride

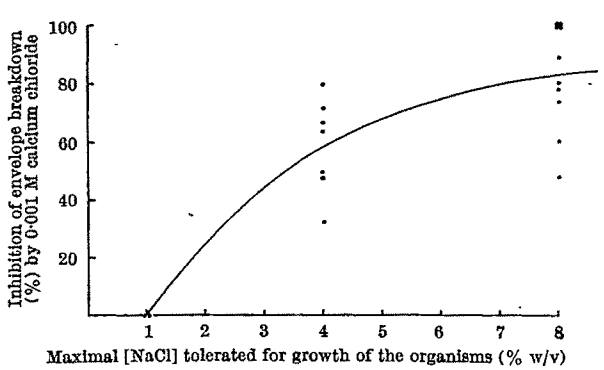


Fig. 1. Relation between inhibition of envelope breakdown by calcium chloride (0·001 M) and maximal concentration of sodium chloride tolerated during growth. Each point represents a single organism and in most cases is the mean of lysis values for two preparations. Of the four preparations with 100 per cent inhibition, two underwent slight and one extensive aggregation in the presence of 0·001 M calcium chloride. No numerical allowance has been made for this. The curve is drawn through the mean values for each group. Differences between groups were significant at the 0·1 per cent level

(0·5 M) inhibited envelope breakdown in some cases, but the extent of inhibition was unrelated to salt tolerances of the growing organisms. There was, however, a differential effect between Ca^{2+} and Na^{+}; the difference (inhibition by 0·001 M calcium chloride)—(inhibition by 0·5 M sodium chloride) showed a positive relation, significant at the 5 per cent level, with maximal salt tolerance. It is probable that the significance of the effects shown could be increased by refinements in some of the techniques used in this survey. In particular, a more realistic treatment of maximal and minimal salt tolerance rather than the present system of grouping would probably allow graphical treatment of the results. The measure, 'specific lysis', takes some account of the effect of protein content on envelope lysis, but probably not a very accurate one. In the envelope of N.C.M.B. 845 autolysis was proportional to protein in excess of about 35 per cent[3]. It is probable that a similar kind of relation exists in many of the present organisms, in which case greater accuracy would be obtained by deriving, for each organism, an envelope protein versus lysis curve.

In spite of these limitations, however, the use of simple techniques has demonstrated, within a group of Gram-negative bacteria, relations between salt tolerances of the organisms on one hand and some measures of envelope instability and cation dependence on the other. Some experimental evidence has thus been provided in support of the hypothesis that salt tolerance and membrane properties are related.

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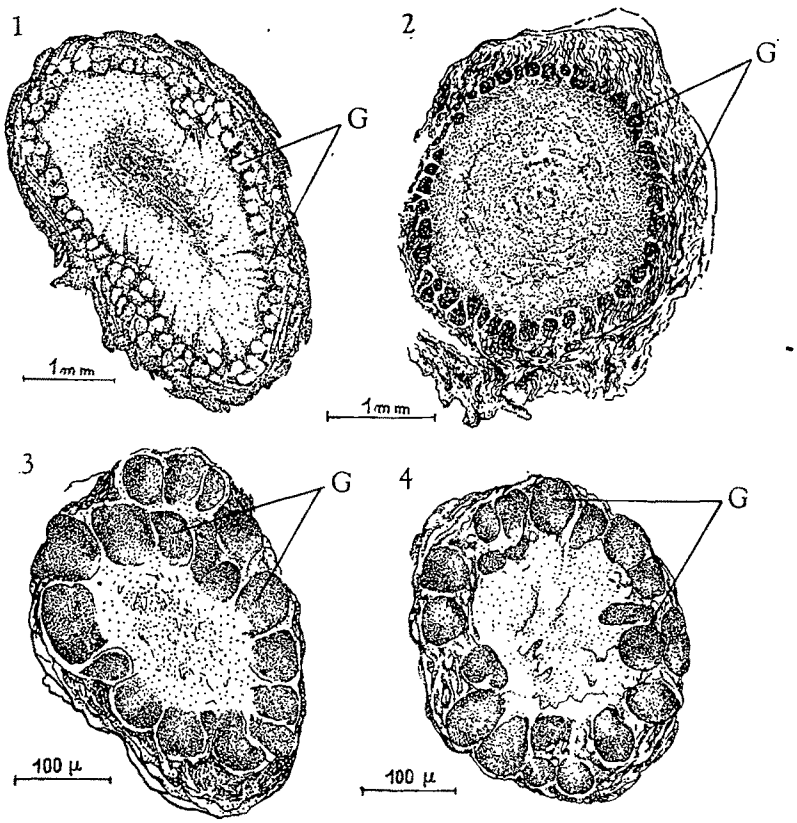


Fig. 1. Cross-sections of the bulbus olfactorius of mammals and of the lobus olfactorius of insects approximately through the central part from somewhat frontal side. Their magnification is given in the same measurements. *G*, glomeruli. (1) *Canis familiaris*; (2) *Erinaceus roumanicus*; (3) *Antophora acervorum* (Hymenoptera); (4) *Periplaneta americana*

ENTOMOLOGY

Structure of the Brain of Insects

Insects, particularly those living gregariously, can perform very complicated activities. These activities are not less complex than those performed by higher vertebrates. It is interesting to know to what degree these activities depend on the structure of the brain. In the higher Hymenoptera the brain is relatively the most complex. The results of my investigations[1,2] show that the part of corpora pedunculata which is called 'calyx' is particularly differentiated. The walls of this calyx in the Aculeata are composed of three layers.

As has already been stressed in my previous reports, it is difficult in insects to associate a more complex structure of the brain with the ability to perform the most complicated activities. For example, the brain of termites[3] (calyx) is simpler than the brain of the higher Hymenoptera. In the walls of the calyx it is impossible to differentiate the aforementioned layers as in the case of Hymenoptera; the brain of the ant is simpler than that of the bee. In ants the third layer is scarcely visible, whereas in bees there is even a sub-layer within the third layer. Nevertheless it is impossible to state that ants and termites yield to bees as regards the performance of the manifold activities.

Both the size of the corpora pedunculata and that of the whole brain are undoubtedly of great importance here. The male of insect communities has a smaller brain than the workers. The queen of these insects also has a smaller brain in spite of its sometimes bigger body and it cannot perform such complex activities as the workers. The size of the brain of insects, as has already been reported by Goossen[4], may increase with the reduction of size of body. However, this is not correlated with increased ability of the complex activities. In this respect there is some similarity to the vertebrate brain[5,6]. That part of the brain of insects most similar to that of the vertebrate brain is the lobus olfactorius analogous to the bulbus olfactorius. There occur, however, some differences which have not been found in vertebrates—namely, those connected with sex[7]. In the lobus olfactorius of the male there occur in some parts big glomeruli which are not found in the female[8–10]. The visible differences in these refer to the glomeruli the size of which in relation to the size of brain is bigger in insects than in mammals; but the glomeruli are more numerous in mammals (Fig. 1).

When comparing the brain of insects with the brain of vertebrates, there may be observed a different structure of its most important part, the corpora pedunculata, on which the association and regulation of stimuli depend.

Some investigators comparing the brain of insects and vertebrates have attempted to make an analogy between the corpora pedunculata and cerebral cortex. Others, like Zwarzin[11], were of the opinion that a comparison is possible with the corpora striata of vertebrates. He

spring-mounted moving partition cell of Yphantis and Waugh. The concentration of enzyme remaining above the partition at the end of the run divided by the concentration in an uncentrifuged control sample (the *Q* ratio) was used in conjunction with the photograph of the meniscus and partition to calculate the radial movement of the enzyme boundary. This value was converted to an *S* value by use of an 'effective centrifugation time' at full speed (including acceleration and deceleration periods) obtained from photographs of a visible moving schlieren peak.

The enzyme produced by heterocaryon 23–39 is heat-labile and was therefore assayed by difference (before and after pre-incubation for 15 min at 43·5° C) in a mixture with wild-type enzyme centrifuged in the same cell simultaneously. The 45–48 enzyme was indistinguishable from wild in thermolability and other properties and was centrifuged separately.

Although the comparisons of two heterocaryons with the parental wild-type by the movable partition method failed to establish any differences, it was decided to compare the same and other heterocaryotic materials in density gradient tubes, since the latter method provides a much more extensive fractionation of each sample (as well as a different medium). The additional heterocaryons tested by this method were chosen because of the relatively normal properties of the adenylosuccinase they produce[3], since a general effect was being sought. Aliquots of 0·2 ml. of crude extracts were floated on a sucrose gradient in the Spinco *SW* 39 swinging bucket rotor and centrifuged at approximately 37,000 r.p.m. for 10–12 h. Samples were then collected by upward displacement with 25 per cent sucrose, in 33–35 fractions of four drops each, by means of a special tube holder with hypodermic needles at inlet and outlet.

On assay of the fractions (from either single or mixed extracts), single symmetrical bands of enzyme were found, with a width (above the half-maximal concentration level) of 5–6 fractions (15–20 per cent of the whole gradient). There was no indication of any other bands, of fractional or multiple molecular weights, nor of any significant differences among the six heterocaryons and the wild-type (74*A*) examined. No effect on sedimentation of active enzyme of wild-type or heterocaryon 45–48 was apparent after partial inactivation by heat treatment or in the presence of thioglycerol. The results are summarized in Table 2. Variation in peak position between experiments is attributable to variations in rotor speed, time, and temperature. However, such variation is compensated for by the inclusion of at least one sample from each experiment in at least one other experiment. Recovery of activity varied from 90 to 106 per cent in the partition cell, and from 70 to 100 per cent in the swinging bucket tubes, for both wild-type and mutant heterocaryon enzymes.

Table 2. SEDIMENTATION OF ADENYLOSUCCINASE OF *ad*-4 HETEROCARYONS AND WILD-TYPE (74*A*) IN SWINGING BUCKET

Exp. No.	Strain‡	Speed (r.p.m.)	Time (min)	Peak activity fraction§
1	74*A*	36,500	720	27–28
	,,	,,	,,	26
	,,	,,	,,	25
2	74*A*	36,800	572	20–21
	45–48	,,	,,	20–21
	45–48+74*A*	,,	,,	21
3	74*A*	36,800	680	21–22
	45–48+74*A*	,,	,,	22
4*	74*A*	36,800	660	22
	45–48	,,	,,	20
5†	74*A*	35,600	627	20
	45–48	,,	,,	20
6	74*A*	36,800	603	21–22
	48–92	,,	,,	21
	82–92	,,	,,	22
7	23–39	35,800	616	23–24
	82–103	,,	,,	24
	48–103	,,	,,	24
8	74*A*	37,000	640	27–28
	82–103+74*A*	,,	,,	26–27

The extracts (0·20 ml.) were layered on sucrose gradient (4·60 ml., 5–20 per cent); both in phosphate buffer (0·05 M, *p*H 8·0).
* 3×10^{-3} M thioglycerol in gradient.
† Extract pre-heated 5 min at 49° C.
‡ Re origin of strains, Nos. 23, 39, 45, 48 (ref. 20). 82, 92, 103 (82, ethylmethanesulphonate; 92 and 103, nitrous acid; M. Case and N. H. Giles, unpublished).
§ Proportional to sedimentation coefficient[21].

These investigations have not provided evidence for any definite mechanism of complementation between allelic adenylosuccinase mutants. However, one possible class of mechanisms is inconsistent with the results obtained, that is, that requiring a permanent association between mutant enzyme molecules of the size normally found active in the wild-type extracts. If the heterocaryon enzymes are mixed aggregates, the active wild-type enzyme must also exist only as an aggregate of similar size, under the various conditions utilized, since there appears to be no heterogeneity in hydrodynamic properties among any or all of the active enzymes.

In a similar investigation, Braymer and Woodward[19] found evidence for heterogeneity in the sedimentation patterns of adenylosuccinase from certain *ad*-4 heterocaryons (not tested in this investigation), but not for that of wild-type or the majority of the heterocaryons. In some cases a small amount of activity was recovered from material sedimenting more slowly than the normal enzyme (personal communication). These results do not appear to be inconsistent with the foregoing conclusion.

Further evidence will be required to distinguish among complementation mechanisms involving temporary or permanent aggregation of whole molecules or parts of molecules, or retention, elimination, protection, or activation of defective portions of mutant enzymes.

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Genetic Variation of Phosphatase in Cattle Serum

A COMBINATION of starch-gel electrophoresis and histochemical staining technique was used to study a variation in the phosphatase of cattle serum. The starch-gel electrophoresis was performed as described by Gahne[1]. The starch gel was cut in three slices; the bottom slice was used for protein staining and the middle slice was stained for phosphatase in the following solution: 100 ml. acetate buffer, 0·1 M, *p*H 5·0, 100 mg sodium α-naphthyl phosphate, 100 mg *o*-aminoazotoluene diazonium salt, 10 drops

of 10 per cent solution of magnesium chloride, 10 drops of 10 per cent solution of manganese chloride, 0·5 g polyvinylpyrolidone, 2 g sodium chloride. The solution is practically the same as that used by Lawrence *et al.*[2]. The gels were incubated 16 h at room temperature. Background staining was removed by washing the gels with methanol/water/acetic acid (50 : 50 : 10). The phosphatase activity was recognized by darkly stained zones.

In some serum or plasma samples no activity of phosphatase appeared, but in other samples one or several zones were observed at the anodic side of the gel. A fast moving phosphatase fraction observed in the serum of some individuals gave rise to the most distinct zone. It was designated by the symbol *A* (Fig. 1). Its position corresponded to the α-globulin area. Two other zones appeared in the transferrin area or just in front of it. Sometimes enzyme activity was found close to the starting line and in the area between the starting line and the $S\alpha_2$-globulin.

Thus, several different zymograms were observed in different individuals. However, in investigations of 48 monozygous cattle twin pairs no differences were found within the pairs while a pronounced variation existed between pairs. In some pairs the individuals of the same pair were in different stages of pregnancy and lactation but did not differ in the enzymatic pattern. Identical zymograms including a distinct *A* zone were also found in a pair, 18 months of age, where one member was castrated as a young calf, while the other member remained intact. The zymograms of four monozygous pairs are shown in Fig. 1. The results of the studies of the monozygous twin pairs indicate a genetic basis for the variation of the cattle phosphatase.

However, the age of the tested animals has a certain influence on the enzymatic pattern. In young calves a phosphatase zone seems always to be present in the transferrin area, while the zone *A* seems to appear first in animals older than 9–10 months. These problems, as well as the nature of the phosphatase zones, will be subjected to further study. The present investigation will be restricted to the inheritance of zone *A* in animals older than one year.

The results to be presented here suggest that the variation of cattle phosphatase is determined by a genetic system symbolized by *F*, and that one allele called F^A determines the presence of the enzyme zone *A*, while another allele or alleles designated F^O give rise to zymograms without the zone *A*. The intensity of the zone *A* varies somewhat between individuals. However, it is not known whether this may be due to a difference between the homozygotes (F^A/F^A) and the heterozygotes (F^A/F^O). The symbols *A* and *O* will be used for the two phenotypic classes.

The distribution of the phosphatase types was studied in the offspring (older than one year) from 263 matings in eight herds with Swedish Red and White cattle (SRB) (Table 1). In 128 matings of the type *O* × *O* all offspring were of the type *O*. Also in the other types of matings there was a good agreement with expectancy according to the proposed genetic hypothesis.

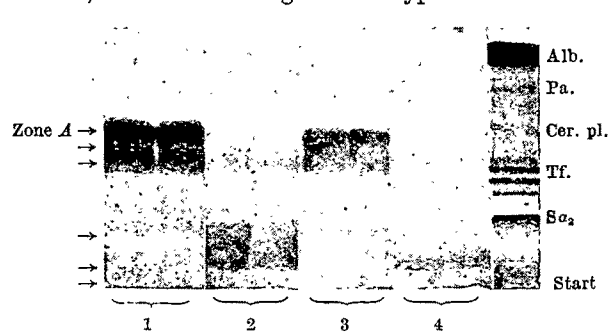


Fig. 1. Phosphatase zymograms of sera from four monozygous cattle twin pairs. Two pairs are of the type *A* (Nos. 1 and 3). Zones with enzyme activity are marked with arrows to the left. The result of amido-black staining after starch-gel electrophoresis of cattle serum of the transferrin type *A/D* and the post-albumin type *A/A* is shown to the right. Alb., albumin; Pa., post-albumin; Cer.pl., ceruloplasmin; Tf., transferrin; $S\alpha_2$, $S\alpha_2$-globulin

Table 1. Distribution of Phosphatase Types of 263 Offspring having Sire and Dam of Known Type

Phosphatase type of				No. of matings	No. of sires
Sire	Dam	Offspring			
		A	*O*		
A	*A*	14	6	20	7
A	*O*	18	18	36	
O	*A*	45	34	79	18
O	*O*	—	128	128	
Total		77	186	263	25

Table 2. Observed and Expected Number of 380 Dizygous Twin Pairs in which the Co-twins have Different or Identical Phosphatase Types

	Phosphatase types			Total
	Different	Identical		
		A	*O*	
Observed	83	57	240	380
Expected	76·0	61·0	243·0	380·0

Additional support for the genetic hypothesis was obtained in studies of 439 female cattle twin pairs. The blood samples were collected by Rendel[3] for some special purposes and the animals were randomly distributed among herds of the SRB breed. Among 380 pairs, which were older than one year and were classified as dizygous in tests for erythrocyte antigens[3], transferrins[1] and post-albumins[1], there were 83 pairs in which the members of the same pair had different phosphatase types, while 57 pairs were classified as *A* and 240 pairs as *O*. Within seven other pairs the phosphatase types differed, while the erythrocyte antigen, transferrin and post-albumin types were compatible with monozygosity. However, the morphological diagnosis indicated that these seven pairs were also dizygous.

The following gene frequencies were obtained by taking one individual at random in each twin pair, $F^A = 0{\cdot}140$ and $F^O = 0{\cdot}860$. If genetic equilibrium is supported the gene frequencies can be used to estimate the expected number of dizygous pairs in which the co-twins should have different or identical phosphatase types. The following formulæ[1] were used (p = frequency of F^A, q = frequency of F^O): (*a*) the probability that the members of a dizygous twin pair have different phosphatase types = $pq(2q^2 + 1{\cdot}5pq)$; (*b*) the probability that the members of a dizygous twin pair have both the type $O = q^2(q + \frac{1}{2}p)^2$.

The observed distribution of the female twin pairs agrees well with expectancy (Table 2).

The *A* type seems to occur in both sexes at equal frequency. A material comprising 225 SRB bulls sold at the official breed sales at an age of 18–30 months was investigated. The following gene frequencies were obtained, $F^A = 0{\cdot}133$ and $F^O = 0{\cdot}867$. These are almost exactly the same frequencies as in the random sample of female twins.

As a whole it can be concluded that the individual differences which occur in the zymograms for phosphatase in cattle seems to be under genetic control.

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PSYCHOLOGY

Parameters of Electric Shock relevant to Electro-convulsion Therapy

In an earlier communication[1], I reported a finding indicating that the power dissipated in the subcutaneous tissues, where the receptors lie, is the parameter most relevant to the determination of threshold electric shocks. This led to the assertion that it would be more reasonable and safer to control wattage rather than voltage or current when using electro-convulsive therapy. As a result of correspondence with Dr. G. R. Hawkes, of the U.S. Army Medical Research and Development Command, I am persuaded that this assertion is misleading. A more accurate conclusion would have been that, although the standard practice of controlling voltage is less safe than controlling the wattage, it would probably be safer still to control current under the conditions obtaining in a mental hospital.

Two assumptions need to be borne in mind to reach this conclusion. First, by far the greater part of the impedance of a biological circuit occurs in the stratum corneum, very little being met in the subcutaneous tissues. Secondly, this impedance is a function of the nature of the contact made between the electrodes and body surface. As was pointed out in the original communication, the use of electrode jelly reduces this source of impedance to a marked extent.

Given these conditions, the relative merits of the three types of stimulation are readily compared. With a constant voltage stimulus a halving of the skin impedance virtually doubles the current. If the impedance of the subcutaneous tissues remains constant, then the power dissipated in these tissues will be quadrupled. Under constant power conditions, halving of the skin impedance produces an increased current and a reduced voltage, so that the power dissipated in the subcutaneous tissues is only doubled instead of quadrupled. With a constant current stimulus changes in the skin impedance cannot affect the power dissipated in the subcutaneous tissues, provided that the impedance of this part of the biological circuit remains unchanged.

If the skin impedance remains constant and that of the subcutaneous tissues varies, then a different picture emerges. A doubling of the impedance in the subcutaneous tissues will virtually double the power dissipated in these tissues under constant voltage, constant power, or constant current conditions, leaving little to choose between them in this respect.

All the foregoing, however, is predicated on the assumption that the impedance of the subcutaneous tissues forms an insignificant fraction of the total impedance of the biological circuit. Most evidence so far favours this assumption, although there may be special circumstances in which it does not hold. Since we know very little about the distribution of energy within a biological circuit when an external E.M.F. is applied via the intact skin a note of caution is in order. In particular, the capacitative changes that occur under these conditions are of uncertain magnitude and locus. It is, therefore, possible that with some kinds of a.c. input, and on the make-and-break with a d.c. input, there may be brief periods in which this assumption is invalidated.

If at any time the impedance of the subcutaneous tissue does form a preponderant part of the load, and if this impedance varies while skin impedance remains constant, then the constant power stimulus would provide better control of the power dissipated in the subcutaneous tissues than either a constant voltage or constant current stimulus. The odds are that these special conditions prevail less frequently when electro-convulsive therapy is being used than those conditions contingent on the two basic assumptions discussed. It follows that the patient is more likely to be safeguarded by a constant current stimulus than by a constant power stimulus, although it remains true that the constant voltage stimulus in general use is the least satisfactory of all in this respect.

If, under constant voltage conditions, electrode jelly is used either more copiously or in fresher condition in a later session of a series of electro-convulsive therapy treatments, then the voltage that was appropriate earlier in the series will be too great when the load is reduced at the skin surface. If dry electrodes are used, then the degree to which the patient is sweating will introduce a similar uncontrolled variable and expose the patient to the same danger of an unintentionally high level of shock in terms of the power dissipated in the brain.

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Electrical Stimulation of Pain and Touch Systems

To take best advantage of cutaneous channels for communication, information must be translated into a form compatible with perceptual properties of the touch sense. To find whether such channels are effective other than as providing mere warning information or slow transliteration of speech, one needs systematically to vary cutaneous stimuli along temporal and spatial dimensions to determine the resolving power of the nervous system for complex patterns of cutaneous stimuli. In contrast to vibration, direct electrical stimulation of the skin has several advantages, but the disadvantage that pain ordinarily accompanies electrical stimulation of hairy body regions. The purpose of this investigation was to determine conditions of painless electrical stimulation of touch, by defining the stimulus conditions which affect pain and touch thresholds.

Touch and pain thresholds as peak current were measured in two experiments as a function of: (1) the number of brief electric pulses, from one to 20; (2) the rate of pulse repetition, 10–250 pulse/sec, on eight body regions, including hairy and hairless tissues[1]. The anodal pulses, delivered through a constant current stimulator, were 0·5 msec in duration at half-peak.

Pain thresholds were rendered relatively reliable by training procedures. One result was relaxed, attentive observers unreactive to threshold pain, in effect, a functional separation of cutaneous pain thresholds from effects of pain reaction. Also, thresholds were made stable by sufficiently long interstimulus and inter-threshold times, which minimized cumulative effects of stimulation.

Pain thresholds and touch thresholds were a decreasing hyperbolic function of the number of pulses in a stimulus train, indicating nearly linear integration of current pulses at different rates by pain and touch. Also, pain and touch thresholds fell at different rates with increasing repetition rates from < 10–100 pulses/sec. It was concluded that stimulation of touch with 0·5 msec current pulses can be reliably pain-free over much of the surface of the body given low repetition rates and low pulse number, because of the shorter time course of integration for touch than for pain.

Pain threshold current in hairy tissues was markedly different on electrode sites with high pain sensitivity relative to touch compared with those with low relative pain sensitivity; corresponding touch thresholds were nearly the same. Thus differences in the likelihood of stimulating pain on hairy tissues were primarily due to pain sensitivity differences, rather than touch sensitivity differences.

The perceptual nature of threshold pain was often described as sting on hairy tissue, dull pain on hairless regions. It was concluded that sting does not depend on the presence of hair or associated musculature, but is

related to the presence of non-myelinated free nerve endings in such tissue.

Pain thresholds in hairy tissues were raised considerably 48 h after the first session; touch thresholds were unchanged. Pain thresholds in hairless tissues were the same or somewhat lowered, thereby indicating that a general adjustment to pain does not underlie the rise. Since, in the first session, only six painful stimuli, and sometimes more than one hundred touch stimuli, were delivered to a given electrode site, pain thresholds in hairy tissue are raised solely from effects of the passage of current.

Pain thresholds on hairy tissue obtained with stimuli of varying repetition rates and pulse numbers were converted to average current per millisecond of stimulation[2]. An approximately rectangular hyperbolic relation was obtained between average current per time unit and stimulus duration, for durations as long as two seconds. Thus pain thresholds on hairy tissues are properly specified in units of average current flow and total time, not power. This finding, together with the importance of long rest intervals to sting threshold stability, points to a chemical intermediary.

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Placebo Reactor

It is often believed that there exist, in the community, certain individuals who are 'placebo reactors', and that these individuals must be excluded from a controlled clinical trial if the 'true' effects of the drug in question are to be correctly assessed. Glaser[1] expressed the extreme view when he wrote "a dummy" [that is, a pharmacologically inert substance] "identifies those patients who can be disregarded, either because they need no treatment or else because they can be cured by psychological influences, and this is as reasonable a procedure as the exclusion from experiments with anti-asthmatic drugs of patients who do not have attacks of bronchial asthma".

The story of the 'placebo reactor' can largely be traced back to Jellinek[2], who reported an investigation of headache in which he found that a proportion of the sufferers obtained relief from a placebo; he further observed a U-shaped distribution of individuals, with regard to this placebo reaction, so that some 'definitely' responded while others 'definitely' did not, only very few being partially relieved. It has since been shown[3–6] that this U-shaped distribution is by no means characteristic of all drug responses, and that the incidence of placebo reactions can vary greatly with the circumstances of a trial. Nevertheless, the mythical person who 'once a placebo reactor' is 'always a placebo reactor' dies so hard that an extra nail for his coffin will perhaps do no harm.

There is, of course, no doubt that some individuals are more amenable to suggestion than others, and in a given situation this increases the likelihood of their deriving some satisfaction from a placebo[7]. Also, the cause of the symptoms is of obvious importance, as Jellinek[2] himself recognized when he wrote "the difference in response to placebo must reflect a difference in the nature of headaches". It was, indeed, a shrewd physician who said "the treatment of scarlet fever depends on what is the matter with the patient"[8].

Apart from the variability of the individual and his symptoms, the design of a drug trial may influence the number of 'placebo reactors' who are discovered, particularly in a complex situation in which several methods of grading the response to the drug are available. This is well shown in my own work on post-operative pain, with which—unlike headache—'complete relief' and 'complete non-relief' rarely occur. The design and purpose of these investigations are explained elsewhere[9]; their present interest lies in the fact that they have provided several different criteria against which 'relief of pain' may be deemed to have occurred after treatment with morphine and a placebo (normal saline).

Table 1. Incidence of 'Placebo Reactors' after Upper Abdominal Surgery

	Improvement of pain alone (%)	Improvement of pain, movement and coughing (%)	Vital capacity (%)	Peak expiratory flow rate (%)
Change of 1 grade or more = 'relief'	45	50	22	12
Change of 2 or more grades = 'relief'	0	0		

Using an arbitrary pain score, only three out of 21 of my patients failed to achieve an improvement of at least 1 grade after morphine; but if this were to be accepted as 'relief', 8 of 18 patients were relieved by saline. If an improvement of two grades was required for 'relief', no patients were relieved by saline and only 2 by morphine. Movement and coughing are especially painful after upper abdominal operations; when ability to move and cough was taken into account, an improvement of one grade or more was achieved by 19 patients after morphine (91 per cent) and by 9 after saline (50 per cent), while an improvement of two or more grades was obtained by 10 of the 21 morphine cases and by none of those who had saline. Finally, an indication of the painfulness of deep breathing and coughing was obtained objectively by measuring vital capacity and peak expiratory flow rate: the vital capacity improved in 10 of the 12 patients after morphine, and in 2 of 9 after saline; peak expiratory flow rate improved in 4 of 12 cases given morphine and in 1 of 8 given saline. Allowing for a moment that percentages can legitimately be derived from such small numbers of individuals, the incidence of patients who obtained 'relief of pain' from an injection of saline, according to these several criteria of 'relief', was as shown in Table 1.

All these figures relate only to pain after upper abdominal surgery; other types of case yielded different responses to saline. Thus, the percentage of 'placebo reactors' among my patients could be made to vary from 0 to 50 according to the stringency of my criteria of 'pain relief'. As a greater improvement is required before 'pain relief' is deemed to have occurred the number of 'placebo reactors' falls; but, at the same time, the 'effectiveness' of morphine diminishes. The investigator is at liberty to choose whether he has 'placebo reactors' or 'morphine non-reactors' among his patients.

All this makes it difficult to understand how so-called 'placebo reactors' can reliably be identified and excluded from a clinical trial, and how the 'true' effects of a drug can be dissociated from its psychological effects. In contrast to Glaser's views[1], one might observe that bronchial asthma itself not infrequently responds to psychological influences, and that "there must be something the matter with a man who comes to a doctor when there is nothing the matter with him"[10]. It is perhaps not even too much to suggest that given the appropriate circumstances each one of us has the makings of a 'placebo reactor'.

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FORTHCOMING EVENTS

Tuesday, July 23—Wednesday, July 24

BRITISH SMALL ANIMALS VETERINARY ASSOCIATION AND THE UNIVERSITIES FEDERATION FOR ANIMAL WELFARE (at the Middlesex Hospital Medical School, London, W.1)—Symposium on "Anaesthesia of Small Laboratory Animals".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANTS (university graduates or holders of Diploma in Technology) IN (a) HIGH POLYMER CHEMISTRY; (b) APPLIED PHYSICS; and (c) METALLURGY—The Vice-Principal, Lanchester College of Technology, Coventry (July 25).

ANALYTICAL CHEMIST (Assistant Experimental Officer aged 18–27, with at least G.C.E. in five subjects including "A" levels in chemistry and one other subject) IN THE DEPARTMENT OF MINERALOGY for the analyses of minerals, rocks and meteorites, and research into analytical methods—The Secretary, British Museum (Natural History), Cromwell Road, London, S.W.7 (July 26).

HEAD (with good academic qualifications with teaching and/or industrial experience) OF THE ELECTRICAL ENGINEERING DEPARTMENT—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (July 26).

RESEARCH ASSISTANT (science graduate, preferably with some knowledge of genetics) to take part in a programme designed to study genetics and cytogenetics—The Secretary, Institute of Cardiology (University of London), 35 Wimpole Street, London, W.1 (July 26).

DEMONSTRATOR IN PHYSICS—The Registrar, The University, Nottingham (July 27).

LECTURER IN THE DEPARTMENT OF PHARMACOLOGY—The Secretary, The University, Edinburgh (July 27).

LECTURER (medically or non-medically qualified, with teaching and/or research experience in physical anthropology, human biology or genetics) IN PHYSICAL ANTHROPOLOGY IN THE DEPARTMENT OF ANATOMY—The Registrar, The University, Liverpool, quoting Ref. No. CV/357/N (July 27).

LECTURER OR ASSISTANT LECTURER IN CHEMISTRY (Inorganic or Physical) IN ST. SALVATOR'S COLLEGE—The Secretary, The University, College Gate, St. Andrews, Fife (July 27).

LECTURER (with a good honours degree and industrial and research or teaching experience) IN THE DEPARTMENT OF APPLIED PHYSICS to teach instrumentation and/or automatic control—The Academic Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff (July 27).

SENIOR ASSISTANT ENGINEER (Distribution Research) (with a degree in engineering or physics and wide experience of power engineering, including the design and planning of distribution systems) IN THE COMMERCIAL AND DEVELOPMENT DEPARTMENT, Headquarters in London—The Assistant Secretary (Establishments), The Electricity Council, 30 Millbank, London, S.W.1, quoting Ref. N./251 (July 27).

ASSISTANT (graduate in botany, agriculture, horticulture, or equivalent qualifications, and preferably some experience in plant pathology) TO THE MYCOLOGIST, for work involving testing the susceptibility to disease of crop varieties and at present is mainly concerned with the cereals—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (July 29).

JUNIOR LECTURER IN PHYSIOLOGY—The Registrar, Trinity College, Dublin 2, Republic of Ireland (July 29).

LECTURER (with higher qualifications) IN PSYCHIATRY IN THE DEPARTMENT OF PSYCHOLOGICAL MEDICINE—The Secretary, The University, Edinburgh (July 29).

ASSISTANT EXPERIMENTAL OFFICER (with a degree in chemistry, physics, agricultural chemistry or equivalent) to carry out chemical and physical analysis of soils—The Secretary, Rothamsted Experimental Station, Harpenden, Herts. (July 31).

BIOCHEMIST (with an honours degree) IN THE PATHOLOGY DEPARTMENT, General Hospital, Aberystwyth—The Group Secretary, Mid-Wales Hospital Management Committee, "Gorseland", North Road, Aberystwyth, Cards. (July 31).

KATHLEEN SCHLESINGER FELLOWSHIP (preferably candidate proposing to investigate degenerative diseases of the brain and central nervous system including biochemical abnormalities) tenable at the National Hospital for Diseases of the Nervous System, Queen Square, London, W.C.1—The Secretary, Medical Research Council, 20 Park Crescent, London, W.1 (July 31).

LECTURER IN MECHANICAL ENGINEERING—Head of the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7 (July 31).

LECTURER OR ASSISTANT LECTURER (preferably prepared to lecture mainly in pure mathematics) IN MATHEMATICS—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (July 31).

LECTURER OR ASSISTANT LECTURER (with a degree in agricultural chemistry and an interest in human nutrition) IN THE DEPARTMENT OF NUTRITION—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 31).

LECTURER (Ph.D. standard) IN BIOPHYSICS to take part in a programme of biophysical and biological research on cilia, under the general direction of Professor Sir John Randall—The Registrar, King's College (University of London), Strand, London, W.C.2 (July 31).

SECRETARY/FIELD OFFICER (with a sound knowledge of agriculture with a background of farming experience, an agricultural college qualification or its equivalent, and preferably graduate experience of livestock breeding and genetics) to organize the Secretariat and expand the activities of the Association—The Secretary, The Sheep Development Association, Holton Paddocks, Wheatley, Oxford (July 31).

STATISTICIAN (with a good honours degree in mathematics or science and knowledge of statistical techniques as applied to agriculture or biology, but applications will be considered from students expecting to graduate in 1963) for the Agricultural Research Council's Unit of Statistics (University of Aberdeen)—The Secretary, Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1 (July 31).

LECTURERS (2) IN VETERINARY CLINICAL STUDIES (Farm Animals) at the Veterinary Field Station—The Registrar, The University, Liverpool, 3 quoting Ref. No. CV/361/T (August 1).

LECTURER (with an honours degree in pharmacy or equivalent qualifications or experience, a knowledge of research methods, and preferably industrial research or production experience) IN PHARMACEUTICS—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1 (August 2).

SENIOR LECTURERS (2) IN ANATOMY at the University of Queensland, Brisbane, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 2).

ASSISTANT EXPERIMENTAL OFFICER (with a good S.L.C., S.C.E., H.S.C., G.C.E. or equivalent or university degree) at the Royal Observatory, Edinburgh, for observational work in various fields of astronomy and astrophysics with special emphasis on galactic research—The Establishment Officer, Room 364, St. Andrew's House, Edinburgh, 1 (August 3).

ASSISTANT (with an honours degree in agricultural chemistry or biochemistry, postgraduate experience in agricultural chemistry, and preferably an interest in animal nutrition) IN THE CHEMISTRY DEPARTMENT at Auchincrulve, for advisory, investigational and teaching duties—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (August 3).

SENIOR LECTURER (with high academic and research qualifications) IN ORGANIC CHEMISTRY—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (August 6).

RESEARCH ASSISTANT (with a degree in botany, biochemistry, microbiology or genetics, and a special interest in biochemical genetics, particularly enzymology) IN THE DEPARTMENT OF BOTANY (Prof. D. Lewis)—The Secretary, University College, Gower Street, London, W.C.1 (August 9).

RESEARCH WORKER (with an honours science degree or equivalent and suitable postgraduate research experience) IN THE MEAT RESEARCH DEPARTMENT of the Animal Production Division for work on histological and histochemical aspects of muscle growth—The Director, An Foras Taluntais, 33 Merrion Road, Dublin, 4, Republic of Ireland (August 9).

ASSISTANT LECTURER (preferably honours graduate in zoology or physiology with suitable postgraduate experience) IN ANATOMY AND HISTOLOGY; and a DEMONSTRATOR (preferably honours graduate in animal husbandry or animal physiology) IN ANIMAL SCIENCE at the School of Agriculture, Sutton Bonington, near Loughborough—The Registrar, The University, Nottingham (August 10).

ANIMAL HUSBANDRY OFFICER; and an ANIMAL NUTRITION CHEMIST at the Institute of Agricultural Research, Ahmadu Bello University, Samaru, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 14).

ASSISTANT PROFESSOR IN THE DEPARTMENT OF ZOOLOGY to participate in the cell biology programme and to teach protozoology, cytology, or a related subject at the senior undergraduate level—Dr. C. V. Finnegan, Department of Zoology, The University of British Columbia, Vancouver 8, B.C., Canada (August 15).

LECTURER and an ASSISTANT LECTURER (with an honours degree in physiology or closely allied subject, and preferably some research experience) IN THE DEPARTMENT OF PHYSIOLOGY—The Secretary, The University, Edinburgh (August 15).

LECTURER IN BOTANY at Fourah Bay College, University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 18).

PROFESSOR and HEAD OF THE DEPARTMENT OF VETERINARY MEDICINE; and PROFESSOR and HEAD OF THE DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 18).

PROFESSOR, SENIOR LECTURER, and a LECTURER IN THE DEPARTMENTS OF CIVIL ENGINEERING, ELECTRICAL ENGINEERING, AND MECHANICAL ENGINEERING at the University of Lagos—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 18).

SENIOR LECTURER OR LECTURER IN THE DEPARTMENT OF BACTERIOLOGY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, Senate House, University of London, London, W.C.1 (August 19).

SENIOR LECTURER (with research experience in at least one of the basic medical sciences and familiar with modern research methodology) IN EXPERIMENTAL SURGERY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 23).

DEPUTY DIRECTOR to be head of a research section as well as carrying administrative responsibilities; HEAD OF EPIDEMIOLOGY SECTION dealing with field surveys of virus incidence, and organization and assessment of vaccine trials; and HEAD OF GENERAL VIROLOGY SECTION which includes virus-cell studies in tissue culture, viral genetics and the biophysics and biochemistry of viruses—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey (August 31).

DIRECTOR OF THE UNIVERSITY OF SHEFFIELD INSTITUTE OF EDUCATION—The Registrar, The University, Sheffield (September 7).

LECTURER OR ASSISTANT LECTURER IN PHILOSOPHY; and a LECTURER OR ASSISTANT LECTURER IN PURE OR APPLIED MATHEMATICS—The Secretary to the Trustees, Magee University College, Londonderry, Northern Ireland (September 11).

PROFESSOR OF PHYSICS in the School of Physical Sciences of the University of Adelaide at Bedford Park—The Registrar, University of Adelaide, North Terrace, Adelaide, South Australia (September 16).

CHAIR OF ADULT EDUCATION—The Registrar, The University, Manchester (September 20).

JUNIOR LECTURER (preferably qualified in education psychology and/or comparative education) IN EDUCATION—The Registrar, Trinity College, Dublin 2, Republic of Ireland (September 20).

ASSISTANT PROFESSOR or an ASSOCIATE PROFESSOR (physiologist or biochemist, M.D. and/or Ph.D.) IN THE DEPARTMENT OF PHYSIOLOGY—Prof. M. Beznak, University of Ottawa, Ottawa, Ontario, Canada.

ASSISTANT (with special interests in inorganic or physical chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Professor, Department of Chemistry, University College, Upper Merrion Street, Dublin 2, Republic of Ireland.

BACTERIOLOGICAL ASSISTANT (with a degree in a biological subject, and preferably previous bacteriological experience) IN THE ENTERIC REFERENCE LABORATORY—The Director, Central Public Health Laboratory, Colindale Avenue, London, N.W.9.

CHAIR OF PURE MATHEMATICS—The Registrar and Secretary, The University, Bristol.

LECTURER IN BIOCHEMISTRY AND ANIMAL NUTRITION—The Bursar, Harper Adams Agricultural College, Newport, Shropshire.

LECTURER (with at least a good honours degree in chemistry, some teaching and research or industrial experience, and an active interest in research) IN INORGANIC CHEMISTRY to teach to Grad.R.I.C., B.Sc. General, and H.N.C. standards—The Principal, South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex.

PHYSICAL OR INORGANIC CHEMIST (Ph.D., with up to five years experience) for fundamental studies in catalysis—The Director of Research, Research Council of Alberta, 87 Avenue, 114 Street, Edmonton, Alberta, Canada.

PHYSICIST and a PHYSICAL CHEMIST (of Ph.D. standing, preferably with research experience in a related field) to join a team engaged in basic long-term research into the photoconducting behaviour of organic materials—The Director, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Midlothian.

POST-DOCTORAL FELLOW for research on the biological control of glycogen metabolism (aspects involving both enzymology and metabolism are under investigation)—Dr. N. B. Madsen, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

POST-DOCTORAL RESEARCH FELLOW in Solid State Physics, for work on the transport and optical properties of insulating solids—The Registrar, The University, Leicester.

RESEARCH ASSISTANT (honours graduate in engineering or physics) IN ESTUARINE HYDRAULICS, to study the regime of the Clyde Estuary, with special reference to sediment movement—The Professor of Civil Engineering, Royal College of Science and Technology, George Street, Glasgow, C.1.

RESEARCH ASSISTANT (preferably with experience in cytology) to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield, 10.

RESEARCH ASSISTANT (with a Ph.D. or considerable research experience in metallurgy, physics, or chemistry) IN METALLURGY, for investigations concerned with physical and chemical reactions occurring in thin oxide films formed on surfaces of metals—Dr. J. S. Ll. Leach, Metallurgy Department, Imperial College of Science and Technology, London, S.W.7.

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (graduate in physics or physical chemistry) IN THE BIOPHYSICS DEPARTMENT, to become a member of a team investigating the structure and properties of viruses and of virus-antibody complexes—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey.

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with at least a first- or good second-class honours degree with a special knowledge of entomology and preferably experience of tropical stored products problems), for research on biological problems of tropical stored products—The Director, Tropical Stored Products Centre, Pest Infestation Laboratory, London Road, Slough, Bucks.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

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Science Museum, London. Science Library Bibliographical Series, No. 785: Intranuclear Lipides. Pp. 5. (London: Science Museum, 1963.) 1*s.* 3*d.* [215

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The Three Cultures. By Prof. H. Irving. (An Inaugural Lecture delivered in the University of Leeds on 3 December 1962.) Pp. i+26. (Leeds: The University Press, 1963.) 2*s.* 6*d.* [245

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The Murphy Chemical Co., Ltd., Summer Spraying for Red Spider, Codling and Tortrix. Pp. 4. (Wheathampstead, St. Albans: The Murphy Chemical Co., Ltd., 1963.) [295

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University of California Publications in Geological Sciences. Vol. 42, No. 3: Petrology and Structural Analysis of Metamorphic Rocks in the Southwestern Sierra Nevada Foothills, California. By Myron G. Best. Pp. 111–158 (5 plates) 1.25 dollars. Vol. 42, No. 4: Structure of Metamorphic Rocks at Mineral King, California. By M. N. Christensen. Pp. 159–198 (4 plates). 1.25 dollars. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1963.) [275

World Health Organization. Public Health Papers, No. 18: Medicine and Public Health in the Arctic and Antarctic—Selected Papers from a Conference. Contributed by K. Lange Andersen and 9 other authors. Pp. 169. 6 Sw. francs; 10*s.*; 2 dollars. Technical Report Series, No. 253: Conference on Medicine and Public Health in the Arctic and Antarctic, Geneva, 28 August–1 September 1962—Report. Pp. 29. 1 Sw. franc; 1*s.* 9*d.*; 0.30 dollars. (Geneva: World Health Organization; London: H.M. Stationery Office, 1963.) [275

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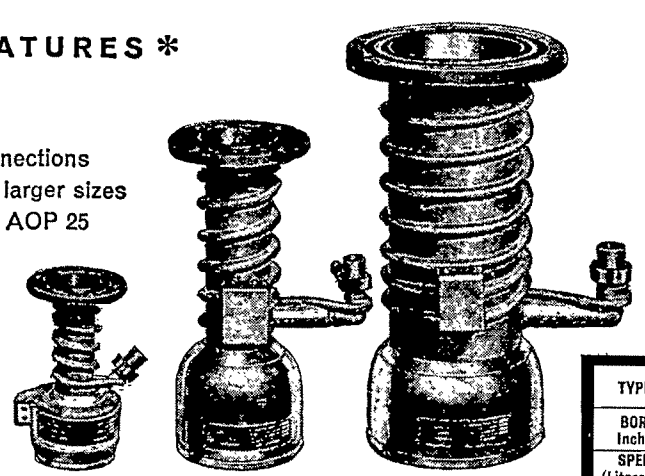
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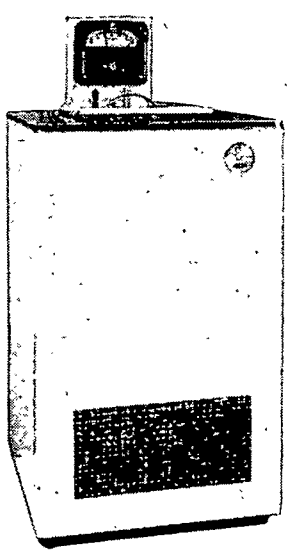
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APPOINTMENTS VACANT

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Experimental Officer in the Department of Metallurgy. Duties will commence as soon as possible after October 1, 1963. The officer will be responsible for the maintenance of X-ray equipment for undergraduate teaching and for research. He will take part in the development of those research projects which rely largely upon X-ray techniques and should be able to give some service assistance to other projects. Candidates should be familiar with standard X-ray diffraction apparatus and techniques, preferably with some knowledge of counter methods, low angle scattering, fluorescence analysis, or texture determination. Some experience with electronics would be an advantage. Qualifications in physics or metallurgy are desirable—for example, graduate membership of the Institute of Physics or L.I.M. Salary range: £880 by £40 to £1,200 per annum. The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than August 10, 1963, to the Registrar, The University, Manchester, 13, from whom further particulars and forms of application may be obtained.

UNIVERSITY OF EDINBURGH
DEPARTMENT OF PHYSIOLOGY
LECTURESHIP AND ASSISTANT LECTURESHIP

Applications are invited for the posts of Lecturer and Assistant Lecturer in the above Department. Salary scales: Lecturer, £1,250 by £105 to £1,670; bar: £1,790 by £120 to £2,150 per annum; Assistant Lecturer, £1,000 by £50 to £1,150 per annum; with placement according to qualifications and experience, and with superannuation benefit and family allowance where applicable. For both posts an honours degree in physiology or closely allied subjects is essential, and research experience is desirable. Facilities exist for research in nervous system, endocrinology and human metabolic problems, but special facilities are available for research on central nervous mechanisms concerned with vision. The successful candidates will be expected to take up duty on October 1, 1963.

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees, should be lodged not later than August 15, 1963.

CHARLES H. STEWART,
Secretary to the University.

RESEARCH INSTITUTE (ANIMAL VIRUS DISEASES), PIRBRIGHT, SURREY, which has hitherto concentrated on foot-and-mouth disease, is extending its activities to include other animal viruses and is re-organizing research work into three or four main sections. Applications are now invited for the following posts vacant in January, 1964:

(a) Deputy Director, who will be head of a research section as well as carrying administrative responsibilities. Appointment in S.P.S.O. (£2,800 to £3,150) or D.C.S.O. (£3,275 to £3,600) grade according to age, qualifications and experience.

(b) Two posts in S.P.S.O. grade:

(i) Head of Epidemiology Section dealing with field surveys of virus incidence, and organization and assessment of vaccine trials. It also includes World Reference Laboratory on Foot-and-Mouth Disease sponsored by F.A.O. and O.I.E.

(ii) Head of General Virology Section which includes virus-cell studies in tissue culture, viral genetics and the biophysics and biochemistry of viruses.

Application form and further particulars from Secretary, to whom applications should be submitted by August 31, 1963.

Monsanto

INDIA

SALES TECHNICAL SERVICE MANAGER

RUBBER CHEMICALS

Expansion of activities in India has created the need for a rubber chemicals Sales Technical Service Manager in the Monsanto Chemicals India (Private) Ltd. organization.

Primarily the position is to create, manage, and control a rubber chemicals sales technical service laboratory. The duties will include responsibility for leading a small team of technical staff, maintaining close contact with technical developments in the rubber industry in India, and supervising the production of suitable technical literature. The position is located at Khopoli, near Bombay, and will involve some travelling.

Applications are invited from men aged up to 40 years of Indian nationality who have an honours degree in chemistry or physics obtained in a British university, or an equivalent qualification. Associateship of the Institute of the Rubber Industry will be an additional desirable qualification. A minimum of two to five years practical experience of rubber technology is essential.

The successful applicant will be a man of imagination, ambitious to take this outstanding opportunity to establish himself in India as a recognized technical expert in this field.

Excellent starting salary and career prospects, pension scheme and other benefits. Passage to India at Company's expense.

Please send details of experience, qualifications, age, etc., to:

The Staff Officer (DL 235),
Monsanto Chemicals Limited,
Monsanto House,
10/18 Victoria Street,
London, S.W.1.

Cadbury's

MATHEMATICIANS/PHYSICAL SCIENTISTS

We offer interesting work in a new section of the organisation concerned with Operational Research, the development of quality control systems, and quantitative assessment of sensory characteristics. Candidates should be under 27 years of age and have an honours degree in either Mathematics or a Physical Science. A knowledge of statistical methods would be an advantage but is not essential.

Write to :

THE EMPLOYMENT MANAGER (EMC)
CADBURY BROTHERS LIMITED,
BOURNVILLE, BIRMINGHAM

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Experimental Officer in Spectroscopy in the Department of Chemistry in the Faculty of Science. Previous experience in at least one branch of the subject is desirable. The person appointed will have the opportunity to apply a wide range of spectroscopic techniques to chemical problems. Salary range: £880 by £40 to £1,200 per annum. The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than August 3, 1963, to the Registrar, The University, Manchester, 13, from whom further particulars and forms of application may be obtained.

POST-DOCTORAL FELLOWSHIP ($6,000, tax-free stipend) for research on the biological control of glycogen metabolism. Aspects involving both enzymology and metabolism are under investigation.—Apply Dr. N. B. Madsen, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

UNIVERSITY OF SHEFFIELD

Applications are invited for a post of Research Assistant to take part in a programme designed to study human chromosome abnormalities and associated disorders. Experience in cytology desirable, but not essential. Suitable for candidates graduating this year. Salary £700 to £900 per annum, according to qualifications and experience.

Further details available from the Secretary, Genetics Department, the University, Sheffield, 10.

GRADUATE OR PERSON QUALIFIED OR experienced in the rubber and/or plastics field required in the Information Division of the Rubber and Plastics Research Association of Great Britain for abstracting scientific literature and answering technical inquiries. Salary according to age and experience within the range £620 to £1,075. Five-day week. Canteen.—Apply in writing to Secretary, R.A.P.R.A., Shawbury, Shrewsbury, Shropshire.

PRODUCT DEVELOPMENT METALLURGIST

The Electrolytic Zinc Company of Australasia Limited have a vacancy for a Senior Physical Metallurgist at their Works at Risdon, near Hobart, Tasmania.

The Company is extending its activities in the fields of product development and technical service with particular reference to present and potential uses of zinc, zinc alloys and zinc products. The successful applicant will be required to take part in and supervise the research and development aspects of this work. He will be expected to initiate investigations into new uses for zinc as well as implement programmes for improvement of existing applications.

QUALIFICATIONS: Applicants should preferably have a University training in physical metallurgy, and some years of experience in metallurgy—either in research or production. They should be capable of supervising metallurgical research, both fundamental and applied.

Appointment is subject to medical examination including chest X-ray.

SALARY: Will be dependent on qualifications and experience and will be at a rate of up to £A3,000 per annum. In addition, a discretionary bonus scheme is in operation based on the prosperity of the Company.

BENEFITS: A Staff Provident Fund is available to which the Company subscribes three-quarters of the contribution.

Contributory Hospital and Medical Benefit Schemes are operated by the Company and Dental Treatment is available at a nominal charge after completion of one year's service.

Accommodation can be made available for a married applicant on a rental basis if required. Assistance can be given to single men in finding accommodation.

REMOVAL EXPENSES: Fares and travelling expenses will be paid for the successful applicant and his family. A liberal contribution will also be paid towards the cost of transporting personal effects.

APPLICATIONS: Should contain full details of courses studied, results obtained, and of subsequent experience and should include the names of at least two referees. They should be forwarded to:

The General Superintendent,
Electrolytic Zinc Co. of A/asia Ltd.,
G.P.O. Box 377-D,
HOBART, Tasmania.

UNIVERSITY OF LAGOS MEDICAL SCHOOL

Applications are invited for (a) Lectureship or (b) Junior Lectureship or Junior Research Fellowship in Pharmacology in Department of Physiological Sciences. Candidates should have a higher degree, with some teaching and research experience. Medical qualification desirable but not essential. Appointment to commence October 1, 1963, or as soon thereafter as possible. Salary scales: (a) £2,200 by £100 to £2,600 per annum; (b) £1,200 by £100 to £1,700. Entry point depending on qualifications and experience. Passages for appointee, wife and up to five children under 11 years on appointment, leave, and termination. Children's and car allowances. Superannuation scheme. Part-furnished accommodation at rent not exceeding 7 per cent of salary. Free medical attention.

Detailed applications (six copies), naming three referees, by August 15, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF CAMBRIDGE

Applications are invited for a Senior Assistantship in Research in Causal Morphology and Fine Structure in the Department of Botany, from October 1, 1963. Candidates should have experience of the application of electron microscopy to plant material. Pensionable stipend in scale £1,250 by £60 to £1,670, plus children's allowance.

Further particulars from Dr. H. L. K. Whitehouse, Botany School, Downing Street, Cambridge. Closing date for applications: August 31, 1963.

THE AGRICULTURAL RESEARCH COUNCIL require an Assistant Experimental Officer at their Ditton Laboratory for work of essentially practical nature on the storage and handling of potatoes. Applicants should have a pass degree in agriculture and an interest in applied research. Salary according to age in scale £590 at age 20, £858 at age 26 or over, rising to £1,053. The post entails considerable travelling.—Forms of application may be obtained from the Secretary, Ditton Laboratory, Larkfield, nr. Maidstone.

Monsanto

RESEARCH CHEMICAL ENGINEER

Monsanto Chemicals Limited require a Research Chemical Engineer with an interest in reaction kinetics to work on chemical engineering aspects of high pressure polymerization reactions. This work will be carried out in our research establishment at Fawley, Hampshire, on the edge of the New Forest.

Applicants should have a good honours degree in chemical engineering with post-graduate or industrial research experience.

Good starting salary and career prospects, non-contributory pension scheme.

Please send applications, giving details of age, education, and previous experience to:

The Staff Officer (DL 236),
Monsanto Chemicals Limited,
Monsanto House,
10/18 Victoria Street,
London, S.W.1.

MEDICAL RESEARCH COUNCIL

LABORATORY OF MOLECULAR BIOLOGY

A vacancy exists for a Graduate in Physics with experience of biological materials, or in a biological science with experience of physical methods, and at least two or three years of postgraduate work, to join small group, under Dr. H. E. Huxley, F.R.S., working on the mechanism of muscular contraction, and to assist in low-angle X-ray diffraction studies of muscle and muscle proteins. Experience with X-ray diffraction work advantageous but general experimental ability more important. Salary scale: £850 to £1,050 or £1,150 to £1,350 according to experience and qualifications.—Applications, giving the names of two referees, should be addressed to Dr. H. E. Huxley, F.R.S., Medical Research Council's Laboratory of Molecular Biology, Cambridge.

UNIVERSITY OF MANCHESTER

Applications are invited for the posts of Lecturer and Assistant Lecturer in the Mechanics of Fluids in the Faculty of Science. Applicants, who will be required to teach Mathematics and Aeronautical Engineering students, may have special qualifications in any branch of fluid mechanics or aerodynamics, but one post may be filled by an applicant with special interests in low speed fluid mechanics and aeroelasticity. The salary scales are as follows: Lecturer, £1,250 to £2,150 per annum; Assistant Lecturer, £1,000 to £1,150 per annum. Membership of F.S.S.U. and Children's Allowance Scheme. Status and initial salary (at the grade of Lecturer) would depend upon qualifications and experience.

Applications should be sent not later than August 15, 1963, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained.

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS

Assistant Experimental Officer to carry out chemical and physical analysis of soils. Degree in chemistry, physics, agricultural chemistry or equivalent. Starting salary between £641 (age 21) and £858 (age 26 or over) on scale rising to £1,053. Superannuation.

Applications, naming two referees, to the Secretary by July 31.

RESEARCH INSTITUTE (ANIMAL VIRUS Diseases), Pirbright, Surrey, invites applications from graduates in physics or physical chemistry for a post in the Biophysics Department. The successful applicant will become a member of a team investigating the structure and properties of viruses and of virus-antibody complexes. The work involves studies by a variety of physical methods (X-irradiation, electron microscopy, ultracentrifugation, etc.) and biological assay procedures. A suitably qualified candidate could work for a higher degree. Appointment in Scientific Officer Grade, £791 to £1,309, or Senior Scientific Officer Grade, £1,438 to £1,772. Starting salary depending on age, experience and qualifications. House available for married man. Superannuation under F.S.S.U.—Applications to Secretary.

WELSH COLLEGE OF ADVANCED TECHNOLOGY

DEPARTMENT OF APPLIED PHYSICS

Applications are invited for a post as Lecturer to teach Instrumentation and/or Automatic Control in the above Department. The appointment will be from September 1, 1963, or as soon as possible thereafter. Applicants should have a good honours degree and industrial and research or teaching experience. The person appointed must be able to plan and organize undergraduate courses in Instrument Technology and/or Automatic Control, in particular for students in the final year of the Dip.Tech. Course in Applied Physics and later at postgraduate level. Successful candidates will be expected to pursue some research or development project and every encouragement and assistance will be given to this end. Salary scale: £1,600 by £40 to £1,800 (under review by the National Incomes Commission).

Further particulars and application forms, to be returned by July 27, 1963, quoting reference number 176 BC, may be obtained from the Academic Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff.

D.S.I.R.

NATIONAL PHYSICAL LABORATORY, TEDDINGTON, MIDDX.

Senior Scientific Officer/Scientific Officer Physicist with an interest in Radiation Dosimetry is needed. After an initial period of training in existing work and techniques involved, research would be undertaken to extend the existing energy range of absolute dose measurement, to include very high energy X- or gamma-radiation, and electrons, using microcalorimetry as well as ionization methods. Qualifications: first- or second-class honours degree in physics or equivalent; for the senior post at least three years' post-graduate research experience is required together with evidence of originality. Salary: Scientific Officer, £831 to £1,359; Senior Scientific Officer, £1,488 to £1,832.

Application form from Director at above address, quoting **120XY**. Closing date August 6, 1963. (5)

UNIVERSITY OF THE WEST INDIES

Applications are invited for Senior Lectureship in Microbiology. Duties include instruction in microbiology for students working for medical degrees of London University and routine clinical microbiological work for University College Hospital. An appointment on secondment would be considered. Salary scale: £2,400 by £100 to £3,400 per annum. Child allowance £150 per annum first child, £100 per annum second, £50 per annum third. F.S.S.U. Housing allowance of 10 per cent of salary, or, if available, unfurnished accommodation will be let by University at 10 per cent of salary. Up to five full passages on appointment, on normal termination, and on triennial study leave.

Detailed applications (10 copies), naming three referees, by August 7, 1963, to Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1, from whom further particulars may be obtained.

GLASGOW ROYAL INFIRMARY

PULMONARY PHYSIOLOGIST

Graduate with honours in physiology with interest in respiratory physiology, and with experience of pulmonary function techniques desirable, but not essential, required for Pulmonary Function Unit now being established. Salary at Hospital Service rate according to experience.

Apply in writing, giving two names for reference, and not later than August 15, 1963, to the Secretary, Glasgow Royal Infirmary, 84 Castle Street, Glasgow, C.4.

UNIVERSITY OF LANCASTER

The University invites applications for a CHAIR OF PHYSICS (other than nuclear physics).

Applications (10 copies), naming at least two referees, should be sent before October 12, 1963, to the Secretary of the University, P.O. Box 78, County Hall, Preston, from whom further particulars may be obtained.

UNIVERSITY OF IBADAN

NIGERIA

Applications are invited for post of Photocopy Technician to work under direction of Senior Technician in University Library microfilm and photocopying section. Good experience in processing and dark-room work, maintenance of equipment and knowledge of processes of documentary reproduction desirable. This is a training post and candidates will be judged on their suitability and willingness to be further trained in appropriate techniques, management of a library photocopying service and maintenance work. Salary scale: £950 by £25 to £1,050 per annum. Passages paid for appointee, wife and up to five children under 11 years, on appointment, approved overseas leave and termination where applicable. Children's and car allowances. Pension scheme. Part-furnished accommodation at rent not exceeding 7 per cent of salary.

Detailed applications (six copies), naming three referees, by August 14, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

AMENDED ADVERTISEMENT

UNIVERSITY COLLEGE OF RHODESIA AND NYASALAND

Applications are invited for three posts of (a) Senior Lecturer, or (b) Lecturer, or (c) Assistant Lecturer in Physics, for one of which applicants should have strong qualifications in geophysics. Appointments on secondment would be considered. Salary scales: (a) £2,100 by £100 to £2,600 per annum; (b) Grade 1, £1,880 by £80 to £2,200 per annum; Grade 2, £1,450 by £70 to £1,800 per annum; (c) £1,200 by £50 to £1,350 per annum. Passages to Salisbury for appointee, wife and dependent children on appointment, with allowance for transport of effects. Installation grant of £100 for persons recruited from outside Africa. Superannuation and medical aid schemes. Unfurnished accommodation at rent of 15 per cent of salary. Assistance given for triennial visits overseas.

Detailed applications (six copies), naming three referees, by August 8, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

GOVERNMENT OF UGANDA

Biochemist required to be in charge of the Biochemistry Division of the Laboratory services located in Kampala and serving all parts of the country and all races. Most of the work originates from Mulago Hospital. Excellent library facilities and opportunities for research. Candidates should possess an honours degree in chemistry, be Associates of Royal Institute of Chemistry and have at least two years postgraduate experience in medical biochemistry. They must also be nationals of and permanently resident in Great Britain or the Republic of Ireland. Appointment on contract for one tour of 21-27 months. Salary between £1,221 to £2,448 a year according to qualifications and experience. Gratuity, taxable, of 25 per cent of total emoluments on satisfactory completion of contract. Education allowances at varying rates. Mid-tour passages for children in U.K. and for whom education allowance is being paid. Quarters with heavy furniture available at a rental not exceeding £102 a year. Return passages for officer, wife and dependent children. Approximately four months' home leave on full pay or total gratuity of 45 per cent on satisfactory completion of contract. Local leave of 16 days a tour.

Further information and application forms from the Director of Recruitment, Department of Technical Co-operation, 3 Sanctuary Buildings, Great Smith Street, London, S.W.1. (Please state full name and quote RC 205/153/026.)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF BIOPHYSICS

Applications are invited from candidates of Ph.D. standard for the post of Lecturer in Biophysics. The successful applicant will be expected to take part in a programme of biophysical and biological research on cilia, under the general direction of Professor Sir John Randall.

Salary according to age and experience within the scale £1,250 by £60 per annum to £1,670 then by £80 per annum to £1,830; then subject to review by £80 to £2,150. A London allowance of £60, family allowances and F.S.S.U. benefits will also be payable.

Applications, giving full details of experience, should reach the Registrar, King's College London, Strand, W.C.2, not later than July 31

PILKINGTON BROTHERS LIMITED

Glass Manufacturers, require

A PHYSICIST

at their new Lathom Research Laboratories for problems covering basic research work and its application to process plant.

Theoretical and experimental work in fluid dynamics would include model studies in viscous and non-viscous flow, heat transfer in high temperature furnaces; and the design and analysis of experiments at small-scale and pilot-plant level for which a knowledge of statistics would be useful.

The requirements are: age about 30 years, with a good honours degree in Physics and some experience of a high temperature process industry.

Please quote RES./M3 in replying to L. P. Laidlaw, Group Staff Department, Pilkington Brothers Limited, Grove Street, St. Helens, Lancashire.

THE ROYAL COLLEGE

NAIROBI

Applications are invited for Senior Research Fellowship in Parasitology in Department of Zoology. Candidates should have a Ph.D. or equivalent postgraduate research experience and should give an outline of the research they would prefer to pursue. Appointment for three years. Salary £1,350 per annum. F.S.S.U. medical scheme. Subsidized accommodation. Return passage to Nairobi for appointee. Baggage allowance.

Detailed applications (six copies), naming three referees, by August 18, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

ROWETT RESEARCH INSTITUTE

(ANIMAL NUTRITION AND ALLIED SCIENCES)

Biochemist or Chemist for Lipids Biochemistry Department. Salary scales: Scientific Officer/Senior Scientific Officer £791 to £1,309 or £1,438 to £1,772, with placing according to age and experience. Superannuation F.S.S.U. Qualifications: good honours degree, or its equivalent, and research experience.

Apply with fullest particulars by August 15, naming three referees, to the Secretary of the Institute, Bucksburn, Aberdeen.

WELSH NATIONAL SCHOOL OF MEDICINE

(UNIVERSITY OF WALES)

APPOINTMENT OF TRAINEE BIOCHEMIST

Applications are invited for the above appointment. The post is intended for a person having a degree or equivalent qualification in chemistry, with experience in or wishing to take up clinical biochemistry. Salary within the scale £720 to £1,020 per annum; point of entry according to qualifications and experience. Pension scheme.

Applications, in writing, within three weeks of the appearance of this advertisement, to the Professor of Pathology, Cardiff Royal Infirmary, from whom further information may be obtained.

UNIVERSITY OF LAGOS MEDICAL SCHOOL

Applications are invited for Associate Professorships in Virology and Bacteriology (two posts) in Department of Microbiology. Candidates must have postgraduate qualifications and considerable experience in the speciality. Medical qualifications advantageous. Duties include teaching and clinical and research work. Appointments carry honorary consultantships to Lagos University Teaching Hospital. Preference given to candidates with experience in teaching hospitals. Appointments to commence October 1, 1963, or as soon thereafter as possible. Salary £3,200 per annum. Passages for appointee, wife and up to five children under 11 years on appointment, leave and termination. Children's and car allowances. Superannuation scheme. Part-furnished accommodation at rent not exceeding 7 per cent of salary. Free medical attention.

Detailed applications (eight copies), naming three referees, by August 15, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY COLLEGE OF RHODESIA AND NYASALAND

Applications are invited for post of Chief Technician in Zoology Department from candidates with university or equivalent experience. Salary scale: £1,200 by £100 to £1,700 per annum, entry point depending on qualifications and experience. Superannuation and medical aid schemes. Passages to Salisbury for appointee, wife and dependent children on appointment, with allowance for transport of effects. Installation grant of £100 in addition for persons recruited from outside Africa.

Detailed applications (six copies), naming three referees, by August 13, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF IFE

NIGERIA

Applications are invited for (a) Senior Lectureships, (b) Lectureships, or (c) Assistant Lectureships in: (i) Animal Nutrition; (ii) Animal Breeding and Genetics; (iii) Animal Health (registrable veterinary qualifications essential); (iv) Soil Microbiology; (v) Tree and Economic Crop Agronomy. Salary scales: (a) £2,275 by £75 to £2,575 per annum; (b) £1,200 by £75 to £1,650/£1,725 by £75 to £2,175 per annum; (c) £950 by £50 to £1,100 per annum (or if over 28 or holding a higher degree £1,050 by £50 to £1,150 per annum). Initial appointments three years, to commence as soon as possible. Secondment for a period of not less than one (but preferably two) academic sessions considered. Passages for appointee, wife and up to five children on appointment, leave and termination. Children's, car, and outfit allowances. Superannuation scheme. Part-furnished accommodation.

Detailed applications (eight copies), naming three referees, by August 10, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 20 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

MONASH UNIVERSITY

VICTORIA, AUSTRALIA

Applications are invited for appointment to the following positions: (a) Reader or Senior Lecturer in Microbiology; (b) Lecturer in Microbiology. Applicants should be free to take up duty in December, 1963, or January, 1964. The Department of Microbiology will be accommodated in newly built and equipped laboratories at the Monash University Medical School, Alfred Hospital. Undergraduate teaching in microbiology for the M.B., B.S. degree starts early in 1964. Research interests of the department will be concerned mainly with viruses and organisms in the borderland between viruses and bacteria. Further information about the department and its research programme may be obtained from Professor B. P. Marmion. Salary ranges: Reader, £A3,000 to £A3,300 per annum; Reader (if medically qualified), £A3,100 to £A3,400 per annum; Senior Lecturer, £A2,480 to £A3,000 per annum; Senior Lecturer (if medically qualified), £A2,600 to £A3,300 per annum; Lecturer, £A1,660 to £A2,450 per annum, with superannuation on the F.S.S.U. basis.

Information regarding application procedure, conditions of appointment, superannuation, travel and removal allowances, study leave and housing, is available from the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University, P.O. Box 92, Clayton, Victoria, Australia. Closing date: Applications close in Australia and London on August 30, 1963.

FIELD STUDIES IN EDUCATION

IN 1960 the Nature Conservancy set up a strong Study Group under the chairmanship of E. M. Nicholson to examine the role of field studies in education. The investigations of the Group were extensive, the work of many hands, so that the report, which has now been published, will serve to mould and to crystallize opinion for some time to come*.

In the report, field studies are taken as comprising the out-of-doors aspects of biology and the earth sciences, geology and geography; and while in the discussion activities of students at field centres occupy a central place, consideration is given to the educational needs of children of any school age, members of youth organizations and, to a lesser degree, the public at large. In almost all respects the Group finds that present facilities fall seriously short of requirements; however, it is expecting a big drive forward in the next few years, associated with reforms in biology teaching which are now in train. Looking on the present situation as fluid and dynamic, the Group invites the co-operation of all people who have constructive proposals to offer. It is in this sense that the following comments are made.

The Group is at its best in presenting a comprehensive survey of facilities for field studies which are now available. It is less convincing in discussing their educational mission. It is a minor point that it makes no reference to the personal observation of the weather, of the expanse of the night sky viewed away from the lights and bustle of the towns, and of the experience of being alone with Nature between dusk and dawn.

More seriously, the Group assumes that children possess a special, innate curiosity about living things and that field studies should serve as a gateway to science. These assumptions are open to question. Altogether, the waxing and waning of interest in things at different stages of life deserve more attention than they have so far received. By the age of fifteen, most boys and girls feel that primroses and blackbirds count for little in their lives, yet at later ages they may become keen about flower gardening. That is why the teaching of natural history below the grammar school needs to be based on individual interest, for in the time available instruction in health and human physiology has more cogent priority.

Love of Nature is a complex emotion, sometimes imbued with patriotism. As the report shows, the organized study of wildlife in Holland is meagre; all the same, public support for conservation is massive, and in intensity of culture. Dutch farming is about the best in the world. Besides, in these days, evidence for the evolutionary origin and unity of all living matter has acquired such compelling validity that it provides grounds for our own reaction to Nature which, rising above æsthetic satisfaction, may attain to reverence of a spiritual kind.

In relation to field studies, as the report shows, the Forestry Commission is helping. However, it might well do much more, for besides possessing large areas of almost unspoiled country, the Commission could provide students with some comprehension of how in certain sectors biological science is being developed and applied. This is truer still of agriculture, in which context the work of the Association of Agriculture has been overlooked. Farming, for example, can present exemplifications of soil science, plant and animal nutrition, genetics, etc., which find little counterpart in the wild. Along these lines, agricultural colleges and farm institutes could be put to more imaginative use, and the National Agricultural Advisory Service is well placed for taking on a more educational role. For comparable reasons, efforts should be made to give some biology students at first hand a better understanding of the life and economic resources of the seas.

In the teaching of biology at all levels, emphasis on finding out will have been pressed too far if it prevents students from gaining some knowledge of the ways in which the application of science contributes to the welfare and culture of man. Of this, medicine is a striking example. Among sixth-form biologists attending field centres, would-be doctors probably make up the largest contingent. There is next to no flow of training in the reverse direction. In other words, the time has arrived when thought should be given to the expansion of field studies into a wider range, and then integrated with a still greater educational whole.

* *Science Out of Doors: Report of the Study Group on Education and Field Biology.* Pp. xvi+240. (London: Longmans, Green and Co., Ltd., 1963.) 12*s.* 6*d.*

FROM ELECTROMAGNETIC WAVES TO CHROMATOGRAPHIC PEAKS

Comprehensive Biochemistry

Edited by Prof. Marcel Florkin and Prof. Elmer H. Stotz. Vol. 1 : Atomic and Molecular Structure. Pp. xi+253. 62*s.* Vol. 3 : Methods for the Study of Molecules. Pp. xiii+324. 80*s.* Vol. 4 : Separation Methods. Pp. xiii+282. 70*s.* (Amsterdam and New York : Elsevier Publishing Company, 1962.)

IF it is becoming necessary for the editorship, let alone the authorship, of a scientific treatise to be shared, it is surely presumptuous for one person to assume the role of critic. It is therefore with hesitation and humility that I attempt to survey the remaining three volumes of Section 1 of *Comprehensive Biochemistry*.

One's first impression is of a disciplined economy of style by an eminent and international team of authors. Volume 1 opens with accounts by W. Parker Alford and H. H. Jaffé of the modern concepts of atomic structure and valency, which, although not exhaustive, probably contain as much as the average biochemist has the time or will to absorb. The reader may find it advantageous to read two short chapters by G. J. Bullen at the beginning of Volume 3 before embarking on the long chapter by J. D. Bernal on the structure of molecules in Volume 1. It is curious that the editors should have considered it desirable to discuss molecular structure before dealing with crystallography and X-ray diffraction methods. Even the last-mentioned two topics could have been brought together in a single chapter. Fortunately, there are a number of cross-references. J. D. Bernal's chapter is divided into two main sections. The first deals with the general features of molecular structure, such as the types of interatomic forces which operate between non-convalently bound atoms, the classification of molecular shapes, and the physical properties of polymer aggregates. The second section considers biochemical molecules from simple amino-acids to proteins and nucleic acids. There is a short section on cellular structure, but the technique of electron microscopy is not described, although it receives passing mention in

Volume 3. There are two unfortunate features of this chapter. Unlike the other chapters in these volumes, which each concludes with a bibliographical section, references here are found partly in the text and partly in tables. Conformity of style would have been appreciated by the reader. Secondly, diagrams 2(*b*) and 2(*d*) (pp. 118–9), which illustrate early stages of the elucidation of the structure of vitamin B_{12} when the presence of a porphyrin ring-system was suspected, should have been omitted or adequately explained. They are rather misleading as they are presented. The final chapter of Volume 1 contains a good but very condensed account of stereoisomerism by K. Mislow.

In Volume 3, following the two chapters by Bullen mentioned here, attention is mainly directed towards the use of spectral methods of determining structure. The absence of any discussion of mass spectrometry is noted with regret. N. H. Nachtrieb briefly describes the technique of emission spectroscopy as applied to metal analysis in biological material. R. A. Morton gives a much longer account of spectrophotometry in the ultra-violet and visible regions. To some extent this chapter is coloured by Morton's own special interests in carotenoids, vitamin A and the visual pigments; it is a pity that room was not found for newer fields such as the use of difference spectra for the study of the conformation and denaturation of proteins. On the other hand, the details for checking and calibrating a spectrophotometer are welcome. There follows a chapter on infra-red spectroscopy by L. J. Bellamy, which is deliberately slanted for the biochemist. There is practically no theoretical treatment, and the cited examples of the use of infra-red spectroscopy are carefully selected to encourage its wider use in biochemical laboratories. A short, clear account of fluorescence, including quenching and polarization, and phosphorescence is given by A. Ehrenberg and H. Theorell; this chapter includes a brief explanation of singlet and triplet states and their relation to absorption and emission of radiation. Two chapters on electron paramagnetic (S. I. Weissman) and nuclear magnetic resonance (C. D. Jardetzky and O. Jardetzky) complete the spectroscopic section of Volume 3. The final chapter, by C. Sadron and M. Daune, on the behaviour of macromolecules in solution reveals another example of the curious arrangement of the subject-matter of these volumes. It is complementary to the two chapters by W. D. Stein in Volume 2 and should have followed them.

Volume 4 is devoted to separation methods, and opens with a chapter by L. C. Craig on countercurrent distribution. This chapter is good so far as it goes; but one is left wondering why steady-state distribution was omitted, particularly now that commercial equipment is available. There follows a long discourse on chromatography by E. Lederer and M. Lederer; much of this is a précis of their well-known text-book and now looks rather *passé*. Far too much space is devoted to adsorption chromatography; too many 'gimmicks', which never became popular, are given another airing; important new techniques such as thin-layer chromatography, gel filtration, the use of ion-exchange derivatives of cellulose and dextrins are either not mentioned or given inadequate coverage. The final chapter by P. Chovin, on gas chromatography, starts with 3 pages of symbols and ends with 1½ pages of applications of the technique, a treatment that is not likely to win any newcomers to this type of chromatography.

To summarize, the first section of *Comprehensive Biochemistry* will satisfy to a large extent the long-felt need for a fundamental treatise of biochemistry, but it could have benefited from better planning by editors and authors alike. The magnitude of the task, the need for rapid publication and the multi-national composition of the contributors are difficulties which should not be underestimated, but then neither should the price.

D. T. Elmore

ADVANCES IN VETERINARY SCIENCE

The Veterinary Annual, 1962

Edited by W. A. Pool. Fourth Year. Pp. xx+419+24 plates. (Bristol: John Wright and Sons, Ltd., 1963.) 45*s*.

THIS is the fourth year of the attempts made by the editor to gather together into one volume some account of the advances made in veterinary science. Veterinary science is a vast subject and many allied disciplines impinge on it. With the help of about 35 men and women drawn from veterinary schools, research institutes and Government departments this fourth *Annual* is published. Within the past couple of years the editor has included special articles which, according to his introduction, are chosen for various reasons. They may show the outlook in a particular country, a particular type of veterinary activity, or the outlook on an advanced type of research on a subject of general importance.

Actinomycosis has been selected this year for special consideration; it is written by a surgeon, and there is apparently little new knowledge on this subject available. The other special articles deal with problems on animal breeding, with cattle practice in England, with foot-and-mouth disease in the Americas, with the impact of ornithology on veterinary practice and research, and with some statistical information. Three deal with related matters; one with veterinary affairs in Africa south of the Sahara, another with veterinary education in underdeveloped countries, and the third with veterinary services in inland Australia. These are not confined to factual accounts and the personal views of the authors emerge; there is a common factor and that concerns the necessity for an improvement in methods of animal husbandry if the hungry people of the world are to be fed an adequate amount of protein. The problems come within the province of the veterinary surgeon, for animals cannot be raised or maintained economically unless they are healthy and free from disorders associated with infection and with nutritional, genetic and environmental errors.

About three-quarters of the *Annual* is given to reviews of present literature. Unfortunately the space granted to each section and sub-section precludes adequate reviews. Veterinary physiology takes about five and a half pages, and one of them is concerned with a list of references. These lists of references are most valuable for they are nearly all recent ones and concern papers published for the most part during 1961. It is not possible to review a subject in which there are 50–60 references and a little over four pages of text. This is a general criticism for, whether the authors are considering the recent advances in diseases related to infective agents, to nutritional, metabolic and reproductive disorders, to public health or to relevant legal decisions and legislation, the list of references is long and extensive and the criticism meagre.

In addition to an index there are two brief sections devoted to new drugs and appliances and to new publications. The short descriptions of the new drugs and appliances have been derived from information supplied by the makers and the maker's name and address are appended. The list of English veterinary works and new editions published during the preceding year contains popular and scientific books and pamphlets.

The editor is courageous and persistent in his yearly attempts to include into about 400 pages so much on such a wide field. It is questionable whether these efforts are a success; the publication of the fourth *Annual* suggests that it is worth while. On the other hand, there is a danger that the uninitiated may be guided along unusual and dangerous paths by directing attention too closely to the text displayed. The book is too short to be critical when consideration is given to the amount and size of the field of relevant knowledge. The special articles are interesting if read with a critical mind. The lists of

references are useful for the research worker who wants a lead to the literature and will not be content unless he reads the original contributions. The veterinary surgeon in practice will, if selective, obtain help. To each reader and user of the *Annual* there is the warning of the need for caution and care. R. LOVELL

ANALYSIS OF ANIMAL ECOLOGY

Animal Ecology

Aims and Methods. By A. Macfadyen. Second edition. Pp. xxiv+344+6 plates. (London: Sir Isaac Pitman and Sons, Ltd., 1963.) 42*s*. net.

THE old days when ecology was largely descriptive seem far away. Because of modern advances in techniques and methods of analysis, animal ecology is now in a very interesting stage of development, which makes a general ecological synthesis by no means an easy task at the present time. Field workers are slowly elaborating specialized methods for the investigation of different classes of problems and tend to get deeply immersed in these technicalities so that it is hard for them to take a broad view of the whole subject the methods of analysis of which and even methods of thought are at present so diverse.

In *Animal Ecology: Aims and Methods*, Mr. Macfadyen has made a very interesting and valuable survey of ecological methods. He is a specialist in the investigation of the fauna of the soil and has made considerable advances in the understanding of energy relationships. These aspects of ecology were emphasized in his successful book of 1957 and the present book is an enlarged and extensively revised second edition. Within the limits of a moderate-sized book full coverage of all aspects of ecology cannot be expected and this is a selective rather than a comprehensive work. Although some examples are quoted from freshwater and marine investigations and chosen papers on mammals, birds, fishes and invertebrates are reported and discussed, the emphasis lies quite firmly on the terrestrial ecology of temperate regions. Tropical ecology is virtually omitted. The work of the large teams of agricultural and medical entomologists investigating the ecology of locusts, tsetse flies and mosquitoes gets almost no mention. However, the emphasis on temperate terrestrial ecology is an important reason for the book's popularity with British and North American students who can use it in connexion with class exercises.

The first part of the book deals with the ecology of individuals and relates the animal to its physical, plant and animal environment. There is a chapter on distribution (but migration is scarcely mentioned) and one on animal metabolism. The second part of the book is on single species populations. Here the reader is introduced to collecting methods and the estimation of animal numbers, demography, population regulation and the biomass and metabolism of populations. The third part, on the ecology of animal communities, occupies half the book and is divided into seven chapters of which the largest is on the application of population dynamics. Here the author selects a dozen miscellaneous ecological investigations, provides a detailed report on each and discusses their general significance in relation to theories of population regulation. One could have wished for a rather more critical approach here, because the author has not reduced all his examples to a common system of terminology and concept and some confusion in their interpretation remains.

Unfortunately the book is marred by a few errors, which have slipped through unchanged from the first edition, such as the minus sign in the equation on p. 70, and the misleading statement about the advantages of saturation deficit as a method of expressing atmospheric humidity. One also regrets the needlessly restrictive definition of K as a population *maximum* on p.133. Had it been defined as an *equilibrium* population which in fact it is, the arguments about population change which follow could have been far more logically stated. These defects are minor ones and the book can be recommended as probably the most useful modern text of ecology for its size.

G. C. VARLEY

LEARNING BEHAVIOUR IN ANIMALS

Learning and Instinct in Animals

By Dr. W. H. Thorpe. Second edition. Pp. x+558. (London: Methuen and Co., Ltd., 1963.) 63*s*. net.

DURING the six years that have elapsed since the first edition of this book went to press a great deal of experimental work has been published, and several provocative theories have been proposed by members of the ethological school as well as by representatives of other disciplines interested in the study of animal behaviour. This is, therefore, an appropriate time for the publication of a revised version of the original work. The author has taken his self-assigned responsibility seriously, with the result that the second edition represents a considerable enlargement and re-casting of the initial presentation. The bibliography includes three hundred new references.

Part 2, entitled "Learning", has been increased by sixteen printed pages, and Part 3, "The Learning Abilities of the Main Animal Groups", has been lengthened by thirty pages. The section dealing with cephalopods has been extensively re-written to incorporate recent work dealing with relationships between the nervous system and behaviour in these organisms. Similarly, the section dealing with bird orientation has been completely revised and brought up to date.

Dr. Thorpe's general philosophical orientation, as reflected in the chapter entitled "Directiveness and Purposefulness", remains unchanged. The presentation of "The Nature of Drive and its Place in the Theory of Instinct" retains the original point of view, but is considerably enhanced by an illuminating consideration of physiological mechanisms, other than those involved in learning, which may influence behaviour—as, for example, the coding in the gene in terms of deoxyribonucleic acid.

The treatment of conditioning and trial-and-error learning has been expanded to include references to work done in the U.S.S.R. In some cases the references are to secondary sources but in others primary reports are cited. The treatment of latent learning now includes a discussion of new work dealing with concepts of expectancy and exploratory behaviour.

The chapter on mechanisms of learning contains a great deal of new material, including a review of theories involving the role of ribonucleic acid in connexion with the memory trace. This chapter is also improved by additions to the introductory section in which various neurophysiological theories of learning are discussed.

All in all, Dr. Thorpe's book remains a unique reference source for readers who wish to familiarize themselves with both the historical and modern ethological approaches to an understanding of animal behaviour. It is of additional value because of the careful and detailed attention directed to the experiments and theories which have been published by non-ethological workers.

At the present time there is no competitive volume to *Learning and Instinct in Animals*. It is safe to predict that no successful competitor will be forthcoming for a considerable span of time. This is an excellent book which deserves a place in the library of every serious student of the science of animal behaviour. F. A. BEACH

WILD LIFE AND THE CAMERA

People of the Forest

By Hans Lidman. Pp. 191 (plates). (Edinburgh: Oliver and Boyd, Ltd., 1963.) 42*s*.

THE camera has long proved its worth in the portrayal of wild life, in particular of bird life; indeed, bird photographers in Britain to-day are a large and for the most part a skilled body. But the book now before us is not concerned with British birds. It is by a Swedish naturalist, Mr. Hans Lidman, and is concerned with the wild life of a Scandinavian forest. Mr. Lidman has used his camera with uncommon skill to show us the birds and beasts of this beautiful but stern environment. His pictures are most impressive whether they are of mammals, birds, reptiles, insects, or the world in which they live. There is a landscape, wind-blown snow and tall pines, that suggests storm and winter cold more intensely than any picture I have seen; and there is a view of a few rushes and some sticks rising from the still misty waters of a forest-girt lake which conveys an impression of early morning stillness and peace hard to surpass.

But, of course, it is the studies of birds and beasts that are the great feature of this book. We may justly term it a picture book, though the text is by no means unimportant, consisting of charmingly written descriptions of the forest life and forest creatures under changing conditions, in mid-winter, spring, summer and autumn. It must be remembered that the letterpress is a translation from the Swedish and it is ever difficult to convey the spirit of the original in an alien tongue. So far as one can judge, the translation is successful, bringing before the reader vivid impressions of the vast forests, impressions made the more real, not to say intense, by the photographs.

Where practically all the illustrations are good it is invidious to pick out particular pictures for remark, but the stoat, transformed by its seasonal change of coat into a pure white ermine (p. 10), seems to me an outstanding snapshot. The two common cranes dancing in courtship-display, facing each other with spread wings and in elegant pose, will rouse the envy of all camera-minded ornithologists, and the portrait of a crane at its nest is a superb study.

But in dealing with the subjects of these magnificent pictures, in the matter describing them and their environment, it is regrettable so little use has been made of scientific names. A fine butterfly of swallowtail type is left unidentified, and a stoat, presumably *Mustela erminea*, is called a weasel, though this term is usually reserved for the tiny *Mustela nivalis*. It is true that in Ireland, where the weasel does not occur, the stoat is given this name; all the same, a stoat is generally a stoat and a weasel a weasel to most of us.

Where there is so much to admire we must not carp at such details, possibly due to translation difficulties, but turn back to the illustrations, such as that of a black-throated diver on her nest, lifting her sleek, almost reptile-like head aloft and looking disdainfully around. This volume would make a fine present for a boy or girl with leanings towards Nature. FRANCES PITT

EARTHWORM PHYSIOLOGY

The Physiology of Earthworms

By M. S. Laverack. (International Series of Monographs on Pure and Applied Biology: Zoology, Vol. 15.) Pp. ix+206. (Oxford, London, New York and Paris: Pergamon Press, 1963.) 45*s*.

AS the author emphasizes in his preface, there has been no general account of earthworm physiology since Stephenson's monograph on the Oligochaeta was published more than thirty years ago. Dr. Laverack's book fulfils a need felt by both physiologists and zoologists for an account of the work done since then, much of which is in obscure journals. Earthworms are almost ubiquitous and easily procured, so that it is perhaps not surprising that we know far more of their physiology and structure than we do of other oligochaetes. The subject of the book is, therefore, wisely limited to a review of work done on earthworms, and yet there are occasional digressions, for example, some information is given on respiration of the fresh-water *Tubifex*. There are chapters on digestion and metabolism, on the calciferous glands, the axial field, nitrogenous excretion, water relations, respiration, the physiology of regeneration, neurosecretion, the nervous system, and, lastly, behaviour. There are simple diagrams summarizing the course of digestion, excretion, water-relations and calcium-transport, which are useful in directing attention to what is established and what remains to be demonstrated. The first chapter is a biochemical potpourri; a frankly heterogeneous collation of such interesting topics as the composition of the cuticle, muscle structure, and pigments. In this last section, attention is directed to the presence of protoporphyrin in the skin, a subject mentioned again later (p. 134) in connexion with darkening in light; but the reader is left wondering what other pigments are present, and the reference to Kalmus and others' work (p. 11) without further explanation is annoying.

Such lapses are few. The author has no aversion for colloquialisms and these may be irksome to some readers. The constant use of Latin abbreviations strewn through some paragraphs I find irritating, though some chapters are almost free of them. The description of the chloragocytes and the present view of their function is most useful, but the contact that such cells have with blood vessels is an important point not emphasized. The sense in which the terms 'lipid' and 'fat' (p. 57) are used is not clear, and it may be pointed out that neither the periodic acid–Schiff nor the performic acid–Schiff is a specific test for phospholipids as the student might infer. Later in the book the two types of light-receptor are mentioned and figured (Figs. 57, 58), but there is no mention of the alleged difference in their spectral sensitivity or of the response of one to light increase, the other to decrease, reported by Unteutsch (*Zool. Jb., Abt. 3*, 58, 69; 1937). There are a few typographical errors of which only *Harmathoë* for *Harmothoë* may be mentioned as it may not be obvious to readers; while in Tables 5 and 6 the rate is presumably in hours, and oxygen-uptake is meant, and in Fig. 29 the oxygen-concentration is presumably in ml./l. The only other criticism is perhaps a personal one—the use of oxygen-*tension*—so prevalent among physiologists to-day.

These are all minor points and do not detract from the great usefulness of this book, which undoubtedly forms an essential addition to libraries of departments of zoology and comparative physiology. Dr. Laverack is to be congratulated on presenting such a concise review. It is well bound, clearly printed and is a worthy addition to this series from Pergamon. R. PHILLIPS DALES

FLOWERS OF THE GARDEN

Garden Flowers

By R. D. Meikle (The Kew Series). Pp. 479+15 plates. (London: Eyre and Spottiswoode, 1963.) 30*s*.

IT is largely the effect of the black-and-white illustrations in this latest volume of the Kew Series that sends one's mind racing back to the early herbals, but the feeling soon begins to dawn that the text is very much in keeping. The illustrations are mostly insets into the text and within their rectangular areas plants defy many of the ordinary laws of growth: the slanting Christmas rose (p. 64) and cyclamen (p. 291), the enwreathed flame flower (p. 134), the almost horizontal tubers of Roscoea (p. 390) are merely examples. Then the text, with its

paragraph of description and another of general notes, could well have been modelled on Clusius.

The advertisement on the jacket says "the authors have not shirked the difficulties of technical terms and problems: they have set out to elucidate them while providing enough scientific information to give the student that essential grounding in the classification and nomenclature of plants that is necessary to him before he can proceed usefully to advanced botanical studies". It is true that there are a couple of introductory pages explaining the barest elements of classification and nomenclature: it is true that technical terms are used and are explained in the glossary (perhaps one or two illustrations of dissected flowers would have been helpful): but it is difficult to detect anything that will lead the student on to advanced botanical investigations, or which is necessary groundwork for them. No attempt has been made to include biological information on seedlings, floral functions, seed production or perennation which might entice an interested reader into the paths of botany.

Perhaps it is well to forget any idea that this is a book for potential students. Its public surely lies among those gardeners who take an intelligent interest in wild species. With Harrison's companion volume *Garden Shrubs and Trees*, it gives a very reasonable coverage of garden plants (excluding bulbs) in a more attractive way (and more cheaply) than the standard works of reference like the Royal Horticultural Society's *Dictionary of Gardening*. Its more restricted scope permits a valuable and relatively simple, yet effective, key to the genera. Another feature which enables this book to be recommended is the generally high standard of accuracy in plant names and descriptions. However, two items require comment. Under *Incarvillea* an unnecessary manuscript name has unfortunately been taken up (*I. macrantha* Grierson); the plant in question is to be called *I. mairei* (Lévl.) Grierson. In the glossary 'zygomorphic' is given a correct explanation but two inaccurate synonyms: such a flower is neither 'irregular' nor 'asymmetrical', it is bilaterally symmetrical. B. L. Burtt

DEVELOPMENTS IN PLANT ANALYSIS

Modern Methods of Plant Analysis

Vol. V. Founded by K. Paech and M. V. Tracey, continued by H. F. Linskens and M. V. Tracey). Pp. xxvi+535. (Berlin—Göttingen—Heidelberg: Springer-Verlag, 1962.) DM. 98.

THIS fine work is a continuation of the series *Modern Methods of Plant Analysis*, which is already well known to most workers on plant biochemistry and metabolism. The untimely death of Prof. K. Paech robbed the series of its initiator and co-editor, but the new editorial combination of H. F. Linskens and M. V. Tracey has maintained in this fifth volume the high standards set by the earlier parts. It is now more than seven years since the previous volumes appeared, and in the meantime there have been many developments in techniques for dealing with plant materials. The present volume is intended to supplement and bring up to date the original Volume 1, which was concerned with analytical methods of general application. This aim has been admirably accomplished and the new volume fully justifies the description "Modern Methods".

So far as a single reviewer is able to judge, all the articles are of very high quality. Although they are principally concerned with practical details and applications of the techniques, sufficient theoretical background is included to permit intelligent use of the methods described. The newer techniques, now widely used by workers on plant biochemistry and metabolism, are treated in full. Thus D. J. David deals with "Emission and Atomic Absorption Spectrochemical Methods", K. Biemann with "Mass Spectrometric Methods", H. Moor with "Gefriertrocknung", and B. Breyer with "Polarography and Tensammetry". Chromatography and allied techniques are covered by articles on "Ion-exchange Chromatography" and "Molecular Sieving other than Dialysis" by N. K. Boardman, "Dünnschicht-Chromatographie" by E. Stahl, "Paper Chromatography on a Preparative Scale" by F. A. Hommes and H. F. Linskens, and "Vapour Phase Chromatography" by S. P. Burg. Other articles mainly concerned with macromolecules include "Determination of Size, Shape and Homogeneity of Macromolecules in Solution" by I. J. O'Donnell and E. F. Woods, "Optical Rotatory Dispersion. Its application to Protein Conformation" by E. F. Woods and I. J. O'Donnell, "Diffuse Röntgenkleinwinkelstreuung" by O. Kratky, and "Immunological Methods" by J. A. van der Veken, D. H. M. van Slogteren and J. P. H. van der Want. There are also more specialized articles by H. Prat on "Méthodes Calorimétriques pour l'Analyse les Végétaux", by A. E. Dimond on "Surface Factors affecting the Penetration of Compounds into Plants" and by J. V. Possingham and P. S. Davis on "Fallout Contamination in Plants".

Two articles in particular have a considerably wider field than that of plant analysis and will be of great interest to many plant physiologists. The contribution of W. L. Butler and K. H. Norris on "Plant Spectra: Absorption and Action" deals with light-scatter phenomena, absorption spectra, action spectra and fluorescence excitation spectra. This article includes an account of the discovery and properties of phytochrome; also the role of spectra in the investigation of photosynthesis. The concise but comprehensive account by A. C. Hildebrandt on "Tissue and Single Cell Cultures of Higher Plants as a Basic Experimental Method" reveals that this technique is a useful experimental method and no longer merely a botanical curiosity.

No laboratory concerned with plant biochemistry or physiology should be without this valuable book. Although it is a German publication, many workers on both sides of the Atlantic will benefit from the fact that of the eighteen articles fourteen are written in English. As a final comment, it should be said that the volume is beautifully printed and produced. A. Allsopp

DEVELOPMENT OF HEATH SOILS

The Development of British Heathlands and Their Soils

By G. W. Dimbleby. (Oxford Forestry Memoirs, No. 23.) Pp. 120+8 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 60*s*. net.

MANY thousands of acres in Britain have been planted with conifers by the Forestry Commission; most of these plantations are on heath soils where fertility is known to be low, yet the very existence of these trees depends on the maintenance of a minimal level of nutrients.

This *Memoir* investigates the development of heath soils at selected sites, chiefly in Yorkshire and in the vicinity of the New Forest, using as data soils which have been buried for varying periods by ancient earthworks. Archæological and other evidence is used to date these soils, and comparisons are made with soils of similar date from other parts of Europe. The main body of the work is followed by a substantial appendix in which detailed information on pollen spectra and soil characteristics from the 32 sites investigated form the experimental evidence for the conclusions.

In a brief discussion on methods, reference is made to a previous work by this author for the details of pollen analysis of relatively recent deposits. This is a highly controversial subject and it would not, I feel, have been out of place to have included some reference to the inherent weaknesses involved, such as the movement of pollen from one horizon to another and the variable resistance of pollen to bacterial and other action. A section on the development of soil profile indicates the various types of podzol which have been found at the different sites; the

author concludes that a certain amount of podzolization did, in fact, take place under forest, but much of the degradation is secondary. Of the possible degrading agents involved, climate is clearly important, as is vegetation cover, but rather conflicting evidence suggests that neither of these is of overriding importance, and the greatest influence seems to have been the nature of the parent material. A very interesting section is that in which the transition from forest to heath is related to various agencies and especially to the part played by Neolithic Man, though it is stressed that the use of fire by Mesolithic and possibly also by Palæolithic Man may have had a greater effect than is often realized. Some comments on the regeneration of degraded soils show that this relatively uncommon phenomenon is usually associated with a grass cover and, therefore, with agriculture, but the depth to which such regeneration occurs suggests that trees may also play an important part. Rather curiously, there is no mention of the extensive work by Ovington on the circulation of nutrients in conifer plantations, especially on the heaths of the Breck.

The author is clearly concerned that the policy of planting conifers on already degraded heath soils can result only in further degradation, though he stops short of stating what positive steps could be taken to overcome this. It is implicit in his general conclusions that some soils could become so exhausted that they would cease to support a continuous vegetation cover at all.

It seems a pity that a *Memoir* of this type could not be bound in a hard cover; but there can be no excuse for neither the title nor the author's name appearing on the spine. While the printing of the text is fully up to the standard expected of the Oxford University Press, the same cannot be said for the coloured plates. I sympathize with the author's contention that soil profiles are best illustrated by colour photographs, but the reproductions provided give only the most general impression of the various horizons.

R. B. Ivimey-Cook

VEGETATION AND HYDROLOGY

Vegetation and Hydrology

By Dr. H. L. Penman. (Technical Communication No. 53 of the Commonwealth Bureau of Soils, Harpenden). Pp. v+124. (Farnham Royal: Commonwealth Agricultural Bureaux, 1963.) 20*s*.

THE scientific problems underlying water conservation have been bedevilled by the diversity of interests and opinions pertaining to the hydrology of a land surface. The available evidence is often derived from technical investigations by agriculturists, foresters or water-engineers, working from basically different assumptions, while the approximate nature of the field techniques used has contributed greatly to the confusion. Hence this publication may be welcome, not only because it gives a survey of the pertinent literature, but also because it attempts to establish modes of approach to the underlying problems and to define them in general and scientific terms.

If climate, and especially rainfall, is determined by the movement of air masses, the only site factor that may control evaporation in the water-cycle is the vegetation cover. Assessments of the relative importance of this factor illustrate extremely well the insecurity of the premises on which alternative views depend and, in particular, the extreme differences in outlook which result from under-estimates of the importance of turbulence and of 'edge' effects in evaporation; the contrast, in short, between estimates based on small sample plots or potted plants, and those derived from a complete and extensive vegetation cover. The survey of the evidence suggests that decisive experiments in this field have not yet been achieved, so that the working hypothesis used in the final chapter may still be the safest approach. It is in essence Rakhmanov's conclusion for catchment area investigations that forest cover does not accelerate evaporation, which depends, therefore, mainly on weather. Dr. Penman leaves the data to speak for themselves. He has, however, indicated the arguments which must be applied to the meteorological approach, has dealt with the difficulties of accurate rain-gauging, the effects of vegetation in intercepting rain, and in increasing the infiltration of water while lessening run-off and soil erosion. He has given also a good discussion of the main problems involved in estimating potential evaporation. This is, therefore, much more than a technical summary, and is a work which will appeal to those with wider interests.

W. H. Pearsall

COMMONWEALTH UNIVERSITIES YEARBOOK

Commonwealth Universities Yearbook 1963

A Directory to the Universities of the British Commonwealth and the Handbook of their Association. Edited by J. F. Foster and T. Craig. Pp. xxxii+1826. (London: Association of Universities of the British Commonwealth, 1963.) 105*s*.; 15 dollars.

"EDUCATION may be said to be permanently in a state of transition and this is to the good. Without change there is no development; without change there is only decay." Thus wrote the reviewer of the second edition (1915) of what was then called *The Yearbook of the Universities of the Empire*, a directory compiled and published by the University Bureau of the British Empire (*Nature*, 96, 317; 1915). In the years that have followed there have been such great developments that the *Yearbook*, which was then 717 pages, dealt with 54 universities and affiliated colleges, and cost 7*s*. 6*d*., now, in its fortieth edition, extends to 1,826 pages, deals with 150 universities and affiliated colleges and costs 105*s*. Moreover the Bureau, founded in 1913, has very recently become the Association of Commonwealth Universities and the directory *The Commonwealth Universities Yearbook*. The fortieth edition thus appears in the Jubilee year of the founding body.

The *Yearbook* is published as a directory to university institutions in all countries of the Commonwealth and is not restricted to membership of the Association. Essentially it is divided up into 18 sections dealing with the countries of the Commonwealth. Each of the sections has a "national introduction", primarily intended as a functional guide for people who wish to attain a general background on university education in that particular country. Then follow the separate university entries and, department by department, their academic staff. At the end of each university section are given details of administrative and other staff, affiliated colleges and halls of residence and general information. In so doing the *Yearbook* fulfils the function of providing basic data to assist a student proposing to go to a Commonwealth country. In addition to the particulars, for example, of "first degrees" and "higher degrees", there is a detailed chart of "Subjects of Study".

In all there are seven appendixes. Appendixes 1 and 2 contain full details of the universities of the Republic of Ireland and the Republic of South Africa. Appendix 3 is a summary of the admission requirements of universities in Australia, Canada and the United Kingdom. Appendix 4 deals with information pertaining to students from other countries in United Kingdom universities. Appendix 5 deals with British academic institutions outside the Commonwealth, and Appendix 6 gives a short bibliography. Details of the Commonwealth scholarship and fellowship plan are dealt with in Appendix 7, and finally, Appendix 8 gives a full account of the Association of Universities of the British Commonwealth. There are excellent general and name indexes.

Man and Beast

By Major C. W. Hume. Pp. 222. (London : Universities Federation for Animal Welfare, 1962.) 15*s*.

THIS is a compilation, by Margaret Pattison, from the writings of Major Hume on matters of animal welfare, and is part of the campaign waged by the Universities Federation for Animal Welfare to ensure that the subject is brought to the attention of all concerned with the prevention of cruelty in general and with the use of animals for the purposes of man. It is well to provide in one small book the essentials of the arguments so well brought out over many years and many of the themes deserve the emphasis of repetition.

There is no doubt about the value of such a body as the Universities Federation. Even the most dedicated research worker can reasonably be reminded that he must constantly give consideration to the ethical and humanitarian aspects concerning animal experimentation. Human nature is such that constant reminders about responsibilities must be accepted. In the exploitation of domestic animals for human food, materials and for work the well-being of the animal is important. Habituation to the more unpleasant features of the necessary practices involved in the exploitation of animals can blunt the susceptibilities of the individual, and actual cruelty can be condoned.

In the past half-century or so much has been done in many countries to prevent suffering that can arise from certain practices. The compulsory use of anæsthesia for certain routine operations has done much in this direction. It is, however, well to emphasize that some legislation in this direction covered practices which a past generation of veterinary surgeons had accepted because animals had from time immemorial been given little consideration on matters related to pain; when enlightened members advocated the necessarily more elaborate procedures now used there had been some lack of support. When a strong trade union has an influence on a regular, seasonal, peripatetic procedure, such as sheep shearing, where profit is closely bound with rapid turnover of the operation and time an all-important factor in profit, insistence on rigid routine and on the necessary local facilities and labour ready for the visiting 'team', the animal concerned might receive less consideration than desirable. With the developments of 'factory' methods for the rearing of birds and animals for the table, where thousands and not tens may be the units, there was never greater need to emphasize that the individual animals have their rights.

This book will be an important reminder that humanity, as well as profit, is as desirable as ever. W. A. Pool

Rutherford at Manchester

Edited by J. B. Birks. Pp. x + 364 + 7 plates. (London: Heywood and Co., Ltd., 1962.) 60*s*.

THE fiftieth anniversary of the discovery of the nucleus by Rutherford and his collaborators in Manchester was celebrated in 1961 by an international conference. This volume records the historical portions of this conference in addition to much other material relating to Rutherford's Manchester period. About half the book is taken up with reprints of the papers by Rutherford, Bohr, Moseley and others which first reported these discoveries. Almost all the remainder consists of reminiscences by Rutherford's students and collaborators. The result is rather heterogeneous. but in the manner of a cinematic montage is quite effective in creating a human picture of the great physicist.

The 'cult of personality' in physics has always been prominent, and is not necessarily a bad thing. Indeed some physicists would say that progress in physics is from personality to personality. The tendency is bad, however, when the influence of one powerful personality holds back the emergence and recognition of the next. That Rutherford himself would have deplored any such effect is clear from a reading of this book. Though he enjoyed being a world figure and could be ruthless in getting results, yet it is clear that he also enjoyed nurturing and developing young physicists with fresh ideas and was completely fair in giving them full credit. Great physicists have not always shown such tolerance as they grew older and it is clear that Rutherford was a great man as well as a great physicist.

It is also clear that although Rutherford used rather simple and cheap apparatus for much of his work, he was delighted with every new technical development which opened up new experimental fields. He had no interest in technical developments as ends in themselves, but he still seized on the best equipment available for studying his interest of the moment.

This book is valuable not only for the factual history which it preserves but also for the inspiration and encouragement it may give to to-morrow's 'personalities' of physics. E. B. Paul

Atomic Theory and the Description of Nature

Four Essays, with an Introductory Survey. By Prof. Niels Bohr. First printed 1934. Pp. vii + 119. (Cambridge: At the University Press, 1961.) 8*s*. 6*d*. net; 1.65 dollars.

OF the four essays by the late Prof. N. Bohr two were originally published in *Nature* in 1925 and 1927, the third in the *Naturwissenschaften* and *Fysisk Tidsskrift* in 1929. First issued in one volume in 1934, they were reprinted in 1961 and a preface was added by Prof. Bohr. Most physicists will be grateful that this little volume is available again. The interest of the four articles is mainly due to the insight one can obtain of the fundamental changes of outlook introduced by the new quantum mechanics. By 1929 Dirac, Heisenberg, Schrodinger, Born and others had provided the mathematical formalism which made it possible to obtain the truly sensational results which the new theories provided. However, the necessity for "a radical departure from ordinary pictorial description and accustomed demands of physical explanations" was not accepted by all physicists (for example, Einstein).

The articles are free from mathematical formulæ, but nevertheless demand close attention to appreciate the wide sweep and the depth of the arguments; they ought to be made compulsory for the young theoretical physicists who are so often virtuosi in the manipulation of algebra (and proud of it) but occasionally shaky in deeper questions of what they are really doing. E. Bretscher

World List of Forensic Science Laboratories

Edited by Dr. D. Patterson. Pp. iii + 30. (London: Forensic Science Society, 107 Fenchurch Street, 1963.) 7*s*. 6*d*.; 1.50 dollars.

THIS excellent publication meets a long-felt need both by forensic scientists and by those who desire their services. It comprises 280 entries from 42 countries and lists such details as the title of each laboratory, its address and telephone number, and the name of its director. No attempt is made to define 'forensic science', but it is evident that the interpretation is wide. While it is not in any sense an 'approved' list, much of the information has been derived from official sources.

The very existence of a forensic 'Who's Who' is certain to foster greater co-operation internationally. Perhaps the first manifestation of this will be by way of the response to the Editor's invitation to assist in the preparation of an even more comprehensive list in due course. In this connexion, laboratories in Germany are conspicuous by their absence, and the Communist countries have but one representative—the Scientific Research Institute of Forensic Medicine, Moscow.

R. A. Outteridge

MECHANISM OF POLYRIBOSOME ACTION DURING PROTEIN SYNTHESIS

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POLYRIBOSOMES or polysomes are clusters of ribosomes which are held together by RNA. They can be seen in the electron microscope as linear arrays of ribosomes which are linked by thin strands, 10–15 Å in diameter[1,2]. Very low concentrations of ribonuclease convert these ribosomal clusters into single ribosomal units[3]. It has been shown that the polysome is the site of protein synthesis in investigations with reticulocytes[3,10], liver[4], HeLa cells[5], and slime moulds[6].

When polyribosomes in reticulocytes were initially discovered, it was assumed that the RNA holding the ribosomes together was the messenger RNA which contains the nucleotide sequence information necessary for the synthesis of the completed polypeptide chain[3]. This assumption was a natural outgrowth of previous experiments which showed that the synthetic messenger, polyuridylic acid, could form aggregates with bacterial ribosomes[7,8,8a]. Recent experiments in both normal and polio-infected HeLa cells have confirmed this initial assumption[5]. Reticulocytes manufacture primarily a single protein molecule, hæmoglobin. It has been shown that these polysomes are predominantly clusters of five ribosomes, although there is also a normal component of tetramers and hexamers in the cells[1]. This natural distribution was interpreted in terms of a mechanism whereby ribosomes become attached to one end of the linear polyribosomal cluster and then gradually move along the messenger strand while the polypeptide chain increases in length. At the end of the messenger chain the ribosomes are believed to detach and the polypeptide chain is released. Models of this type have also been considered by Gilbert[9], Gierer[10], and Noll *et al.*[11]. This mechanism provides a ready explanation for the naturally occurring distribution of polysomes in reticulocytes, that is, predominantly pentamers, with associated tetramers and hexamers, since random attachment at one end and detachment at the other end of the messenger strand would produce this distribution. Other evidence supporting an interpretation of this type was the observation made in electron micrographs that the ribosomes in the polyribosomal clusters are separated by gaps of 50–150 Å (refs. 1 and 2). This makes it unlikely that the polypeptide chains could be made by growing first on one ribosome and then somehow jumping from one to another to complete the chain.

A schematic model of this concept of polysome function is shown in Fig. 1. An oncoming ribosome without a polypeptide chain (diagonal shading) is shown attaching at one end of the polysome, while at the other end a ribosome with its completed polypeptide chain is released and the chain is freed. The diagram shows five ribosomes, which would be found in the case of a messenger RNA long enough to code for a 150 amino-acid polypeptide chain the size of hæmoglobin. However, similar models could be drawn involving longer messenger chains with more ribosomes which would be active in the synthesis of longer polypeptide chains.

In this article we describe three types of experiments which were designed to test this model of polyribosome function. In these experiments we used HeLa cells which contain a broad distribution of polyribosome size[5]. Electron micrograph investigations have shown that the larger clusters contain approximately forty ribosomes[12]. One of these experiments is a test of ribosomal attachment. Another demonstrates the release of polypeptide chains. The last shows that all polyribosomes are active in protein synthesis and discharge their ribosomes during *in vitro* incubation.

Ribosomal attachment experiments. HeLa cells were grown in suspension culture, and cytoplasmic extracts prepared by a slight modification of the method described by Penman *et al.*[5]. The cells were chilled, centrifuged, washed and re-suspended at 10^8 cells/ml. in a standard buffer (0·01 M *tris* buffer, *p*H 7·8, 5×10^{-3} magnesium acetate, 5×10^{-3} M potassium chloride and 6×10^{-3} M mercaptoethanol). The cells were then broken open with a Dounce homogenizer, and the nuclei and mitochondria removed by centrifugation (10 min at 4,500*g*).

For the ribosomal attachment experiments HeLa cells were grown for approximately one generation in medium supplemented with tritiated uridine. Tritiated single 74 *S* ribosomes were isolated as described in Fig. 2. During this isolation procedure, the single ribosomes were separated from polysomes by centrifuging them twice on sucrose gradients. Only the centre fractions from the 74 *S* peak were used.

In this experiment the tritiated ribosomes were added to an unlabelled cytoplasmic extract and incubated for 3 min under various conditions as shown in Fig. 2. Following this incubation, the materials were chilled, placed on a sucrose gradient, centrifuged and analysed for optical density and radioactivity. In Fig. 2 the direction of sedimentation is to the left. There is a sharp peak in both

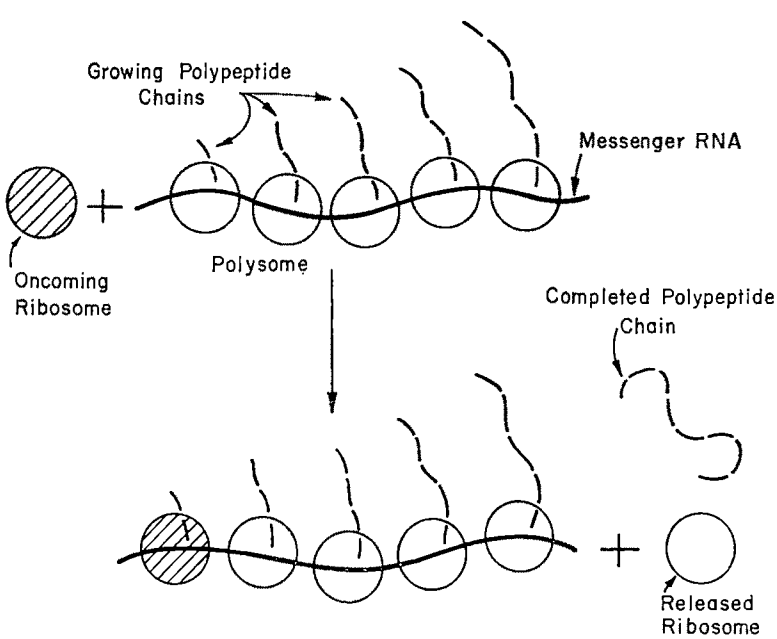


Fig. 1. A schematic model of polysome function

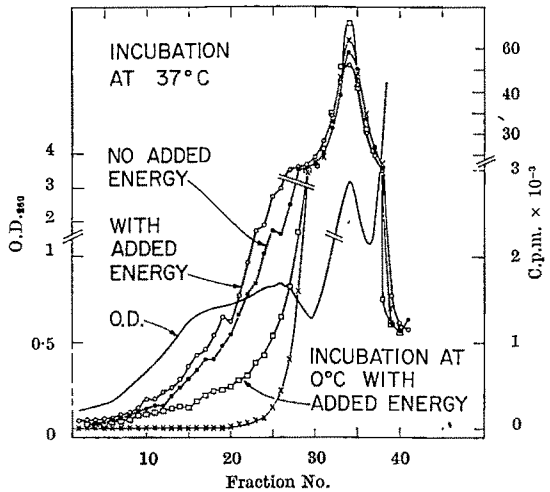


Fig. 2. Attachment of tritiated 74 *S* ribosomes to polysomes. 0·2 mg of 74 *S* tritiated ribosomes were added to 1·2 ml. of cytoplasmic extract and then incubated for 3 min. After incubation, the samples were chilled, and layered on a linear 15–30 per cent w/w sucrose gradient (containing 0·1 M *tris*, *p*H 7·8, 0·05 M potassium chloride and 0·005 M magnesium acetate). The material was centrifuged for 105 min at 25,000 r.p.m. in a Spinco *SW* 25 rotor. The bottom of the tube was punctured and the contents of the gradient were continuously analysed for optical density at 260 mμ in a Gilford spectrometer. Fractions were collected and counted in a scintillation counter. The curves are as follows: ribosomes alone (×), incubation at 37° C with no added energy (●), and incubation at 37° C with added energy (○), incubation at 0° C with added energy (□). Forty-one fractions were collected. The peak tube 34 is the 74 *S* ribosomes. The components added for energy were 2·5 μM/ml. ATP, 0·3 μM/ml. GTP, 12·5 μM/ml. phosphoenolpyruvic acid and 50 γ/ml. crystalline pyruvate kinase. The tritiated ribosomes were isolated by preparing cytoplasmic extract from cells grown with tritiated uridine (2·6 c./mM; 1 mc./ml. of culture medium) and then preincubating for 30 min to release single ribosomes from polysomes. The preincubation system contained 10 μM/ml. *tris* buffer (*p*H 7·8), 5 μM/ml. magnesium acetate, 5 μM/ml. potassium chloride, 6 μM/ml. mercaptoethanol, the energy system listed above and 0·07 μM/ml. each of 20 naturally occurring amino-acids. Following preincubation, the ribosomes were centrifuged (2 h, 105,000*g*), the pellet resuspended in unlabelled supernatant and then centrifuged for 325 min on a sucrose gradient. The 74 *S* peak was collected and dialysed overnight against standard buffer. The ribosomes were again centrifuged into a pellet and the 74 *S* fraction was again isolated on a sucrose gradient

optical density and radioactivity near tube 34, which is associated with single 74 *S* ribosomes[13]. The material to the left of this peak represents the polysomes. The optical density shows a maximum that sediments about 2·5 times as fast as the 74 *S* ribosomes. This maximum is a five ribosomal peak, and further down the gradient there is a long tail of optical density which represents polysomes of increasing length. This distribution is characteristic of a mammalian cell which is manufacturing a large number of different proteins[5]. The tritiated ribosomes alone sediment as a 74 *S* peak with a diffusion base, as indicated in Fig. 2. However, if the tritiated ribosomes are added to the cytoplasmic extract, it can be seen that some of the tritiated ribosomes now sediment with the polysomes. Incubation at 0° C results in some displacement of these ribosomes. However, incubation at 37° C in the cytoplasmic extract results in a considerable increase in the number of tritiated ribosomes which are attached to the polysomes, and the addition of more energy results in a further increase. These experiments show that the ribosomes have become attached during this short period of incubation so that they now can migrate through the sucrose gradient with the more rapidly sedimenting polysomes. In repetitions of this experiment, the addition of energy to the cytoplasmic extract resulted in up to a 100 per cent increase in the number of tritiated ribosomes attached as compared with the number attached in the absence of added energy.

Experiments of the type shown in Fig. 2 can be interpreted quantitatively. In particular, we can ask what is the relation between the number of attached labelled ribosomes and the number of polysomal or messenger RNA ends as a function of polysomal size. We can calculate the number of polyribosomal ends at any point in the gradient. As shown in the schematic diagram in Fig. 1, the number of available ends must be inversely proportional to the number of ribosomes found in the polysomal chain. We have been able to determine the number of ends in the following manner.

The optical density at any particular point in the sucrose gradient is a measure of the number of the ribosomes which are found in each fraction[13]. The position of a particular fraction in the sucrose gradient is related to the number of ribosomes in the polyribosome chain. This number has been determined in several ways. In some gradients it is possible to see fine structure or contours in the optical density profile which go out to as many as 10 or 12 ribosomal units. This method of counting is an extrapolation of the type of result which was described in the hæmoglobin polysomal cluster where the peaks of optical density were found associated with a fixed number of ribosomes as determined by electron microscopic examination[3]. In addition, it is possible to calculate an approximate sedimentation constant for polyribosomal clusters by assuming that they exist in the form of an ellipsoid of revolution[10]. Finally, the number of ribosomes have also been determined in HeLa gradients directly by electron microscope studies[12].

Thus knowing the number of ribosomes in a fraction and the length of the polyribosome chain, it is possible to plot a curve, as shown in Fig. 3, of the number of polyribosome ends as a function of the number of ribosomes in the polysome chain. This curve is shown in Fig. 3, together with a curve showing the number of ribosomes found in the various fractions. By using the known specific activity of tritiated ribosomes, we can interpret an experiment such as that shown in Fig. 2 in terms of the number of labelled ribosomes which are attached to the various polyribosomal clusters. These experimental points are shown by the solid circles in Fig. 3, and, with a suitable adjustment of ordinate, it can be seen that these points fall almost precisely on the calculated solid

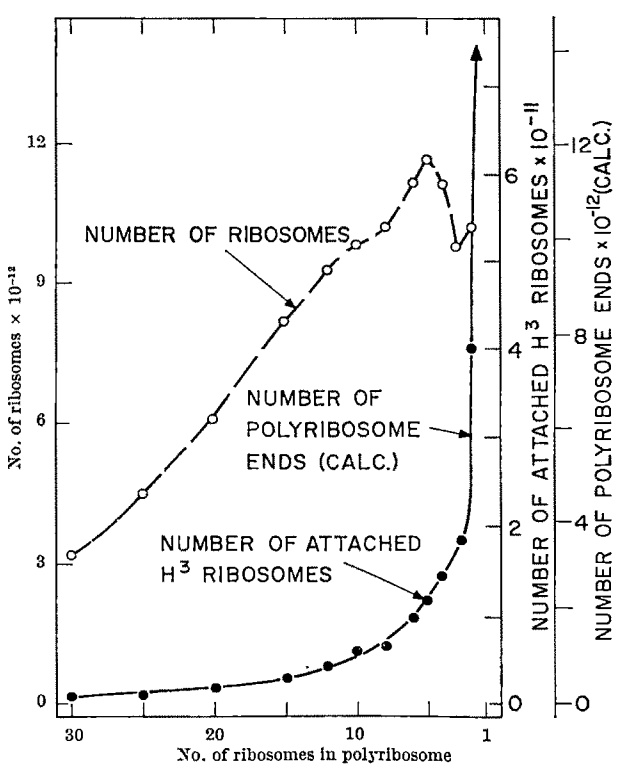


Fig. 3. The relation between the number of polyribosome or messenger RNA ends and the number of attached tritiated 74 *S* ribosomes after 2 min of incubation at 37° C in cytoplasmic extract with added energy. The dashed line shows the number of ribosomes in various fractions as a function of the size of the polyribosome. The solid line is the calculated number of ends and the solid circles are the experimental points

curve showing the distribution of polysome ends. In the experiment in Fig. 3, approximately 5 per cent of the polyribosome ends have a labelled ribosome attached to them after 2 min of incubation at 37° C in the presence of added energy. Since the number of labelled single ribosomes was approximately one-third the number of unlabelled single ribosomes in the cytoplasmic extract, it can be estimated that approximately 20 per cent of the polysomes have added a ribosome during this short time period. However, the concentration of both ribosomes and polyribosomes in the cytoplasmic extract is less than one-tenth that which prevails inside the cells *in vivo*. Therefore, it is quite likely that this amount of attachment represents only a small fraction of what is going on *in vivo* during the same time period.

The ribosome attachment experiments in Fig. 2 show that it is possible to obtain some attachment to a polysome at 0° C, even though there is no concomitant synthesis of a polypeptide chain at that temperature. Raising the temperature results in the synthesis of a polypeptide chain, and the attachment of more ribosomes. This suggests that the protein synthetic mechanism may be divided into two parts, one of which may involve the attachment of a ribosome to the end of the messenger RNA chain. This reaction can occur at 0° C even though the enzymatic reactions necessary for protein synthesis are not proceeding. In this regard it should be pointed out that ribosomes from *E. coli* have been shown to attach to polyuridylic acid at 0° C in a similar manner[8].

This experiment shows that the polysomes are able to have single ribosomes attached to them and, furthermore, the number attached is independent of the length of the polyribosome. This may be interpreted as indicating that all polysomes have the same number of attachment sites, since the number of attached ribosomes is proportional to the number of ends. Considering the polarity of the messenger RNA chain, we believe this indicates a single attachment site as shown in Fig. 1.

Protein detachment experiments. In order to demonstrate the detachment of ribosomes and release of completed polypeptide chains, another type of experiment was devised. The polyribosomes were labelled *in vivo* by exposing HeLa cells to a short pulse (1·5 min) of amino-acids labelled with carbon-14. Following this, the cytoplasmic extract was prepared, and the ribosomes and polyribosomes were then centrifuged into a pellet and the supernatant decanted. This pellet, with the attached labelled nascent protein, was re-suspended in an unlabelled supernatant of the original volume. Thus the system was reconstituted with unlabelled amino-acids and *s*RNA, and the only radioactivity present in the system was that which had been pelleted with the ribosomes.

This material was then incubated *in vitro* with energy at 37° C and samples were collected at various time periods, as shown in Fig. 4. At zero time it can be seen that most of the counts are associated with the polyribosomes. The fact that the optical density peak in the polysome region is different from that of unpelleted extract as shown in Fig. 2 indicates that the polysomes have suffered a certain amount of breakdown due to the pelleting and re-suspension[3].

Further evidence for this breakdown is a small peak of radioactivity in the single ribosome region. This peak would not be there unless there had been some breakdown. However, it is clear that the bulk of the counts are associated with the polysome region. As incubation proceeds, it can be seen that the radioactivity in the polysome peak gradually disappears and shifts to the right, that is, it is associated with smaller

Table 1. Distribution of Radioactive Protein during *in vitro* Incubation

Time (min)	Total counts	Counts in various fractions (per cent) Polyribosomes	Single ribosomes	Soluble protein
0	15,146	75	7	18
5′	14,276	58	11	31
10′	15,135	42	12	46
25′	17,158	18	14	68

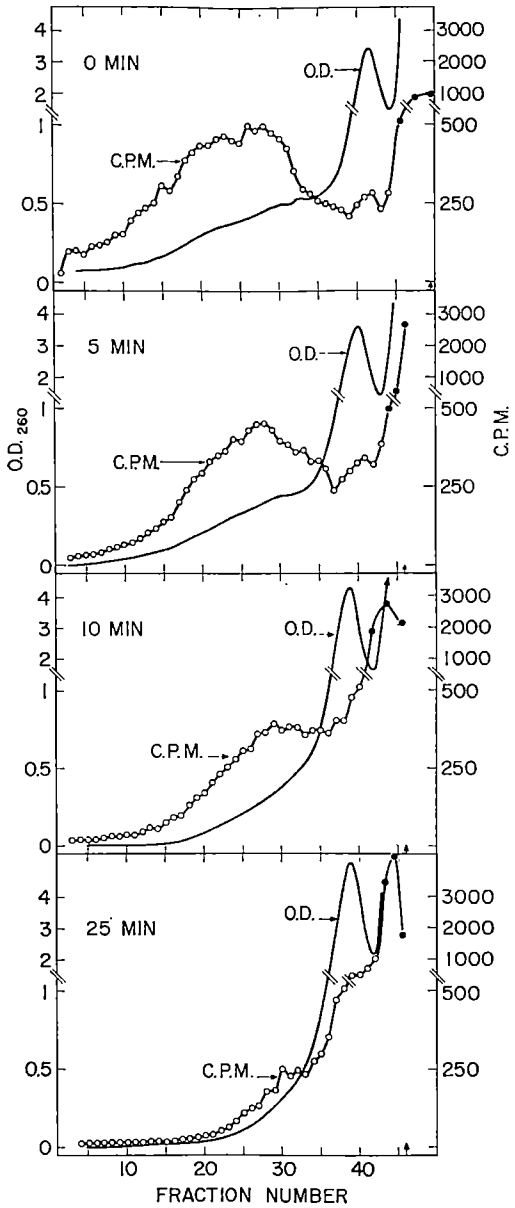


Fig. 4. Sucrose gradients showing the results of an *in vivo* labelling with ^{14}C-amino-acids followed by an unlabelled *in vitro* incubation of the ribosomes. One litre of logarithmically growing HeLa cells (4 × 10^5 cells) were labelled for 1·5 min with 100 μc. of ^{14}C-algal protein hydrolysate (1·6 mc./mg) as described previously (ref. 5). Cytoplasmic extract made from these cells was centrifuged and the pellet was resuspended in 6 ml. of unlabelled supernatant. After the addition of energy, the material was incubated at 37° C and 1·5 ml. samples were removed at 0, 5, 10 and 25 min for sucrose gradient analysis. The fractions were prepared for counting by the addition of 0·5 mg of serum albumin carrier protein and trichloroacetic acid was added to a final concentration of 5 per cent. The precipitate was plated on 'Millipore' filters and counted. The samples in the soluble protein region (●) were prepared for counting by a modification of the method of Siekevitz (ref. 15). The vertical arrow on the base-line indicates the last fraction

polyribosomes. However, accompanying this, is a substantial increase in the amount of labelled soluble protein which is found at the top of the gradient. The distribution of radioactivity during this incubation process is shown in Table 1. At the starting time 75 per cent of the counts are found in the polyribosomes and 18 per cent in the soluble protein (which is probably due to trace amounts of the radioactive supernatant in the pellet). The 7 per cent in the single ribosome peak is due to breakdown associated with sample manipulation. After incubation for 25 min, it can be seen that the polyribosome counts now have only 18 per cent of the total counts, while 68 per cent are found in the soluble protein. Thus

incubation under conditions which favour protein synthesis has resulted in a release of the bulk of the radioactivity associated with the ribosomes and their accumulation in soluble protein.

Earlier experiments with reticulocytes demonstrated that the effect of ribonuclease treatment is to bring about a quantitative transferal of both polysomes and their nascent protein radioactivity to the single ribosomal peak[3]. Thus the increase from 7 to 14 per cent of the counts associated with single ribosomes may be related to residual ribonuclease activity present in this system. However, we have carried out other experiments in an attempt to assay the amount of ribonuclease present in the cytoplasmic extract from HeLa cells. Polyuridylic acid labelled with carbon-14 was added to the incorporating system and its half-life determined during incubation at 37° C. In these experiments it was shown that the half-life is about 50 min. This is in marked contrast to the half-life of less than 1 min during incubation with an *E. coli* cell-free system[8]. Our experiments, therefore, suggest that there is little ribonuclease free in the HeLa system.

The gradual decrease in the size of the polysomes as the incubation proceeds suggests that the amount of attachment of single ribosomes to polysomes is perhaps much less than the amount of detachment. Thus during this incubation a polysome containing, for example, five ribosomes would gradually discharge individual ribosomes and polypeptide chains until finally it is left with the last ribosome attached to the messenger RNA in the process of completing its chain. It is quite likely that some of the radioactivity associated with the single ribosomal peak may be derived from this source.

These experiments demonstrate that *in vitro* incubation with an amino-acid incorporating system results in the liberation of radioactive polypeptide chains which were formed during an *in vivo* labelling. At the same time single ribosomes become detached from the polyribosomes. Results similar to these have been obtained with reticulocyte polysomes[14].

Amino-acid incorporation in vitro. Another kind of experiment has been carred out to show the effect of *in vitro* incubation on polyribosomes. In these experiments unlabelled cytoplasmic extract was made and to it were added amino-acids labelled with carbon-14 and a source of energy. Fig. 5 shows the time course for the incorporation of ^{14}C-amino-acids into protein both on the polyribosomes and in the entire extract. For the first 10 or 15 min incorporation is approximately linear. Following that,

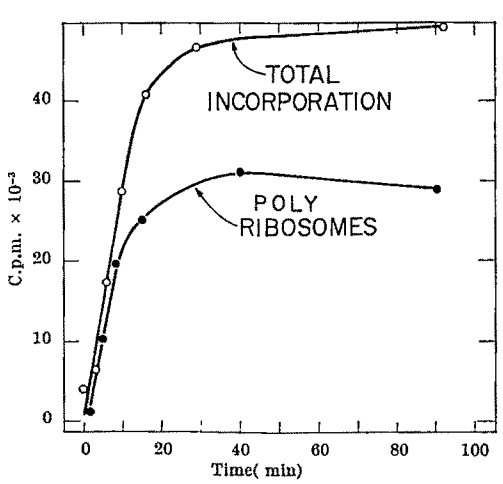
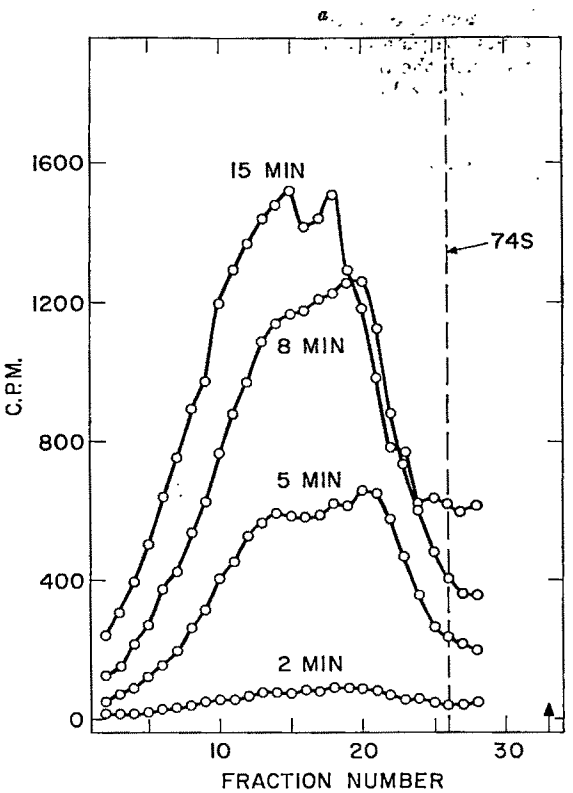


Fig. 5. Kinetics of ^{14}C-amino-acid incorporation into cytoplasmic extract. The incubation conditions were as in Fig. 2, except that 8 μc./ml. of ^{14}C-algal protein hydrolysate was added at zero time. 0·1 ml. samples were removed at various time periods, and the data is presented for the 1·5 ml. samples which were used in Fig. 6. The total incorporation was counted by the modified Siekevitz method (ref. 15). The polyribosome curve was obtained from the sucrose gradients shown in Fig. 6*a*



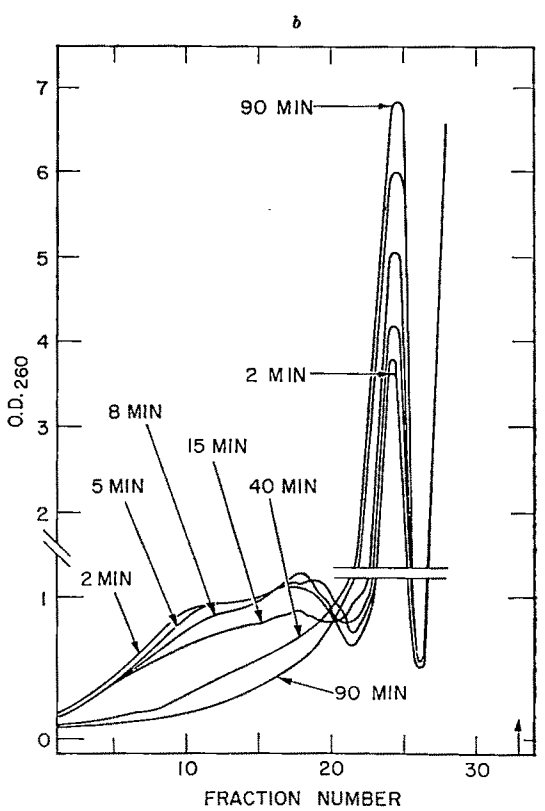


Fig. 6. Sucrose gradient analysis of *in vitro* amino-acid incorporation. The conditions for incorporation are as in Fig. 4. 1·5 ml. samples were removed at various time periods and analysed on the sucrose gradient as described previously. (*a*) The radioactivity associated with the polysome peak for various time periods. (*b*) The optical density of gradients at various time periods of *in vitro* incubation. The peak height in the 74 *S* region rises steadily with time from 2 min to 90 min

there is a decrease in the rate of incorporation of ^{14}C-amino-acids. A plateau is reached in the number of counts associated with the polyribosomes and the total incorporation also plateaux but at a higher level.

Samples were isolated at various intervals during this incubation, chilled and then placed on the sucrose density gradient. The optical density and radioactivity associated with these fractions is shown in Figs. 6*a* and *b*. In Fig. 6*a* it can be seen that the radioactivity is incorporated continuously throughout the entire polysome region. Thus all sizes of polysomes are active in protein synthesis in agreement with the attachment experiments which showed that ribosomes are attached to all the different lengths of the polysomes. In addition, it can be seen that the total radioactivity associated with the polysomes increases with time of incubation. However, a difference can be seen between incorporation into smaller polysomes in contrast to the incorporation into larger polysomes. Between 8 and 15 min the penta- and hexa-ribosomal units (fractions 17–18) incorporate somewhat less than the polysomes of length near 30 (fractions 5–6). This might be expected if the smaller polyribosomes are making smaller polypeptide chains. In the steady state these polyribosomes have less radioactivity than those making longer polypeptide chains. An observation similar to this has been made by Noll *et al.*[11] in their experiments with rat liver polyribosomes.

Fig. 6*b* shows the optical density profile for varying times of incubation. It can be seen that the effect of incubation is to bring about a gradual decrease in the size of polyribosomes as the incubation proceeds. The longer polyribosomes at the left-hand side of the gradient begin to decrease in number and associated with this is a gradual rise in the optical density of the monomer peak. It has been shown that this reaction is energy dependent. This type of result would be anticipated in an *in vitro* system in which the rate of attachment of new ribosomes to a polyribosome is much less than the rate of detachment. Thus the effect of incubation is to bring about a quantitative conversion of polyribosomes into single ribosomes. This effect has also been described with liver polyribosomes[11].

Thus, in summary, the demonstration of ribosomal attachment, polypeptide chain release and the release of ribosomes from polysomes during incubation are consistent with the general scheme of polysomal function during protein synthesis as outlined in Fig. 1.

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THE GALAXY AND THE MAGELLANIC CLOUDS

THE International Astronomical Union symposium No. 20 on "The Galaxy and the Magellanic Clouds" has recently been held in Australia—the first of the Union's symposia to be held in the southern hemisphere. It was co-sponsored by the International Astronomical Union and the Union Radio-Scientifique Internationale, and conducted under the auspices of the Australian Academy of Science. In some ways the symposium was a successor to the Groningen (1953) and Stockholm (1957) conferences on co-ordination of galactic research. However, the choice of papers placed special emphasis on southern problems in the Galaxy, particularly the nearby spiral arms, and the nature of the galactic centre region. Being the first major conference on the Magellanic Clouds, there was a feast of papers on the structure and evolution of our closest neighbour galaxies.

Thirty overseas astronomers, many with particular interests in the southern sky, accepted invitations to attend the symposium, together with twenty local delegates. The first week of the symposium (March 18–21) was held in Canberra and was devoted to galactic problems.

The proceedings began with an excellent review by J. H. Oort of our present knowledge of the large-scale structure of the Galaxy and of the many fundamental questions awaiting solution. Some of the major topics raised were the dynamics of the Galaxy, the structure, content and evolution of spiral arms, the distance to the galactic centre, the expansion of the gas found there, the nature of the galactic corona and the possible role of magnetic fields.

Many of the forty-nine papers presented concerned the concentration of different objects into spiral arms. Such concentrations are found for young galactic clusters (Becker), *O* and *B* stars (Petrie, Thackeray, Bok), long-period cepheids (Kraft and Schmidt), H II regions (Becker, Courtes), interstellar absorbing clouds (Thackeray), *A* stars (Lindblad, Bok) and early *M* stars (Westerlund). Objects of greater age—such as galactic clusters of spectral type later than *B*3, short-period cepheids, late *M* stars and planetary nebulæ (Perek)—show no concentration into arms, so that the life-time of a spiral feature appears to be of the order of 5×10^7 years. Detailed comparison of the distribution of objects of different ages promises to reveal the life-cycle of a spiral feature, and should indicate whether the stars disperse by their random motions or whether the motion of the gas differs systematically from the gravitational circular velocity. R. P. Kraft and M. Schmidt found that the older Cepheids tend to lie closer to the galactic centre than the Sun. As *OB* associations are found in greater numbers farther out than the Sun, there is the suggestion of an outward movement of the region of active star formation.

There was considerable discussion of the different locations of the H I concentrations within 3 or 4 kiloparsecs of the Sun and the segments of spiral arms delineated by optical indicators. The shape of the H I arms agrees roughly with those traced by the optical objects, and H. F. Weaver and others stressed that the disagreement in positions could arise in part from the use of photometric distances for stars and kinematic distances for H I regions. However, B. F. Burke reported a similar lack of correspondence for the southern part of *M*31. In his summarizing paper on galactic problems M. Schmidt gave a lucid exposition of the need for using similar methods for determining the distributions of objects before comparisons are made. He stressed the value of comparing longitude distributions quite apart from any distance

measures, particularly near tangential points. The strength of this method had been seen in the paper by B. J. Bok, showing the *OB* stars and clusters in the Carina arm clearly isolated in longitude, as B. Y. Mills found for the synchrotron emission. The longitude distribution is also a powerful means of delineating spiral structure from the 21-cm emission within the Sun's distance from the centre (Kerr).

An alternative comparison is that based directly on radial velocities, without resort to a rotational model to find distances. Cases were reported of good agreement between the velocities of H II regions, young stars and interstellar absorption lines (Courtes), between 21-cm absorption features and H II regions (Parijsky) and between 21-cm emission features and Ca II absorption lines (Thackeray). Another powerful, but little exploited, method is the ordering of objects in the line of sight by absorption measurements, both optical and radio.

There were a number of papers which increased confidence in the photometric distance scale. A. Blaauw's determination of the distance to the nearby Scorpio–Centaurus association gives an alternative method of finding the distance of the remote stars in H and χ Persei, and agrees within $\pm$ 0·27 magnitudes with the less-direct procedure starting from the Hyades. J. Graham also showed that H_β intensities yield excellent distances ($\pm$ 0·4 mag.) for *O* and early *B* stars, and could be applied to stars of 15 mag. using a telescope of only 50″ diameter.

The kinematic distances determined for H I concentrations rely heavily on our knowledge of galactic rotation and of the Sun's distance from the centre (R_0). The method will fail where there are large departures from circular motion. Maps of the neutral hydrogen spiral arms published by the Leyden and Sydney observers have been based on a value of the Oort constant *A* of 19·5 km/sec kiloparsec, and so a distance to the centre of 8·2 kiloparsec. For some time it has appeared that the photometric distance scale corresponds to an appreciably smaller value of *A*, and many separate determinations were discussed at the symposium. It seems probable that the value of *A* must be about 15 km/sec kiloparsec. Thus it is not surprising that large discrepancies are found between optical features and the published positions of the H I concentrations.

A new galactic rotation curve was presented by F. J. Kerr, based on high-resolution 21-cm measurements along the galactic equator. This confirmed the difference between the velocities for positive and negative longitudes indicated by broad beam surveys. The shape of the northern and southern velocity curves are found to be strikingly similar, suggesting that there are two major spiral arms or rings between the Sun and the centre, with a fine-structure that persists over a wide range of longitude. The north–south velocity asymmetry may reveal an outward motion of the material in the solar neighbourhood as previously suggested by Kerr. However, his latest results show that there is not a general expansion of the gas in the Galaxy, but that large-scale deviations from circular motion (up to 20 km/s) are present. R. M. Petrie's *B*-star velocities indicated strong local departures from circular motion, and a small outward motion of the material near the Sun. Non-circular motion over regions of 1 kiloparsec scale were also obvious in H. F. Weaver's discussion of the velocities of very-early-type stars.

The interpretation of the galactic rotation measurements depends on the solar motion. The accuracy of the standard solar motion was questioned by R. P. Kraft and M. Schmidt, and by O. J. Eggen.

Perhaps the most striking feature of the symposium was the wealth of information yielded by the large radio telescopes which are now in operation at Parkes in Australia, Green Bank in the United States and Nançay in France. F. J. Kerr's investigation of the galactic plane between $l^{II} = 60°$ and $l^{II} = -60°$ has already been mentioned. The work of B. F. Burke, K. C. Turner and M. H. Tuve between $l^{II} = 11°$ and $l^{II} = 50°$ also revealed the extreme complexity of structure of the H I spiral arms. These papers demonstrated that 21-cm investigations of the structure of the Galaxy are still in their infancy.

The high-resolution continuum measurements by E. R. Hill and M. M. Komesaroff at 1,410 Mc/s have yielded a notable improvement in our knowledge of radiation near the galactic plane. Many sources can be identified with H II regions in the *Mount Stromlo Atlas*. Many other sources previously regarded as extended are resolved into groups of discrete sources, which appear to be spread along spiral arms. It is striking that the distribution of the thermal radiation along the plane (for l^{II} between 280° and 354°) remains as a continuum background similar to that found with wide-beam surveys, rather than being resolved into many sources as found in the anticentre region. The very symmetrical distribution of the background thermal radiation about the centre agrees with the previous evidence for a ring of ionized hydrogen (or part thereof) 3·5 kiloparsec from the centre.

One of the challenging developments in radio astronomy has been the measurement of linear polarization of the galactic non-thermal (synchrotron) radiation. Measurements in Australia have confirmed the original Dutch observations of polarization in limited regions of the sky at 408 Mc/s. The recent measurements in Holland were reviewed by G. Westerhout. For polarized extragalactic sources at low galactic latitudes F. F. Gardner has measured considerable Faraday rotation, and a depolarization apparently associated with the fine structure in the electron distribution across the line of sight. These effects could explain the failure to observe polarized radiation from large areas of the galactic belt. For the regions where polarization is observed Gardner finds that the Faraday rotation is small, suggesting that they are very local.

There was only one paper on the fundamental subject of how the spiral structure of the galaxy persists. B. Lindblad demonstrated that the trailing arms produced by gravitational perturbations may become a quasi-steady formation by the circulation of matter between the outermost arms. This implies that the arms are quite massive and contain stars of population I of fairly high age, which calls for detailed investigation of the composition of a typical arm. Expectations that the spiral arms might be maintained against differential galactic rotation by hydromagnetic forces faded when R. D. Davies described the Zeeman splitting of 21-cm absorption features. Although the interpretation of the results was hotly debated, it became clear that the general galactic field is below 10^{-5} œrsted, too weak to resist the gravitational forces.

B. Y. Mills reviewed the evidence for the existence of a corona extending out to a radius of 10 kiloparsec from the centre. J. H. Oort reported observations of discrete H I clouds with large negative velocities at high latitudes, presumably located in the corona. The amount of gas in the corona might be 10 per cent of that in the galactic disk.

The second week of the symposium (March 25–28) was held in Sydney. The first day was devoted to the galactic centre, the majority of the papers discussing recent radio-astronomical measurements. A high-resolution survey at a wave-length of 10 cm by B. F. C. Cooper and R. M. Price showed the same complex of sources near the centre known from Drake's work at 3·75 cm. The central source appears to be mainly thermal in nature; the surrounding sources are partly non-thermal at 10 cm, the non-thermal emission becoming predominant at longer wave-lengths. This result was borne out by Y. N. Parijsky's observations at a number of wave-lengths between 3 and 33 cm. Considering the energy losses of relativistic particles Parijsky argued that the non-thermal emission and the gas flow from the centre are episodical in nature, arising from magneto-hydrodynamic instabilities.

J. H. Oort concluded that the neutral gas flow might be due to the explosion of a very large mass of gas in the galactic nucleus, if the 'explosion' lasted more than 50 million years. Oort's results showed that, despite the marked asymmetry of the gas distribution near the centre, almost equal masses of gas are found in the features in the south-east and north-west quadrants. B. F. Burke reported 21-cm observations over a wider range of longitude showing 3 or 4 distinct features in the moving gas near the centre, one being a branch on the 3 kiloparsec expanding arm. His measurements do not agree with a uniform expansion of the 3 kiloparsec arm. High resolution observations by F. J. Kerr show large H I density fluctuations along the 3 kiloparsec arm, the structure size being several hundred parsecs. Kerr's 21 cm absorption measurements show many small absorbing patches as well as the nearby hydrogen and the 3 kiloparsec arm. The rapidly rotating disk of Rougoor and Oort shows considerable fine structure. The only optical contribution to the session was G. Courtes' description of observations of H II regions near the centre with a high selectivity interference filter giving almost complete elimination of starlight.

The last three days of the symposium were devoted to the Magellanic Clouds, and were accompanied by far more active discussion of the twenty-nine papers than had marked the galactic sessions. In his introductory address, H. C. Arp pointed out that 66 per cent of galaxies may be dwarf galaxies like the Clouds, yet they contribute only about 0·3 per cent of the observed mass. Arp discussed the ways in which a double system like the Clouds might have evolved, the similarity of composition implied, and the possibility of a tenuous link between the Clouds and the Galaxy. The role of magnetic fields in the formation of the Clouds is most important, and calls for careful optical and radio measurements of polarization, particularly in the bar of the Large Cloud. The Clouds provide an unrivalled opportunity to locate the exact places where stars are now being formed.

The most striking results to emerge on the structure of the Clouds were the high resolution radio observations carried out in Australia. R. X. McGee showed that the neutral hydrogen in the Large Cloud can be resolved in position and radial velocity into many separate cloud complexes, on a low intensity background. The positions of these complexes correspond closely with H II regions and with young stellar associations. Some of the complexes have diameters over 500 pc, and masses up to 10^7 solar masses. Very little H I was found in the bar, in agreement with optical evidence that it contains a considerably older population than most other Large Cloud objects. In contrast with the H I complexes in the Large Magellanic Cloud, J. V. Hindman found only a few individual H I clouds in the Small Magellanic Cloud, loosely associated with H II regions. The gas is smoothly distributed over the main region of the Small Cloud, but shows a curious bifurcation in radial velocity. There appear to be five or six discrete gaseous bodies, two of them producing peaks in the 21-cm line profile separated by about 40 km/s. This suggests a large-scale separation of the components but, as pointed out by Oort, separation at such a low velocity could not last. Seeking an alternative explanation, Thackeray and Bolton suggested that self-absorption could produce the bifurcation, but this did not seem generally acceptable; Oort suggested that there is a complicated streaming of the gas in the system. A similar contrast was provided by G. Courtes' observations of H II regions, which had much filamentary structure in the Large Cloud and very regular distributions in the Small Cloud.

Radio continuum observations at 1,410 and 408 Mc/s were reported by D. S. Mathewson and J. R. Healey, showing that many discrete sources were present beside the 'background' radiation from the Clouds. In the Large Cloud 30 Doradus was very prominent and 90 per cent of the other discrete sources were shown to be thermal and identified with H II regions, many also corresponding to McGee's H I regions. One source in the Small Cloud was suggested as a supernova remnant.

B. E. Westerlund reported on the distribution of planetary nebulæ, of *M* stars and carbon stars detected with an infra-red objective prism, and of 400 clusters in the outer part of the Large Magellanic Cloud. The carbon stars show a tendency towards clustering and avoid regions of *OB* stars. Westerlund also found that the number of Wolf–Rayet stars in the Large Cloud is proportionally greater than in the Small Cloud or the Galaxy, about half occurring in associations. The distribution of these very young objects gives an important guide to the processes of stellar evolution in the Clouds.

The basic problem of the elimination of foreground stars of intermediate colour has been resolved by several different methods. Using objective prism techniques, Ch. Fehrenbach has identified more than a hundred members of the Large Cloud by their high radial velocities, although A. D. Thackeray pointed out that a few Population II stars in the Galaxy will have high reflected velocities and need to be eliminated by a luminosity classification. Sir Richard Woolley eliminated foreground stars by their proper motions determined from the chance discovery of a deep astrographic plate taken in 1912. Th. and J. H. Walraven have also had considerable success in identifying Cloud members by means of accurate multi-colour photometry. By such elimination of foreground stars meaningful colour-magnitude arrays can be obtained.

Colour-magnitude arrays were presented for a number of clusters in the Clouds. B. J. Bok discussed the isolated young association *NGC* 1929–37 in the Large Cloud, containing 97 stars with $M \leqslant -4{\cdot}0$ contributing 4,000 solar masses to a total mass of less than $2{\cdot}4 \times 10^4$ solar masses. The association contains much ionized hydrogen and is embedded in a concentration of neutral hydrogen. The total mass of H I is about 5×10^6 solar masses (McGee) and could give rise to several hundred more generations of bright stars. We may here find some of the vital answers to the problems of stellar formation. The deficiency of red supergiants or other evolved stars suggests that the association is not stable, but radial velocities are not yet available to check this. B. E. Westerlund investigated clusters such as *NGC* 602 in the 'wing' of the Small Cloud, finding that the wing is generally made up of clusters of blue stars. During a search for a second population in the Large Cloud Sir Richard Woolley found a faint open cluster of intermediate age, demonstrating the need for investigation of the numerous less-conspicuous clusters in the Clouds.

V. A. Ambartsumian suggested that 30 Doradus should be regarded as a 'super-association', a special class which he has found in a number of super-giant galaxies.

W. G. Tifft discussed the old-population stars found in the Small Cloud halo. The red cluster *NGC* 121 has a colour-magnitude array quite similar to that for galactic globular clusters, but stars of this age are rare in the Clouds. In the inner halo globular-like clusters such as *NGC* 419 of intermediate age are common. A. D. Thackeray commented on the dearth of RR Lyrae variables in Cloud clusters. S. C. B. Gascoigne discussed the colour-magnitude arrays for six red, globular-like clusters in the Clouds. All these contained bluer stars than are found in galactic globular clusters or in *NGC* 121, and most have ages of about 10^9 years. It appears that clusters of all ages are present, but that the main phase of star formation in the Clouds began much later than in the Galaxy.

Long-standing arguments about differences in the metal abundances of the brightest stars in the Galaxy and the Large Cloud were resolved during the symposium by work such as Feast's on *NGC* 330. Agreement was reached that the abundances are the same. D. J. Faulkner produced evidence that the helium/hydrogen ratio in Large and Small Cloud emission regions was 25 per cent

lower than in the solar neighbourhood, while L. H. Aller suggested that the oxygen/hydrogen and neon/hydrogen ratios were also slightly lower. The near equivalence of the element abundances in the Clouds and the Galaxy is somewhat surprising in view of the more advanced evolutionary state of the Galaxy.

S. C. B. Gascoigne presented observations of 14 Small Cloud Cepheids and 13 Large Cloud Cepheids. In the Small Cloud the slope of the period-luminosity relation is steeper than Arp's, while in the Large Cloud it is lower than that found by the observers at the Royal Greenwich Observatory. The small difference in slope might be due to a difference in reddening. It appears unlikely that the Small Cloud Cepheids are actually redder than those in the Galaxy.

M. W. Feast has derived a rotation curve for the Large Cloud from the radial velocities of 40 emission nebulæ. He finds that the position of the major axis agrees with that suggested by de Vaucouleurs, and that the rotation curve is close to that found for the inner regions from 21-cm measurements. There can be no doubt that the rotation centre lies 1° away from the centre of the bar, posing the problem of how the bar has been maintained despite differential rotation.

The symposium was closed by a thorough review of Magellanic Cloud problems by A. D. Thackeray, followed by a session of most active free discussion.

The publication of abstracts of papers before the symposium was one of the many activities of the organizing committee under B. J. Bok which contributed to the success of the symposium. The proceedings will be published at the end of the year by the Australian Academy of Science and the International Astronomical Union, and edited by F. J. Kerr and A. W. Rodgers.

Notable among the visits made during the symposium were those to the 210-ft. radio telescope operated by the Commonwealth Scientific and Industrial Research Organization at Parkes and the optical interferometer of the Universities of Sydney and Manchester at Narrabri. An aerial tour of inspection was made of the field station of Mount Stromlo Observatory on Siding Spring Mountain near Coonabarabran. Other visits were made to Mount Stromlo Observatory, the site of the University of Sydney's Mills Cross near Canberra and the Sydney Observatory.

The symposium was marked by the fruitful combination of radio and optical observations. It was fitting that it should have been held in Australia, where both these branches of astronomy are thriving. Many of the visitors were impressed by the number of young astronomers in the country, and stressed the need for a large optical telescope to assure the development of the subject, to complement the major radio telescopes and to exploit the astronomical potentialities of the Southern Milky Way and the Magellanic Clouds. B. J. Robinson

STRATOSPHERIC PARTICLES AT 20 KM

By S. C. MOSSOP

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RECENT investigations by Junge *et al.*[1–3] have demonstrated the presence of a widespread, persistent layer of particles between altitudes 15 and 25 km. The maximum particle concentration, which occurs at about 20 km, was found to be approximately 0·1 per cm³ and varied only by a factor of 3 among the dozen or so measurements made at various times and latitudes. The particles range in diameter from about 4 to 0·2μ, the lower limit being set by collection methods used. The particles are hygroscopic and consist mainly of sulphate. The altitude distribution of the sulphate points to a stratospheric origin, and Junge suggests that it is formed by the action of ozone on sulphur-bearing gases carried up from the Earth's surface. He has shown that the stratospheric aerosol contains, in addition to sulphur, the elements aluminium, silicon, potassium, calcium and iron.

Friend and Sherwood[4] have found that more than 90 per cent of the total mass of the stratospheric aerosol consists of ammonium persulphate and ammonium sulphate. Eight collections at an altitude of 20 km yielded an average concentration of these particles of 1·1 per cm³ for diameters of 0·16μ and above, the variation being 0·1–3·6. These values depend heavily on extrapolation to the smallest sizes and may be in error by a factor of 5.

Because of this omnipresent layer of sulphate particles, Junge and Manson[3] point out the difficulty of detecting extra-terrestrial particles in this size-range, at altitudes below 25 km.

Higher-altitude collection has since been accomplished by Hemenway and Soberman[5], who report on the examination of micrometeorites recovered by a sounding rocket from heights of over 80 km. These particles they classify as spheres, irregular particles with rounded surfaces, and 'fluffy' irregular particles which appear to be aggregates of much smaller nodular particles each about 0·1μ diameter. Observed clusters of such smaller particles on the collecting surface suggest that some aggregates break up on impact. The particles examined by Hemenway and Soberman generally lie between 0·1 and 1μ diameter, though irregular particles up to 40μ in length are illustrated. A few results of analysis of particles are reported. It is also stated that 'fluffy' particles of about 1μ in greatest dimension have been found in collections made in the lower stratosphere from *U*-2 aircraft.

Particles collected at an altitude of 20 km over Australia by exposing electron-microscope grids covered with a nitrocellulose film in the airstream from a *U*-2 aircraft have been examined by the present author. These consist almost exclusively of hygroscopic particles of the size and concentration reported by Junge *et al.* A typical collection is illustrated in Fig. 1. It has been confirmed that ammonium sulphate is a major constituent of these particles. Within the aggregations of water-soluble material are small, dense, insoluble particles many of which are undoubtedly of extra-terrestrial origin. These are of such a size that most of them would escape collec-

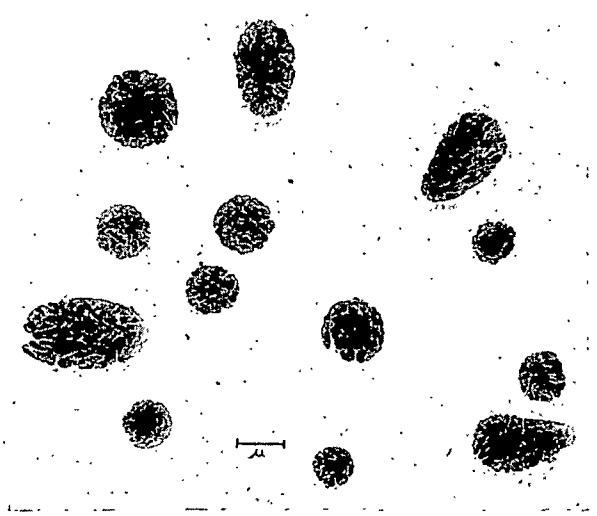


Fig. 1. Electron micrograph of atmospheric particles collected at an altitude of 20 km, February 13, 1963

tion if they were not encased in the much greater mass of sulphate.

In order to reveal these insoluble particles for examination it is first necessary to remove most of the soluble material by floating the specimen grids on purified water, collecting face upward. Dialysis for a suitable length of time leaves only the insolubles surrounded by a residual ring which establishes the position of the original material, as shown in Fig. 2. Occasional rings are devoid of insoluble particles; but as particles of the expected appearance are found on the film nearby, it is believed that each aggregate of soluble material contains at least one insoluble particle. This has been confirmed by exposing several collections of stratospheric particles to an intense electron beam: the sulphate evaporates, leaving behind at least one stable particle for each 'parent' particle.

The flattened shape of the parent particles leads one to believe that they must have been moist when collected: impact occasionally gives rise to a ring of small satellite particles round each main one. The insoluble particles presumably assist the nucleation of the soluble solids which are deposited on them from gaseous reactions. In rare cases the soluble deposit is thin enough for the enclosed dense particles to be visible without dialysis: an example is shown in Fig. 3. This represents a case where the insoluble particles have had a comparatively short sojourn between 15 and 25 km.

Eight collections of particles made at 20 km between latitudes of 20° and 45° S. over the period January–May, 1963, show concentrations of parent particles varying only from 0·04 to 0·09 per cm³ with an average of 0·06. These values are corrected for the theoretical collection efficiency of the sampling probe.

The insoluble particles left behind after dialysis fall into the following categories:

(1) Electron-dense, irregular particles with nodular surfaces. The particles in Fig. 3 are of this type.

(2) Highly irregular, less electron-dense particles which resemble, in all but size, the 'fluffy' particles of Hemenway and Soberman. Their appearance is consistent with formation by the break-up of such aggregates.

(3) Electron-dense spheres and spheroids of diameters 0·5–4μ were found in the collection of May 7, 1963.



Fig. 2. Residue after dialysis of water-soluble material from collection of January 31, 1963

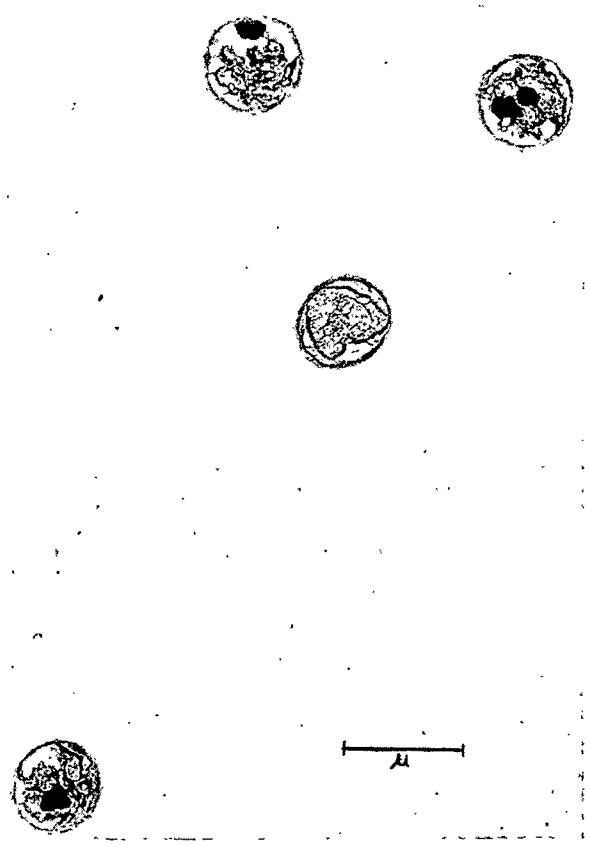


Fig. 3. Particles collected on March 12, 1963, in which denser inclusions are visible even without dialysis

(4) The collection of April 11, 1963, and subsequent collections contain comparatively large angular particles quite different in appearance to the usual types of insoluble particles present. These are undoubtedly volcanic dust from the eruption of Mount Agung, Bali, on March 17. The southward progression of vivid sunsets after the eruption and their persistence, point to the presence of considerable quantities of volcanic dust in the stratosphere at the time and place of these collections.

From the similarity in appearance of particles in categories 1–3 to those found by Hemenway and Soberman above 80 km, it is thought likely that they are extraterrestrial in origin. The particles of the first two categories range in size from 0·02 to 0·5μ with a median value of 0·08. The number found within each soluble particle may be as high as 8, but the average appears fairly constant from one occasion to another at about 2. It thus appears that the average concentration of such particles at an altitude of 20 km is at least 0·1 per cm³. This refers only to those particles which have sojourned at altitudes below about 25 km long enough to grow by deposition of sulphates to a diameter greater than 0·1μ, the theoretical cut-off limit for the collection system used. To collect smaller, newly arrived particles it is obviously necessary to sample well above 25 km.

I thank the personnel of *O.L.*11, 4080th Strategic Reconnaissance Wing, U.S. Air Force, for carrying and operating the particle-sampling apparatus on their aircraft. I also thank the Sydney University Electron Microscopy Unit for electron microscopy and in particular Mr. C. Shorey for his assistance.

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FACTORS DETERMINING THE BEHAVIOUR OF PLANT PATHOGENS IN SOIL

AN international symposium on factors determining the behaviour of plant pathogens in soil, sponsored by the Agricultural Board of the National Academy of Sciences—National Research Council and supported by grants from the National Institutes of Health, National Science Foundation and Agricultural Research Service, was held during April 7–13, at the University of California, Berkeley.

The symposium was organized by W. C. Snyder (chairman), K. F. Baker (co-chairman), R. R. Baker, F. E. Clark, A. W. Dimock, W. A. Kreutzer, J. D. Menzies, L. I. Miller and Z. A. Patrick. There were 35 invited papers, and 300 active workers in this field were asked to participate in the meetings.

Fungi, bacteria, viruses and nematodes, either free-living or dependent on plant roots and stems in soil, cause plant diseases resulting in enormous annual losses in food production. Control of these diseases has been pursued, using a variety of biological and chemical methods with some outstanding successes. 'Take all' of wheat caused by the fungus *Ophiobolus graminis*, once a major threat in most wheat-growing areas of the world, is effectively controlled by rotation of crops. 'Wilt' diseases of many crops caused by the *Fusarium* fungus are partly controlled by crop management and the use of resistant varieties. 'Bunt' of wheat is now almost completely controlled by pretreating seed with chemicals before planting. *Phymatotrichum* root rot of cotton, 'scab' of potato, *Sclerotium* rot of peanut, and diseases of several important tropical crops are being successfully controlled by regulating the activity of the pathogens through manipulation of the organic matter in soil.

These and the many other effective controls already established have been developed, partly by empirical testing methods, but based on an understanding of the behaviour of the causal organisms in soil in relation to the host plants. However, for many important soil-borne diseases affecting the world's crops there is still no satisfactory method of control. Empirical testing of control methods has either failed or resulted in a baffling mixture of success and failure at different times or in different places.

Basic investigations on the behaviour of micro-organisms in soil has expanded rapidly in the past twenty-five years. Concomitant with this expansion, both as cause and effect, is the growing understanding of the soil as a complex environment in which the biological entities, the higher plants, soil microflora and fauna interact with each other and with the physical and chemical factors. Thus for the plant pathologist interested ultimately in the control of economic diseases, important information is now forthcoming, not only from his own basic studies on disease organisms, but also from the fields of plant anatomy and plant physiology, biochemistry, soil physics, soil chemistry, genetics and soil microbiology.

This symposium brought together for the first time leading workers in these fields in a most successful attempt to integrate the present information, to determine the most promising new approaches to biological control of diseases and to define the more important areas in which investigations were urgently needed.

S. D. Garrett sketched the broad background of the symposium in the introductory paper, "Toward Biological Control of Soil-Borne Plant Pathogens". He outlined the broad patterns for decreasing the population or activity of pathogens in soil by introduction of antagonistic micro-organisms to soil, or the modification of the soil environment by the use of added organic matter, the growing of particular crops or selective chemical and physical treatments. He discussed in detail the necessity for critical experimentation on, and analysis of, the present views on soil populations and the factors influencing them.

The remaining papers of the symposium may be considered in four sections.

The six papers following Garrett by A. N. Burges, D. K. McE. Kevan, J. H. Warcup, J. M. Hirst, D. Park and A. S. Sussman dealt with the present background knowledge of the soil microflora and fauna, their distribution, growth and survival. Kevan's comprehensive coverage of the soil microfauna disclosed our ignorance of the relation between these animals and the general soil microflora, but he suggested there may be some possibility of nematode control by other members of the soil fauna.

Burges and Warcup were complementary in that, while both of them stressed the importance of methods for population determination, the former dealt in detail with the complicated ecological succession involving fungi, bacteria and animals in specific situations in soil. Warcup discussed the broad range of fungi occurring in soils, stressing particularly groups such as the Basidiomycetes which had not been adequately sampled by previous methods, and he then considered the activity of the organisms in relation to substrate. Hirst's discussion of dispersal of soil micro-organisms emphasized the neglected evidence that they do disperse, but in the majority of cases at a very slow rate. His broad approach will undoubtedly stimulate more precise experimentation along these lines and his discussion of the importance of resting structures in relation to dispersal in time effectively linked his paper with those of Park on survival and Sussman on dormancy. These last-named speakers surveyed the known factors such as antibiosis, competition for nutrients and adverse soil physical or chemical effects on survival structures of organisms, and the importance of dormancy in these same structures.

The second section of the symposium, consisting of papers by W. A. Raney, H. D. Chapman, Flora M. Scott, H. Burström, A. D. Rovira, H. Katznelson and D. J. D. Nicholas, dealt with the influence of physical and chemical factors of the soil and the presence of higher plants on the microbiological population. Physical factors outlined by Raney have scarcely been investigated for their influence on soil micro-organisms although there are abundant suggestions of their importance in the plant pathology literature. There is more detailed evidence of the influence of chemical factors to the point of partial control of a disease such as *Thielaviopsis* root rot of citrus. The detailed papers by Scott on root anatomy and Burström on root physiology brought most of the symposium participants to a common meeting ground for the first time. The rationalization of root structure and function with each other and with the evidence on root exudates and root infection provided a lively debate. Rovira and Katznelson discussed in detail the nature of the root exudates and their influence on soil micro-organisms, particularly bacteria. Their presentations led to considerable discussion on the importance of these phenomena and the use of model systems in their further investigation. Nicholas extended the incidental remarks of the two previous speakers in a discussion of the rhizosphere influence on plant nutrition in which he considered the supply of and competition for many minerals by both the micro-organisms and the higher plants.

The third section of the symposium, with papers by J. L. Harley, C. H. Cadman, N. T. Flentje, I. W. Budden-

hagen, W. B. Mountain, J. C. Walker, I. A. M. Cruickshank and P. S. Nutman, dealt with host infection, mechanisms of pathogenesis and host resistance. Cadman reviewed the present knowledge of fungi and nematodes as virus vectors and put forward tentative groupings of the soil-borne viruses in relation to either their known or probable characteristics. Mountain comprehensively reviewed the present knowledge on nematode infection of higher plants and subsequent mechanisms of pathogenesis. Nutman, in a detailed discussion of *Rhizobium* legume symbiosis, outlined the mechanism of root-hair invasion by the bacteria and indicated different stages where the invasion or subsequent symbiosis breaks down; he postulated physiological reasons for the breakdown.

Harley and Flentje discussed the infection of plants by fungi and the contrasts between mycorrhizal fungi causing little destruction of host tissue and the recognized pathogens where enzymatic attack or metabolite toxicity causes death or serious malfunction of host tissue. Buddenhagen extended the discussions by the earlier speakers in this section on the evolution of pathogenicity and presented a stimulating discussion of population dynamics of bacteria in relation to survival in soil and spread from one host to another. Walker, in his discussion of disease resistance, reminded the symposium that while we have long-standing examples of disease resistance we still have not found any basic pattern linking mechanisms of pathogenesis with the genetic information on resistance. Cruickshank, however, in discussing the role of phytoalexins, suggested that antifungal materials formed by hosts in response to infection may be an important aspect of disease resistance.

The final section, consisting of papers by F. E. Clark, R. A. Fox, R. M. Jackson, M. G. Boosalis, R. Mankau, R. R. Baker, A. E. Dimond and J. G. Horsfall, Mary D. Glynne, Z. A. Patrick and T. A. Toussoun, T. S. Sadasivan, R. A. Ludwig, G. W. F. Sewell, W. A. Kreutzer and S. Wilhelm, dealt with a range of factors influencing the population of saprophytes and pathogens in soil and the use of these factors in biological control of disease.

Clark outlined the possible factors in soil making for competition between organisms, urging much clearer thinking about the concept of competition. Jackson dealt with the general question of antibiosis in soil, suggesting there was strong presumptive evidence for antibiosis as a factor in competition, but indicating there was still no clear picture of the origin of antibiotics in soil or the factors influencing their presence. Boosalis and Mankau stressed the fact that while parasitism and predation between micro-organisms is well known in laboratory culture there is still little direct information on its occurrence in natural soil. Baker and Dimond linked these discussions on antagonism with their consideration of 'inoculum potential'. Inoculum potential is not a static concept of population of an organism but a dynamic concept of population, biological energy and infectivity. Dimond and Horsfall set out a mathematical basis for estimating the several aspects of inoculum potential.

Fox and Mary Glynne discussed the severity of diseases in Malaya and Britain in relation to long-term crop management, where in both instances disease control has been effective. The associated microbiological investigations in each case are giving new insight into the reasons for these successes. Patrick and Toussoun presented evidence that organic residues so often regarded as beneficial by increasing antagonism of other organisms to pathogens can often be harmful because of toxic materials produced. Ludwig described similar effects from the use of chemicals in soil which in some cases had been dramatically successful but in other cases made the position worse. Kreutzer carried this analysis further, indicating that soil in which most of the organisms were killed was an excellent substrate for re-colonization by potential pathogens, suggesting the most effective treatment was one specific for the particular pathogen. Sadasivan dealt with the effects of mineral nutrition on disease control and presented some interesting results from the use of foliar application of different minerals. Sewell emphasized the fact that although temperature effects on soil organisms and disease were well known because they were easy to study, the effects of oxygen/carbon dioxide relations and other factors were probably of equal importance in influencing pathogen population and activity in soil.

Wilhelm concluded the formal papers with a comprehensive analysis of the biological balance in natural soil. He stressed the fact that the microbial complexity of soil maintains a natural balance which keeps a wide range of disease organisms in check. Our primary task is to swing further the balance against disease organisms.

The formal review of each day's papers by J. D. Menzies, A. Kerr, J. E. DeVay, G. C. Papavizas and A. W. Dimock gave a valuable progressive synthesis of the discussions.

There are sufficient examples of successful biological control of diseases to indicate that concentrated basic investigations in this field would yield important economic results. But there are important gaps in our knowledge that frustrate our present attempts to achieve this. These gaps became obvious during the symposium discussions.

Our knowledge of the soil microflora and fauna is still fragmentary, limited on one hand by the inadequate techniques so far developed and on the other by the immensity of the task. We must, as a basis for much of our future work, intensify our general ecological studies on the soil micro-organisms.

Microbial competition in soil was a question dominating much of the symposium discussions. Is it competition for oxygen, water, space, nutrient supply, or is it escape from or resistance to antibiotics? Clark's provocative analysis went almost unchallenged because the present research fragments are insufficient to make an informative picture. Yet this is one major prop to the working concept of biological control. How can we systematically and effectively increase competition by addition of organisms to soil, by alteration of the physical or chemical factors of the soil unless we clearly define the nature of such competition?

The host plant as a factor influencing the chemical environment of the soil or as a substrate for infection was also a dominating feature of the conference. Extensive debate on the detailed physiology and anatomy of roots, the nature of the root surface, the detail of secondary root emergence revealed our needs for further information on critical aspects. Composition of root exudates is now well documented and there was strong opinion that our future studies in this field should be directed into two main channels. What components of root exudates are most active in influencing the sections of the microflora antagonistic to pathogens? What components of root exudates directly influence the infection of roots by pathogens? There is already evidence that foliar sprays and other treatments can influence root exudates and this line of investigation holds considerable promise for disease control. Such investigations are already meeting other investigations on the detailed analysis of plant infection in relation to the anatomy and physiology of the root. The place of model systems in such investigations was warmly debated, the crux of the debate being the relation between an artificial model system and the natural conditions in the soil, a further reflexion of our present fragmentary knowledge of the ecology of soil organisms and the influence of chemical and physical factors in the soil.

Throughout the symposium the variability of the soil micro-organisms continually intruded into discussion. How applicable are experimental results with one race of an organism to another race? Where such races have been recognized and defined the problems are recognized, but this represents the minority of cases. Wider recognition of race differences in relation to survival in soil and

pathogenicity to different hosts, investigation of the responsible mechanisms of variation and the background genetics of these organisms is essential.

The vigorous discussions during the symposium will undoubtedly influence programmes of work already in progress and shape the framework of much of the future planning. This was its purpose and the indications are that it was eminently successful. Equally, certainly it encouraged our hope for significant extension of the already successful biological control of plant diseases.

The benefit and encouragement from this symposium was expressed in a resolution, endorsed unanimously by the participants: "that a second symposium, along somewhat similar lines, should be held possibly in Britain, in five years time". A steering committee was selected at the symposium to investigate this possibility.

The proceedings of the symposium, including papers, session reviews and general discussion will be published in full. The volume should serve as a valuable reference text in this field. N. T. FLENTJE

TEACHING OF BIOLOGY

AN interim report of the Royal Society—Institute of Biology Joint Committee on the Teaching of Biology has recently been issued.

The Committee has made itself acquainted with a large amount of work being carried out by numerous bodies at present interesting themselves in the improvement of biological teaching.

It feels strongly that biology should not be taught solely as a vocational training, but should take its proper place as a powerful instrument of education for life in the modern world. It has taken particular note of the Biology Section of the Nuffield Foundation's Science Teaching project and its work in devising teaching aids for the age-range 11–16.

Biology in the Sixth Form: University Entrance. The Committee has been informed of the views of professors of botany and zoology on desirable qualifications for entrance to university biology departments. A majority of each expresses a preference for a combination of biology and chemistry at the Advanced Level of the General Certificate of Education with supporting qualifications in physics and mathematics. A willingness to accept other combinations has been indicated, and the more traditional one of botany, zoology and chemistry is preferred by a minority. The Committee agrees that means should be devised to secure the maintenance of a minority stream of 'natural history' biologists through the universities.

Biology Syllabuses. In accordance with the trend already noted many examining bodies are at present producing or revising syllabuses for biology as a single subject at advanced level. The Committee notes with regret that no co-ordination has been attempted between the different examining bodies and that needlessly wide divergencies are arising which will create serious difficulties for sixth-form biology teaching

Relations between Sixth Form and University Biology Teaching. The connexion between school and university teaching is the weakest link in the present chain. It requires careful study with a close co-ordination of the views of those engaged on both school and university aspects of the problem. The Committee proposes to set up a small study-group to obtain the necessary data which should enable it to make proposals within a reasonable time for a rational solution of this critical problem.

Teachers' Refresher Courses. Individual university biology departments, usually with the co-operation of departments of education, have provided valuable help to school teachers in the form of vacation courses of about a week's duration. These have been well supported and much appreciated; but it is thought that not more than 10 per cent of the demand is at present met. To satisfy the demand fully would place an unmanageable burden on the university departments. It has been suggested that university and technical college departments should be asked to provide short (one-day or week-end) courses for teachers from schools in their own neighbourhoods. A sampling inquiry to about a dozen departments has shown much willingness on their part to co-operate. The Committee therefore proposes to circularize biology departments in universities and technical colleges throughout the country with the hope of promoting a nation-wide network of such help for teachers.

Curriculum Studies. A panel of the Joint Committee met and discussed the American Biological Sciences Curriculum Study courses with a number of educationists who have recently visited American schools.

As a result of their discussions it is not felt possible to adapt these courses to British conditions, both because of the considerable differences of suitable biological material, and because of the great divergences of the American and British educational systems into which the biology has to be fitted. The publications associated with the courses are being sent to Britain, and may have their uses as teachers' source books (see *Nature*, **197**, 4; 1963). It does not seem necessary either to encourage or to discourage their entry.

A pamphlet setting out the aims of the Biological Sciences Curriculum Study project and its relevance to British conditions is being prepared and will be published in the Institute of Biology Journal.

Pamphlets on Current Topics. The Committee has welcomed a proposal from the Institute of Biology to publish on a subscription basis a series of booklets on subjects of biological interest yet falling outside the normal text-book range or up-to-dateness. It has undertaken to advise on the choice of topics and a number of titles are under consideration.

OBITUARIES

Prof. F. W. Jane

THE sudden death of Prof. Frank W. Jane on May 6 in Ibadan where, with the permission of the Council of Royal Holloway College, he was acting as temporary professor and head of the Department of Botany in the University of Ibadan, leaves a gap which is going to be very hard to fill. Indeed, because he was such a modest man, it is only now that one realizes in how many different spheres he had achieved distinction and how widely he will be missed.

First and foremost he was a botanist and naturalist. Much of his early research work, while he was at University College as assistant lecturer, lecturer and then reader, dealt with freshwater algae. The results were published between 1937 and 1944 in such journals as the *Transactions of the Hertfordshire Natural History Society* (1937, 1938,

1939), the *New Phytologist* (1941, 1944) and the *Journal of the Royal Microscopical Society* (1944). These include detailed descriptions of *Pteromonas varians*, *Cyclonexis erinus* and *Chlororhabdion diogenes* (all new species) and a revision of the genus *Harpochytrium*. They are a tribute to his excellent microscopy and meticulous technique and brought him the award of a D.Sc. in 1944. Although recently he had little time for active research on algae, his interest in it remained. There was generally at least one research student in the Department of Botany at Royal Holloway College working on an algal topic under his supervision, most recently in relation to the growth of algae in reservoirs. This led to a grant from the Department of Scientific and Industrial Research to promote such research and a close connexion with the Metropolitan Water Board.

From 1949, when he was appointed to the chair of botany at Royal Holloway College (University of London), much of his time and energy went on administration and in work on timber and trees. His book, *The Structure of Wood*, published by Black in 1955 and which he had almost finished revising, is the most authoritative treatment of the subject. It is used not only by academic students but also by students of timber technology. Indeed, he was one of the country's leading authorities on trees. Coupled with his fair-mindedness and impartial judgment this gave him the standing of an expert in lawsuits involving trees and timber and brought him much advisory work on tree management in estates and towns. He had a close connexion with the Institute of Wood Science and the Timber Research and Development Association.

The Botanical Supply Unit of the University of London, started in 1950 at Englefield Green, owes a great deal to his vision and drive. Under his wise chairmanship it has expanded from three to twenty acres and is now a Botanic Garden in which he will long be remembered and one of which the University may justly feel proud.

It was as a naturalist, ornithologist and photographer that Jane spent any leisure he allowed himself, and to his students and colleagues he was a delightful and inspiring companion on any field excursion. It is characteristic of him that here too he made himself an expert. Among other things, he served on the Field Studies Council, had been president of the School Nature Union and the Essex Field Club and was chairman of the local Committee of management of Blakeney Point for the National Trust. In the new booklet of Blakeney and Scolt Head, about to be issued, he was responsible for the sections on Birds, Flowering Plants and Ecology. He was a perfectionist in his photography and used his own coloured slides to illustrate the many stimulating talks he gave to biological and natural history societies throughout the country.

From the age of fifteen, when he left school, he had to work to earn his academic education and he took his degree as a part-time student at Birkbeck College. It was perhaps the memory of these early days that made him so sympathetic to anyone who wanted to learn and gave him such a practical interest in education at all levels. As chief examiner and later moderator in the advanced level of the General Certificate of Education in botany he was able to influence the teaching of botany and biology in schools. He was similarly involved in biological education at training colleges and was particularly interested in the problems raised by the expansion of their course from two to three years. He did invaluable work in planning the training of science laboratory technicians, among whom he numbered some of his personal friends. He was a vice-president and former president of the Institute of Science Technology as well as chairman of the University of London Committee on the Training of Technicians. Unlike some professors, he was always ready to encourage and supervise part-time research students. He will be remembered in Africa particularly for his services to the Departments of Botany of those overseas colleges in special relation with the University of London to which he paid many visits and gave much advice. Their appreciation is summed up by one of them in a letter which says his loss will be felt in no fewer than six of the developing countries, and that it is perhaps symbolic that he should have died in Africa.

His untimely death at the age of sixty-two, at the height of his powers, will be mourned by the many people who have benefited from his help and encouragement. We extend our sympathy to his wife and daughter.

Margaret A. P. Madge

Dr. H. S. Holden, C.B.E.

After graduating with honours in botany from the University of Manchester, H. S. Holden was appointed a lecturer and demonstrator in botany in University College, Nottingham, in 1909. At that time the College occupied buildings in Shakespeare Street. Even in those early days, Harry Holden was a stimulating personality. He initiated and directed vigorous research work in the Department. He was a gifted exponent and, looking back, it seems quite natural that he should have taken an active part in the Workers' Educational Association Extension courses run by and from the College.

He always had a deep interest in micro-organisms and during the First World War served as bacteriologist at the Plymouth Naval Hospital. On his return to University College, Nottingham, he took up his general teaching duties and the advancement of research work. His own particular interests lay in the field of plant anatomy and in microbiology, and this dual interest is reflected in the range of work on the anatomy of plants, on fungi, and on bacteria which came from the Department in subsequent years.

About 1921 he was awarded the degree of D.Sc. of the University of Manchester for work on seedling structure. In 1927 he was appointed senior lecturer in botany and head of the Sub-Department of Industrial Bacteriology. In the years preceding 1928 plans were made to build at Highfields a new University College, and Holden assumed much of the responsibility for the planning of the Department of Biology. On the retirement of Prof. Carr, Holden was appointed head of the Department of Biology in 1928. The growth of this Department in subsequent years and increases in the staff resulted in the re-alignment of Holden's duties, and in 1932 he became professor of botany and head of the Biology Department, and finally in 1934 professor of botany and head of the Botany Department.

His early anatomical studies led him to an interest in palæobotany, particularly of Coal Measure plants, and he collaborated with Dr. D. H. Scott in a series of anatomical studies of fossil plants. He was responsible for the purchase by the University College of the D. H. Scott collection of reprints, which consists of a wide range of pamphlets dealing with all branches of botany.

Holden was a man of great enthusiasm, blessed with an enquiring mind which led him into paths not at that time recognized as the precincts of botanists or biologists. Thus, he was consulted on various occasions by local police authorities in connexion with cases of water pollution. His wide knowledge of microscopy and of plant structure, even in its most minute details, led quite naturally it seems now to the development of applied microscopy and of scientific methods in the solution of crime. His early teaching experience in Nottingham was in the old College in Shakespeare Street. This may have been a portent of the future, because when the East Midland Forensic Science Laboratory was established in 1936 he became its first director. This Laboratory finally occupied well-planned and well-equipped premises in Shakespeare Street, not far from the old College. I believe this Laboratory was the first of its kind in the country, and it is not surprising that some ten years

[redacted]he Forensic Science Service, Holden [redacted]f the Metropolitan Police Laboratory [redacted] Yard.

[redacted]his interest in micro-organisms, it was [redacted]al that even when he became director of [redacted] Science Laboratories in Nottingham he still [redacted] contacts with industrial firms in the neigh- [redacted] and with the discovery of the importance of [redacted]n the early years of the War he was instru- [redacted] bringing together in a Nottingham laboratory [redacted]onsiderable collection of cultures of species of [redacted]*um*.

[redacted]ve vivid recollections of my first meeting with [redacted] Holden in the Department of Botany, University [redacted]asgow, round about 1923. On that day he was sitting [redacted]e assistants' room inking in drawings of a fossil stem [redacted]n the Kidston collection. I was captivated by the [redacted]eness of his draftsmanship, and by the speed with [redacted]hich he worked. He kept up a running fire of com- [redacted]mentary during the whole of the period, and I had a foretaste then of what has remained with me as an abiding memory of Dr. Holden, his immense vitality and deep interest in botany, and his rare genius for communicating with anyone his unbounded enthusiasm. He was a man who could not tolerate inactivity and slackness and who spent his energies in the service of biology, particularly of botany, without stint. He was gifted with considerable organizing ability, and was quick to see and to seize advantages in situations as they presented themselves.

There can be no doubt that Holden exemplified scientific versatility to an amazing degree. His success in the forensic science field bears witness to this, but after a long and active career in this service it gave pleasure to many of his friends that he returned to his palæobotany studies in the last few years of his life, and brought to these the same enthusiasm and vigour which so marked him in his days in the Department of Botany, University College, Nottingham.

C. G. C. CHESTERS

NEWS and VIEWS

Biochemistry at Sheffield: Prof. W. Bartley

DR. W. BARTLEY, who has been appointed to succeed Prof. Quentin H. Gibson in the chair of biochemistry at the University of Sheffield (see *Nature*, 198, 939; 1963), returns to the University where he graduated in 1950 with first-class honours in physiology. In the same year he joined the Cell Metabolism Research Unit of the Medical Research Council in the Department of Biochemistry at Sheffield, and in 1954, on the appointment of the director of the Unit, Prof. H. A. (now Sir Hans) Krebs, to the Whitley professorship of biochemistry at Oxford, he moved with the Unit to the Biochemistry Department at Oxford. In 1959 he transferred to the staff of the Oxford Biochemistry Department as a University demonstrator. His main research interests concern the biochemistry of mitochondria, a field in which he has made important contributions distinguished by careful and systematic experimentation. His interests include the elucidation of the part played by lipids in structure and function in living cells, and generally the relations between structure and biochemical function. He has built up a reputation as an enthusiastic undergraduate and postgraduate teacher who may confidently be expected to add to the distinguished record of the Biochemistry Department at Sheffield.

The 1963 Guggenheim International Astronautics Award: Prof. M. Nicolet

PROF. MARCEL NICOLET, director of the Centre National de Recherches de l'Espace in Belgium, has been awarded the 1963 Daniel and Florence Guggenheim International Astronautics Award. The Award, which carries with it a prize of 1,000 dollars, is offered annually to an individual who has made outstanding contributions to the progress of astronautics during the preceding five years. Prof. Nicolet is well known for his achievements in the fields of aeronomy and planetary atmospheres. He was the first to point out correctly the effect of sunlight and diffusion in modifying the composition of the upper atmospheres of Earth and planets. This work enabled him to predict in the planetary upper-atmospheres the existence of a helium belt which was later shown by space probes to be correct. He was awarded the Triennial Prize of the Agathon De Potter Foundation of the Belgian Royal Academy of Sciences and became a member of the Academy in 1962. For seven years he was secretary-general of the Special Committee of the International Geophysical Year.

The Nuclear Science and Engineering Corporation, Pittsburgh, Pa.

Mr. Francis S. McMichael and Mr. Frank G. Chambers have been elected to the Board of Directors of the Nuclear Science and Engineering Corporation.

F. S. McMichael

Mr. Francis S. McMichael is a vice-president of Mellon National Bank and Trust Co. He is a director of the Jeannette Glass Co., the Keystone Box Co., South Hills Ornamental Iron Co., Division of Mulach Steel Corporation and the Pryce Machine and Manufacturing Co. He is also a member of the Allegheny County Bar Association, the American Society of Planning Officials, the Pennsylvania Planning Association and the Mt. Lebanon Planning Commission.

F. G. Chambers

Mr. Frank G. Chambers is president and a director of the Continental Capital Corporation, a small business investment corporation in San Francisco. He is chairman of the Board of Hazleton-Nuclear Science Corporation, the western affiliate of the Nuclear Science and Engineering Corporation. He is also a director of Maydwell and Hartzell, Guardian Paper Co., Kimball Manufacturing Co. and the *Journal of Commercial Art*. He served as director of the Office of Priorities and Controls, Munitions Board of the U.S. Defense Department.

The Nuclear Science and Engineering Corporation was formed in Pittsburgh, Pa., immediately after passage of the Atomic Energy Act of 1954. It conducts research and development, utilizing nuclear and radioactive tracer techniques on production and research problems of industry and Government. It also provides specialized radioactivity and radiation measurement services, and is considered a leading producer of radioactive isotopes. The four technical departments of the firm include physical sciences, biomedical isotope applications, radiation biology and radioactive materials.

The Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme : Awards

AWARDS under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows: Dr. M. Adhikari, lecturer in applied chemistry, University of Calcutta, to enable him to work on physico-chemical properties of clays and clay membranes at the Imperial College of Science and Technology, London, from July to December 1963; Dr. A. K. Chandra, lec-

turer in chemistry, University of Calcutta, to enable him to carry out research in quantum chemistry at the Mathematical Institute, University of Oxford, for six months from October 1963; Prof. J. N. Chatterjea, professor of organic chemistry, Patna University, to enable him to study fungal metabolites at the School of Pharmacy, London, for six months from September 1963; Dr. E. U. Emovon, lecturer in chemistry, University of Ibadan, to assist him to learn further techniques in gas phase kinetics and photochemistry at the University College of Wales, Aberystwyth, for six months in 1963; Dr. D. M. Moore, lecturer in botany, University of Leicester, to assist him to visit the Falkland Islands and possibly South Georgia for about three months from January 1964 to collect flowering plants there, in connexion with a study of evolutionary and phytogeographical relationships of circum-Atlantic floras; Dr. H. M. Pantin, geologist at the New Zealand Oceanographic Institute, Wellington, to assist him to investigate the small-scale internal structures of marine sediments from the New Zealand area and from the region of the British Isles, at Bristol, for a year from October 1963; Prof. W. G. Spector, professor of pathology, St. Bartholomew's Hospital Medical College, to enable him to carry out an electron microscopic study of certain aspects of the inflammatory reaction, at the University of Melbourne, for three months from December 1963; Dr. C. W. Stearn, associate professor of geological sciences, McGill University, Montreal, to assist him to visit the United Kingdom during February–April 1964, for an examination of Nicholson's collection of Stromatoporoids in the British Museum; Dr. J. R. Turvey, lecturer in chemistry, University College of North Wales, Bangor, to enable him to study new techniques for investigating the structures of glycoproteins at Queen's University, Kingston, Ontario, from July to September 1963; Dr. E. H. Wright, lecturer in chemistry, Fourah Bay College, University College of Sierra Leone, to enable him to study recent developments in surface chemistry at the University of Bristol for three months from September 1963.

The Paul Instrument Fund Award

THE Paul Instrument Fund Committee has made grants as follows: £7,485 and £7,836 to Prof. H. M. Barlow, Pender professor of electrical engineering, University College, London, (i) for the development of a microwave electrostatic wattmeter, and (ii) for the construction of instruments for the measurement of electrical power at very high frequencies by the absorption of the angular momentum of a circularly polarized wave; £4,970 to Prof. P. B. Moon, Poynting professor of physics in the University of Birmingham, for the construction of a linear motion gamma-ray spectrometer; £3,000 to Dr. G. Munday, lecturer in chemical engineering and chemical technology, Imperial College of Science and Technology, London, for the design and construction of a camera having frame rates up to 200,000 p.p.s., capable of synchronization and being a true framing device having automatic shutter facilities, to be used for further experiments on the dynamic deflexion of disks; £950 to Mr. K. G. Nichols, lecturer in electronics, University of Southampton, for the construction of apparatus giving an improved method for the vacuum deposition of thin films of high melting-point materials; £1,800 to Dr. E. E. Schneider, reader in solid state physics, King's College, Newcastle upon Tyne, for the development of superconducting cavities for use in magnetic resonance spectrometers; £730, as a supplementary grant, to Dr. P. M. B. Walker, lecturer in zoology, University of Edinburgh, for the improvement of the microspectrophotometer which he constructed by means of previous grants amounting to £9,730. The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British research workers in Great Britain f design, construction and maintenance or much improved types of physical i apparatus for investigations in pure or ap science".

The Australian Marine Sciences Association

THE inaugural meeting of the Australi Sciences Association was held at Cronulla, N Wales, on May 18 and was attended by 43 o founder members. The objects of the Associa "to promote the marine sciences in Australia provide for the exchange of ideas and information b those concerned with marine science". It was a that ordinary membership should consist of "pe who are or have been engaged in research in a branc marine science and such other persons who seek mem ship whom the Council deems could contribute to t objects of the Association". The first Council of th Association consists of: *President*, Dr. G. L. Kesteven; *Vice-President*, Prof. W. Stephenson; *Secretary*, Dr. J. M. Thomson; *Treasurer*, Dr. J. C. Yaldwyn; *Councillors*, Miss J. Hope MacPherson, Dr. E. P. Hodgkin and Dr. H. B. S. Womersley.

At the associated congress held on May 19 the following papers were presented: G. F. Humphrey, "The Organization of the C.S.I.R.O. Division of Fisheries and Oceanography"; H. B. Wisely, "Fouling Tests and the Rearing of Pelagic Larvæ"; D. J. Tranter, "Zooplankton Abundance in Australian Waters"; D. Vaux, "Fisheries Hydrology in South-eastern Australia"; J. P. Robins, "Water Type Preference of Tuna"; G. L. Kesteven, "Marine Stock Assessment"; J. M. Thomson, "Counting Salmon"; W. G. H. Maxwell, "A Review of Marine Geological Research in Australia"; H. B. S. Womersley, "A Review of Marine Botanical Research in Australia"; J. T. Baker, "The Chemistry of Some Marine Purple Pigments"; D. F. McMichael, "The Australian Museum's Swains Reef Expedition".

Further information concerning the Association can be obtained from the Secretary at P.O. Box 21, Cronulla, New South Wales.

The Arthur D. Little Research Institute

THE annual report for 1962 of the Arthur D. Little Research Institute records a broadening of activities in physics, including initiation of a basic study of the interface between metals and molten glass, and re-orientation of the work of the High Polymer Group, whose work has been primarily concerned with the crystallization behaviour of polymers with the view of obtaining control of the structure and properties of the final product (Pp. 44. Inveresk, Midlothian: Arthur D. Little Research Institute. 1963). Reduction in spherulite size in a given sample of polyethylene on cooling from the melt has been achieved through the discovery of the process of 'seeded' crystallization. An investigation has also been initiated into the mechano-chemical modification of co-polymers. The biochemical and biophysical work on glutens has pointed to interesting properties of gluten-based films, and work has commenced on the utilization of humic acids from peat and anti-tubercular factors in milk whey. New techniques have been devised for preparing 'tailor-made' polyamides and an investigation commenced on carrageenan, the mixture of water-extractable polysaccharide sulphates of certain species of red seaweeds. Investigations continued on the chemical modification of wood and the emission of corrosive volatiles from wood, as well as the chemical modification of cotton fabric to give increased crease-resistance. The nature of the impurities which cause spontaneous polymerization of ethylene sulphide has been determined, and when these impurities are avoided ethylene sulphide has been kept at room temperature for six weeks without deterioration. Work

continued on its reaction with amines and with mercaptans. Investigations in the hydrogen embrittlement of certain steels continued, and also of the reaction between molten aluminium and water vapour. The total staff at December 31, 1962, was 51, including 25 graduates, and a list of publications is included.

Employment of Graduates

THE annual report of the University of Birmingham Appointments Board for the year ended December 31, 1962, records a more selective attitude from employers (Pp. 6. Birmingham: The University, 1963). However, the anxiety of many firms not to become inbred is regarded as having prevented a squeeze on graduates with low honours or pass degrees, and competition is regarded as undoubtedly growing at this end of the academic scale. Of the 804 who obtained first degrees in 1962, 762 registered, of whom 413 men and 33 women were in science, compared with 407 and 37, respectively, in 1961. Of the graduates in science and technology, 6·3 per cent took teacher training and 2·3 per cent teaching posts in schools. Of 199 men who obtained first degrees in science in 1961–62, 83 took up research work or further academic study at home and 6 overseas, 30 teacher training and 70 gained employment by December 1962; for 113 with higher degrees the corresponding figures are: 18, 22, 2 and 51; for 214 men with first degrees in technology, the corresponding figures are: 63, 4, 4, and 127; and of 123 with higher degrees in technology, 7 took up research work or further academic study at home and 72 had gained employment by December 1962.

The Carnegie Institution of Washington

A MOST attractive illustrated brochure issued by the Carnegie Institution of Washington describes concisely but clearly some of the achievements during the past sixty years of the Mount Wilson and Palomar Observatories, the Geophysical Laboratory, the Department of Terrestrial Magnetism, the Department of Plant Biology, the Department of Embryology, and of the Genetics Research Unit. The brochure shows very clearly the character of the Institution's work, which is now concentrated mainly in three broad fields: the form, contents and dynamics of the universe; the structure and evolution of the Earth; and the frontiers of biology (Pp. 20. Washington, D.C.: Carnegie Institution of Washington, 1963).

The Transvaal Museum, Pretoria

IN their annual report for 1962, the Board of Trustees of the Transvaal Museum, Pretoria, still trust that the recommendations of the two commissions of inquiry will be implemented (Pp. 38. Pretoria: Transvaal Museum, 1963). They deplore the present unsatisfactory circumstances both with regard to the Museum in general and the Cultural History Division in particular. Both the finance and space requirements are inadequate. The Museum was instrumental in promoting the Namib Desert Research Station Project. During the year, five large habitat-groups were completed, namely, sable, waterbuck, koedoe, mountain zebra and crocodile. Considerable additions have been made to the study collections, including several new and rare forms, especially among the insects. Some alleviation in the storage of large mammals and historical material was made possible by the renting of two storerooms outside the precincts of the Museum.

Role of Microbiologists in Underdeveloped Countries

IN a speech delivered last November to the American Society of Microbiology on the role of microbiologists in assistance programmes for underdeveloped nations, and now printed in the *Journal of the Washington Academy of Sciences* (April 1963), Dr. C. Lamanna makes three recommendations regarding the contribution of microbiologists, and illustrates their significance by describing briefly the results achieved by such an approach to the control of diarrhœal diseases in the Philippines. First, he recommends that the allocation of resources and the direction of the efforts of microbiologists should fit into a general scheme of balanced activities, aimed at raising living standards as a whole, including efforts to reduce barriers to change and to raise the economic resources of the community. Secondly, he stresses the need to reduce the cost of microbiological activities, both by simplification and the use of local substitutes for imported material where possible. Thirdly, the microbiological research programmes in these countries should evolve by natural progression from efforts to meet practical needs. In this way the social value of microbiology would be demonstrated and the microbiologist himself would be required to accept his social responsibilities.

Microbial Classification

ARRANGEMENTS are being made by the Canadian Committee on Culture Collections (in collaboration with the Numerical Taxonomy Sub-committee of the International Association of Microbiological Societies) to hold a Summer School Conference on Microbial Classification in late August or early September, 1964. Strong emphasis will be placed on the numerical taxonomy of bacteria, and most of the time will be given to discussion of focal topics, including theory and practice of microbial classification, data-processing methods, computers, and implications for culture collections and nomenclature. Further details can be obtained from Dr. C. Quadling, National Research Council of Canada, Sussex Drive, Ottawa 2, Ontario.

Radioisotopes and Ionizing Radiations in Entomology

No. 9 in the bibliographical series published by the International Atomic Energy Agency, Vienna, is divided into two main sections dealing respectively with radioisotopes and ionizing radiations, and their uses in entomology; and there is a short section concerned with nematodes in agriculture (*Radioisotopes and Ionizing Radiations in Entomology*. Pp. 414. Vienna: International Atomic Energy Agency; London: H.M.S.O., 1963. 168 Schillings; 48*s*.; 8 dollars; 32 N.F.; 28 D.M.). The references are arranged under authors' names in alphabetical order within each topic; the topics include ecology, physiology and biochemistry, insecticidal studies, practical applications in control, techniques, etc. Each reference is accompanied by an abstract of the contents. Data on special subjects, such as dispersal and flight range, radiation sterilization, metabolism of insecticides, have been brought together in a series of tables in the appendix. An index to authors and a very detailed subject index are included. The period covered is 1950–60 (very few papers had been published before 1950) and the coverage must be almost complete. As a piece of bibliographical work it is well done (the compilation is by Mrs. M. Binggeli) and well produced. It is, of course, illogical to divide a science on the basis of a technique—but it may be none the less useful for that.

The Department of Geology at the University of the Panjab

A REPORT by Prof. N. R. Martin on the establishment of the Department of Geology at the University of the Panjab, West Pakistan, 1952–62, under the expanded programme of technical assistance, which has recently been published by the United Nations Educational, Scientific and Technical Organization, summarizes the work of the Technical Assistance Mission in establishing this Department, to which contributions were made under the Colombo Plan, from the Leverhulme Trust Fund and by the British Council, as well as by Unesco. The number of agencies which participated in this project is one of the features of the whole project and it is also noteworthy

that it took a full decade to complete. The staff, headed by a professor, includes three readers, five lecturers, five student demonstrators and two technicians, a secretary, clerk and library clerk, with four laboratory assistants and others bringing the total to 30, with a departmental expenditure for 1961–62 estimated at 765,882 rupees. Some 83 students have already graduated as B.Sc. and 49 as M.Sc., and a research programme has now been established. There is a real need for geologists in Pakistan, and the Mission was consistently supported by the Government of Pakistan as well as by Unesco, and was given as much freedom as possible by the University to build and administer the Department. Further expansion is recommended over the next two years by the establishment of a fourth readership (in structural and applied geology), a new lectureship (for hydrogeology), expansion of analytical facilities in the mineralogy section and of laboratory facilities in the applied geophysics section. The Department should be kept as a fully integrated department for at least five years, but by 1950 should be appraised with the view of raising the main sections to departmental status. Concentration until 1968 of all geological studies for the degrees of M.Sc. and Ph.D. work at the Panjab University is also recommended, and further assistance with overseas staff under an aid programme is regarded as unnecessary.

Farm Buildings Research

The first supplement, 1958–61, of Part 3, *Buildings for Poultry*, issued by the Agricultural Research Council, has recently been published (Pp. 71. London: Agricultural Research Council, 1963. 4*s*.). This bibliography of farm buildings research provides important basic information: in the past, much waste has occurred from the provision of unsuitable and uneconomic housing. Over-emphasis on one or another feature can lead to unsatisfactory accommodation and the disease situation is often below par on this account. With insufficient attention to the temperature requirements, to the general comfort of the occupants, etc., costs of ill-health may more than balance economies in management. Abstracts are given of 300 publications dealing with one or other of the important factors that require consideration in the planning of buildings. An indexed summary covers thirty major items, such as watering, nesting and humidity requirements of fowls, turkeys, ducks and geese.

Announcements

Dr. Charles Wilcocks, formerly director of the Bureau of Hygiene and Tropical Diseases, London, has been elected president of the Royal Society of Tropical Medicine and Hygiene in succession to Sir George McRobert.

The International Association of Forensic Toxicologists was formed during the conference on "Legal Medicine, Pathology and Toxicology", held in London in April 1963. The aims of the Association are to promote co-operation and co-ordination of effort among the members and to encourage research in chemical toxicology. The president is Dr. E. G. C. Clarke, and application forms for membership can be obtained from the secretary, Dr. A. S. Curry, 32 Rutland Drive, Harrogate.

A conference on "Metabolism and Physiological Significance of Lipids" will be held in Cambridge during September 15–21. Further information can be obtained from Dr. R. M. C. Dawson, Biochemistry Department, Institute of Animal Physiology, Babraham, Cambridge.

A general discussion on "Fundamental Processes in Radiation Chemistry", arranged by the Faraday Society, will be held in the University of Notre Dame, Indiana, during September 2–4. Further information can be obtained from the Assistant Secretary, the Faraday Society, 6 Gray's Inn Square, London, W.C.1.

A conference, arranged by the Research and Industrial Applications Sections of the Royal Statistical Society, will be held in the University of Southampton during September 14–16. Further information can be obtained from the Secretary, Royal Statistical Society, 21 Bentinck Street, London, W.1.

An international conference on "Microwaves, Circuit Theory and Information Theory" will be held in Tokyo during September 7–11, 1964. The conference is being organized by the Institute of Electrical Communication Engineers of Japan. Further information can be obtained from Dr. Kiyoski Morita, c/o The Institute of Electrical Communication Engineers of Japan, 2–8 Fujimicho, Chiyoda-ku, Tokyo.

A symposium on "Modern Instrumentation in Science and Industry", organized by the London Branch of the Institute of Biology, the London Section of the Royal Institute of Chemistry and the London Branch of the Institute of Physics and the Physical Society, will be held at the Institution of Electrical Engineers on January 17, 1964. Further information can be obtained from the Honorary Secretary, Institute of Biology, 41 Queen's Gate, London, S.W.7.

A colloquium on "The Optics of Solid Thin Layers" is being organized by the Commission Internationale d'Optique and will be held in Marseilles during September 8–15. Subjects under consideration will include: theory of solid thin layers; preparation of layers; structure and optical properties; optical applications. Further information can be obtained from Prof. P. Rouard, Faculté des Sciences, place Victor-Hugo, Marseille 3.

A summer school in "Molecular Biology" will be held in Ravello during September 2–15. Subjects covered by the School will include: the physicochemical methods of studying biological macromolecules; structure, function and biosynthesis of nucleic acids and proteins; mechanism of enzymatic reactions; quantum theory of biochemical structures and reactions. The School will be followed by a symposium on "The Electronic Aspects of Biochemistry", to be held during September 16–18. Further information can be obtained from Prof. B. Pullman, Institut de Biologie Physico-Chimique, 13 rue Pierre Curie, Paris 5.

A conference on "The Liquid State", arranged by the Institute of Physics and the Physical Society, will be held at the Imperial College of Science and Technology, London, during September 9–13. The conference will be divided into four main sections: structure and excitation spectra; equilibrium properties, including simple mixtures and solutions, evaporation and solidification; transport and dissipative processes and fluctuations; electronic properties. Further information can be obtained from the Administrative Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

The fifth international congress on "The Stratigraphy and Geology of the Carboniferous", arranged by Charbonnages de France, will be held in Paris during September 9–12. Subjects under consideration will include: stratigraphy and palæontology; sedimentology, petrography, geochemistry; coal petrography; the microflora of the Palæozoic; the microfauna and its applications to the stratigraphy of the Carboniferous; special geological problems. Further information can be obtained from the General Secretariat, Fifth International Congress on the Stratigraphy and Geology of the Carboniferous, Charbonnages de France, 9 avenue Percier, Boîte Postale, 396–08, Paris 8.

Erratum. In Table 1 of the communication entitled "Progestational Activity of 4-Hydroxy-17-Alpha-Acetoxyprogesterone" by Prof. G. Sala *et al.*, which appears on p. 262 of the January 19 issue of *Nature*, steroid No. 6 should be 9β-10α-pregna-4,6-dien-3,20-dione.

NEW DEVELOPMENT IN UNIVERSITY EXTRAMURAL WORK

THE report of the Universities Council for Adult Education, 1961–62, entitled *New Development in University Extramural Work**, prepared by Mr. A. Parker, director of Extramural Studies, University of Birmingham, is based on the reports of the extramural departments of the universities of the United Kingdom for 1961–62 and on additional information supplied by them. It raises again, at the outset, the question whether the new variety and extent of extramural work was entirely mere opportunism, but Mr. Parker suggests that the past seven years have been formative and that a new pattern is emerging. He considers that diversity between the different forms of course that have become established parts of extramural programmes—the day-release classes, the post-graduate courses, the provision for vocational groups, and work with industry—is less apparent than some years ago. Moreover, he feels that the underlying unity is to be sought in the distinctive kind of teaching that extramural departments should provide.

Several departments have directed attention to an increasing tendency for extramural work to mirror the range of intramural work of universities, and this might provide a simple answer to the question as to the distinctive characteristics of extramural teaching. While Mr. Parker suggests that extramural departments may sometimes be able to make a special contribution to courses arranged in co-operation with university departments of technology, he suggests further that the traditional concern of extramural departments with liberal studies has not diminished in importance. However, it would seem that liberal studies are not necessarily promoted by the traditional means nor necessarily differentiated sharply from professional studies.

If it is accepted that extramural teaching should be of a distinctive kind, it should also follow that this teaching should be open to all adults who are capable of profiting from such teaching. The three-year tutorial class was designed to meet a particular need, and, despite the decline in the number of such classes, in many places it still exemplifies the best extramural teaching. It should be asked, however, whether the spirit of the tutorial class may be preserved in new forms of course designed to meet contemporary needs, and Mr. Parker comments on the remarkable response at times in recent years from industrial workers, both in terms of quality and quantity.

Beyond this, Mr. Parker directs attention to the needs of a new group—the applicants with two or more Advanced-level General Certificate of Education subjects—for whom university places cannot be found, and asks whether traditional policies towards providing extramural courses leading to examination should be revised. He recognizes that extramural courses could not provide a substitute for intramural courses, but points out that, if considered as an adjunct to some of the many proposals for meeting unsatisfied demand for university places, they could provide at least occasional contact between students and good university teachers. Curiously enough, even in this context, the possible contribution or participation of the colleges of technology does not appear to be visualized. Moreover, while Mr. Parker recognizes that some extension of the principle of external degrees may be implied, he does not consider what might be implied in the suggestion of a new kind of external degree examination, specially designed to test the particular abilities of mature students who have the advantages, as well as the disadvantages, of alternating study with employment.

The report also includes a section by Mr. M. Bruce on the work of the Council's Sub-Committee on Broadcasting, set up in 1960, including its comments on the Government's White Paper on the Pilkington Report. The general expansion of programmes is welcomed, and educational programmes for adults are defined as programmes (other than school broadcasts) arranged in series and planned in consultation with appropriate educational bodies to help viewers towards the progressive mastery or understanding of some skill or body of knowledge. This definition is held to include programmes primarily designed for class use, for example, in technical colleges or centres for adult education, as well as those primarily designed for the viewer at home.

* *New Development in University Extramural Work.* Report of the Universities Council for Adult Education: 1961–1962. Pp. 28. (Liverpool: Hon. Secretary, Universities Council for Adult Education, The University, 1963.)

THE C.S.I.R.O. ANIMAL RESEARCH LABORATORIES

THE annual report of the Commonwealth Scientific and Industrial Research Organization Animal Research Laboratories for 1961–62* continues the accounts of much important research work covering a wide variety of conditions and of great importance to other countries. It outlines the work of three divisions dealing with animal genetics, animal health and animal physiology.

Apart from original work relating primarily to Australian conditions there are outstanding examples of the complementary value of work in Australia in the elucidation of important disease problems in many countries. Examples are the 'race', run off in 'heats' nearly half a century ago, on botulism research; the production of infective and toxic mixed culture after years of research from cases of 'lamsiekte' in cattle in South Africa; the discovery that a similar disease of animals in Australia was actually botulism; the triumph of technique in South Africa that enabled pure cultures of the organism to be made; the finding that the basic cause of the condition in that country was deficiency of phosphorus in the soil that led to depraved appetite with bone-chewing. The growth from this work of the importance of other mineral deficiencies that lead to ill-health in farm animals, Australia taking a share in these developments, was a striking development of the decades prior to the Second World War.

The importance of studies in general animal physiology was realized in certain of the newer countries earlier than in Britain, and, possibly with the less-entrenched and organized seats of learning, such work began to receive recognition in these countries at least a decade earlier. With the incentive of utilizing the great areas of potential animal country that is often of less obvious quality than the animal production areas of the old world, plus an open-minded outlook towards such developments, the enthusiasm and enterprise shown has paid large dividends. Work from Australia under the influence of the Commonwealth Scientific and Industrial Research Organization

* *C.S.I.R.O. Animal Research Laboratories.* Annual Report 1961–1962. Pp. 177 (mimeographed). (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1962.)

has consistently proved the value of its policies. The range of genetics research reported runs from purely scientific work on *Drosophila* to studies of the myxomatosis virus, physiology of the coat in cattle concerning sweat glands and hair, to hybrid vigour.

Infective and non-infective diseases important to Australia and disease related to worm and arthropod infestation receive great attention. The geographical position of Australia has provided a great freedom from some of the devastating diseases of other countries with comparable climatic conditions and wise policy has enabled the position, all round, to be maintained. There are great risks of diseases introduced in the processes of commerce. The important work on bovine contagious pleuro-pneumonia, solidly entrenched in northern parts of the continent for a very long time, is continued. The special climatic conditions of the continent have great influence on animal husbandry, and physiological investigations into the means of obtaining the best possible results by attention to the production and management of animals that can thrive and be suitably exploited have been extensively carried out.

PRIORITIES OF HUMAN RESPONSIBILITY

THE following statement of policy concerning "Priorities of Human Responsibility" was issued after the fourth meeting of the International Trustees of the World Wildlife Fund which was held in Switzerland recently:

"The World Wildlife Fund believes that man has responsibilities of trusteeship for the natural world over which we now exercise such sweeping power. How do these responsibilities equate with other human responsibilities? The ending of all forms of human suffering is clearly of paramount importance. We must never waver in the fight against disease, hunger, the threat of war and every other kind of disaster and human misery. We must strive to make a world worth living in for everyone; but is a world without wildlife and wild places worth living in? Even when mankind is free from want and fear, will our children's grandchildren thank us if we have sealed off great wild areas of the earth from the Sun with bricks and mortar, concrete and plastics? Will they have to ask 'What was a wild animal?' or 'What was a wild place?'.

"When there is an unavoidable collision between the survival of man and the survival of wildlife, human interests must clearly prevail. It is our thesis, however, that such collisions are rare—that in most cases a little thought, ingenuity and good will can permit the co-existence of man and wildlife without which man himself is so much the poorer. Most of the species exterminated by man in historical times need not have become extinct and would not have done so if anyone had bothered about it. The survival of the dodo and the great auk would not have impeded human progress in the slightest degree.

"Thus the Fund's campaign is not a case of animals versus man. Conservation is for man, for the long-term benefit of humanity, and to ignore it is short-sighted and improvident. There is a close link between medical science and natural science, and an even closer link between the achievement of freedom from hunger and the conservation of wildlife, because both have to do with the proper use of the land and its priceless treasures.

"But there are also responsibilities for adding something constructive and uplifting to human lives as well as for saving them—responsibilities which in the field of conservation, grow daily more urgent, for the wild creatures cannot protest, and once a species becomes extinct nothing can re-create it. Ultimately, therefore, the concern of the World Wildlife Fund is with the future benefit of man and the spiritual enrichment of his life."

THE INTERNATIONAL EPIZOOTICS BUREAU, PARIS

THE report* for 1961–62 by the director, Dr. R. Vittoz, of the International Epizootics Bureau, Paris, made at the thirtieth annual conference, covers incidence of world-wide disease.

The Bureau functions through officials of the countries represented on its council and has a permanent staff in its Paris Office. Reports of its conferences are published in considerable detail and bulletins are issued giving statistical information supplied to that office. From time to time special conferences are called, in addition to the regular seasonal meetings, when particular internationally important problems arise, such as, for example, the break-out of African horse-sickness from the endemic regions in Africa in quite recent years, with great extension from the Middle East into Pakistan, India, and beyond. There is close association for relevant purposes with other international bodies such as the World Health Organization and the Food and Agriculture Organization of the United Nations. Collectively, these bodies are able to take most satisfactory action in emergency and, according to circumstances, also in the preparation of short- or long-term plans.

The functions and organizations of these bodies each strengthen the value of the others. Their knowledge of local conditions and circumstances everywhere is very good and detailed, and, with the help of the several countries, rapid and efficient action can be taken when required, experts and materials being mobilized and concentrated to the best advantage.

Many examples could be given of the manner in which the functions of the Bureau and of the other organizations with which it works have been utilized. During their existence there have been dangerous extensions of animal disease into countries previously free and, if early and effective action had not been available, losses from devastating disease would undoubtedly have been carried even further than was the case, with very serious results to countries with valuable livestock industries.

With the rapid extension of air transport within the memory of serving officials of all countries, and the risks of the possibilities that may arise from space travel in the future, the dangers of infective disease are far greater than in the past. Diseases that the veterinarian had considered to be comfortably tucked away in Africa and limited to that continent have caused very serious losses within the experience of the present working generation of experts. While known to be of the utmost importance to their endemic areas there had been little fear that their interest would be more than academic to workers elsewhere. Blue-tongue of sheep has invaded the United States and at great cost; African swine fever—quite a different virus to the unfortunately well-known scourge of most countries—has invaded the Iberian Peninsula and Mediterranean countries.

* Report for 1961–62 of the International Epizootics Bureau. Pp. 94. (Paris: Office International des Épizootics, 1962.)

As a result of the activities of these international bodies the serious problems posed by foot-and-mouth disease have been mutually assessed by the countries most nervous about its depredations, and also by the countries, less nervous, but fearing the dangerous possibility that inefficient control measures might permit extensions. An exotic and dangerous type of the virus has penetrated southern European countries and great endeavours are being made co-operatively to prevent its arrival in Western Europe.

The diseases at present causing most concern because of the difficulties about their control are dealt with in this report in great detail, region by region. Where wide-scale vaccination is useful and local means are insufficient, much effort is concentrated on the provision of materials and skilled assistance. W. A. Pool

INTERSTITIAL SOLID SOLUTIONS IN BODY-CENTRED CUBE METALS

By Dr. J. STRINGER and A. R. ROSENFIELD*

Department of Metallurgy, University of Liverpool

THERE is a well-known correlation between the activation energy for self-diffusion ΔH and the melting-point T_m for body-centred cube (b.c.c.) metals[1]:

$$\Delta H(\text{eV/atom}) \approx \frac{3T_m \text{ (deg K)}}{2{,}000} \quad (1)$$

The linearity of this relation arises because the activation energy is required to overcome the binding energy for the ring of atoms through which the diffusing atom has to move. Consider, for example, an atom diffusing from one cube centre to a vacant position at the centre of the adjacent cube. If the lattice parameter is a_0, the hard-sphere atom diameter is $0{\cdot}866\ a_0$, and the tunnel between the four atoms at the corners of the cube face through which the diffusing atom has to move has an inscribed diameter of $0{\cdot}548\ a_0$. During the movement, therefore, the four atoms at the face corners are displaced from their equilibrium positions by an amount $0{\cdot}159\ a_0$. The melting-point is related directly to the binding force between atoms, and thus ΔH increases with T_m.

In general, the activation energy of any solid-state diffusion process for which the rate-determining step is the displacement of matrix atoms will increase with the melting-point of the matrix element and, irrespective of the form of the functional relationship between ΔH and T_m, one boundary condition is:

$$\Delta H = 0 \text{ when } T_m = 0 \quad (2)$$

However, when the activation energy for the interstitial diffusion of oxygen, nitrogen, or carbon in b.c.c. metals is plotted as a function of the melting-point of the solvent (Figs. 1–3), a linear relation analogous to equation (1) is obtained only for those interstitials that exhibit low solubility; those with solubilities greater than about 1 per cent[2] exhibit little variation of ΔH with T_m. Thus:

$$\Delta H = \alpha(x)T_m \text{ for interstitials with small solubility} \quad (3)$$

and:

$$\Delta H = \beta(x)T_m + \gamma \text{ for interstitials with large solubility} \quad (4)$$

In these equations, the values of α, β and γ depend only on the interstitial considered, and in general $\beta < \alpha$. It is interesting to note that recent investigations of diffusion of substitutional atoms in f.c.c. metals show that the activation energy depends markedly on solubility[3,4] although qualitatively the effects are different.

Cottrell[5] has shown that the diffusion coefficient can be calculated from the Portevin–Le Chatelier effect by means of the equation:

$$D_T = 10^{-9}\dot{\varepsilon} \quad (5)$$

where $\dot{\varepsilon}$ is the strain rate at the temperature T at which the effect appears. Since D_0 is always about 10^{-2} cm^2 sec^{-1} for diffusion of interstitials in b.c.c. metals and strain rates used experimentally are generally close to 10^{-4} sec^{-1}:

$$\Delta H = 25{\cdot}3\ kT \quad (6)$$

where k is Boltzmann's constant. The general validity of equation (5) has been demonstrated by Mincher and Sheely[6] and others. Table 1 shows values of ΔH calculated from equation (6). These values, included as squares in Figs. 1–3, agree very well with the values predicted by equations (3) and (4), demonstrating the usefulness of Portevin–Le Chatelier data for predicting activation energies of diffusion for systems for which no direct measurements are available.

Table 1. Calculation of Activation Energies using the Cottrell Equation

Metal	Blue brittleness temperature °C	Ref.	ΔH eV/atom	Interstitial
Cr	300	24	1·26	C
Cr	475	25	1·64	O
W	950	26	2·70	O

The oxygen solubility in a number of transition-metal alloys is related to the electron/atom (e/a) ratio. With $e/a < 5{\cdot}7$, oxygen is soluble, and with $e/a > 5{\cdot}7$ oxygen is insoluble in a range of niobium alloys[7]. Jones *et al.*[8] have shown that the magnetic susceptibility of a number of transition-metal alloys is a minimum at an electron/atom ratio of approximately 5·7, and suggest that either the d-band is split or the density of states curve in the d-band shows a marked minimum at this electron/atom ratio. Bryant[7] has suggested that the vanishing solubility at this concentration can be accounted for on the basis of a Jones–Hume-Rothery mechanism, in which the development of the Fermi surface into a region of k-space in which the density of states is rapidly falling encourages the appearance of a new phase. This explanation is not altogether satisfactory in this case because it fails to explain why oxygen is insoluble in b.c.c. alloys with e/a in excess of 5·7, when the density of states is presumably rising again. This model predicts a solubility minimum at $e/a \simeq 5{\cdot}7$, but not a vanishing solubility at higher concentrations. On the basis of the model, Bryant suggested that oxygen is present as a positive ion in niobium, but this conclusion must be discounted.

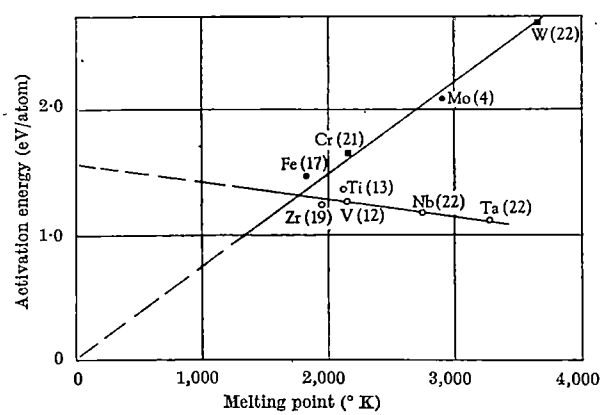


Fig. 1. Variation of activation energy for diffusion of oxygen with the melting-point of the solvent
○, Soluble; ■ ●, Insoluble

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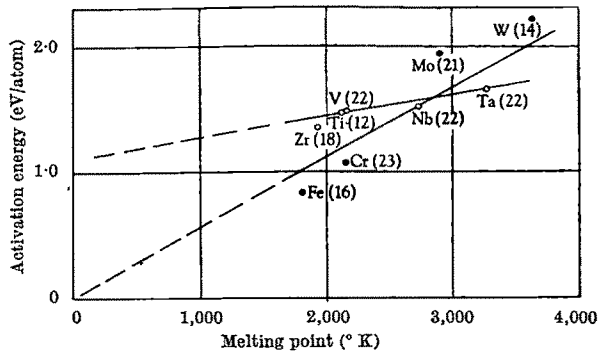


Fig. 2. Variation of activation energy for diffusion of nitrogen with the melting-point of the solvent
O, Soluble; ●, insoluble

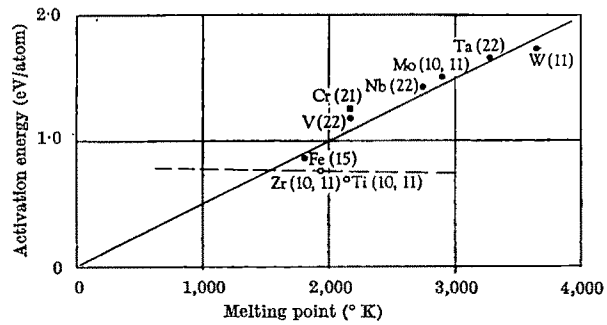


Fig. 3. Variation of activation energy for diffusion of carbon with the melting-point of the solvent
O, Soluble; ■ ●, insoluble

The largest inscribed sphere which can be accommodated in the b.c.c. lattice is situated at positions of the type (0, 1/2, 1/4), and is in contact with the four atoms at the positions 0, 0, 0; 0, 1, 0; 1/2, 1/2, 1/2; and $-1/2$, 1/2, 1/2. These form a distorted tetrahedron, and the inscribed sphere has a radius of 0·1260 a_0, where, as before, a_0 is the lattice parameter. A sphere of this size would not, however, be very tightly bound, since the path to the adjacent site has a minimum radius of 0·0973 a_0, and the diffusion of an atom of exactly the right size would involve a displacement of three atoms by only 0·0287 a_0. There is also a site centred at 0, 1/2, 1/2 in the distorted octahedron formed by the atoms at 0, 0, 0; 0, 0, 1; 0, 1, 0; 0, 1, 1; 1/2, 1/2, 1/2; and $-1/2$, 1/2, 1/2. This site has a very small inscribed sphere, radius 0·0670 a_0, but it has a large volume: for example, a cylinder thickness 0·134 a_0 and diameter 0·548 a_0 can be accommodated, having a volume approximately five times that of the inscribed sphere in the tetrahedral site. In addition, diffusion of an ion having these dimensions would be relatively difficult, involving a movement of two matrix atoms through 0·207 a_0. From consideration of the crystallography of the martensite transformation, it is usually assumed that, in iron, carbon is located in these octahedral sites.

It follows from this geometrical analysis that, at least in those b.c.c. elements in which solubility is fairly high, the interstitial solute is most probably on the octahedral site and is not spherical, but lenticular or toroidal. It is difficult to discuss, even in semi-quantitative terms, the shape of ions in metallic solutions, but it seems likely that the saturated ions such as O^{--} have spherical symmetry. It is also likely that ions having half-full shells, such as O^{+}, will have spherical symmetry, although in this case there are other possibilities. In the free atom the three $2p$ orbitals are at right angles, so that, for example, an ion with four electrons in the p shell might have a configuration in which two of the p states are full and one is empty. The charge distribution would in this case be toroidal. It is also possible that one of the orbitals would be full and the other two half full, in which case the charge distribution would be cigar-shaped. In the case of an ion with five electrons to be accommodated in this shell, the charge distribution will be lenticular. Since the solvent has cubic symmetry, it seems unlikely that, at least for ions in the octahedral sites, the geometry of the orbitals will be very much affected by the matrix, although for these solute elements the possibility of (sp) hybridization forming a tetrahedral arrangement of orbitals cannot be overlooked.

In the free atom the $2p$ state is 6-fold degenerate, but since the octahedral site has only one 4-fold rotation axis, the presence of the lattice field will partly remove the degeneracy, producing a splitting of the levels. On symmetry grounds it can be inferred that the new levels will consist of one 4-fold degenerate level and two non-degenerate higher levels, associated with the p-state parallel to the 4-fold axis. It is, however, possible in the case of large ions that the 4-fold level may split into two doubly degenerate levels, each symmetrical with respect to the 4-fold axis of symmetry. The actual degree of filling of these levels will depend on their position relative to the Fermi surface in the solvent. If all the levels are below the Fermi surface a large spherical ion will be formed that would be expected on geometric grounds to have very low solubility.

On this basis it is possible to suggest an explanation for the dependence of oxygen solubility on electron concentration. For low e/a the highest level at least is above the Fermi surface and consequently unoccupied, producing an ion which can be accommodated in the octahedral site. However, at an electron-to-atom ratio of about 5·7 there is a minimum in the density-of-states curve so that the Fermi surface expands rapidly as the electron concentration increases, submerging the highest level and producing the large insoluble spherical saturated ion. This model successfully explains the disappearance of the solubility and accounts for its coincidence with the minimum in the d band.

The diffusion data can also be explained on this basis. As has been pointed out, the diffusion of a lenticular or toroidal ion is difficult, involving large movements of the matrix atoms. However, during movement of the atom the remaining degeneracy will be removed, and some of the levels will increase in energy. It is then possible for ionization of a lower level to occur, an electron being lost to the conduction band of the metal. (This electron will remain in the general vicinity of the interstitial because of the distortion of the bands locally due to the excess charge on the ion.) This new ion will not only be smaller, but also its shape may change, thus making diffusion easier. If this ionization is the process for which activation is required in the diffusion, it follows that the activation energy for diffusion is unlikely to depend very strongly on the melting-point of the matrix and will certainly not approach zero as the melting-point falls to zero. If, however, the highest-filled level is deeply submerged below the Fermi surface, the ionization energy may exceed the activation energy for the normal lattice distortion mechanism, and consequently ΔH will show the normal dependence on the matrix melting-point. The condition for the appearance of a 'normal' activation energy is thus approximately the same as for the disappearance of appreciable solubility, explaining the difference in equations (3) and (4) for the small and large solubility interstitials.

It remains to determine the probable form of the oxygen ion in the systems with high solubility. On the basis of the previous argument the most likely forms would be those with one or two electrons short of a full shell, but in order to diffuse the ion with two electrons short would require only the energy difference between the filled level and the lower of the upper two levels. The major energy difference would be expected to be between the upper two levels, with the difference between the lower of these and the lower levels relatively small. During displacement, this gap would reduce still further,

and the ion would diffuse simply by changing its configuration and hence its shape. It seems more likely, therefore, that the oxygen is present as ions lacking only one electron from a full shell, that is, the O^- ion. Recent studies of the interaction energies between oxygen in solution and dislocations gives some support to the idea that a single negative charge should be assigned to the oxygen ion in high-solubility systems. Rosenfield and Owen[9] have shown that the yielding of tantalum containing small amounts of oxygen cannot be explained simply on the basis of elastic interaction, but that it is necessary to attribute a charge to the ion to interact with the dislocation dipole. Satisfactory agreement is obtained using a single negative charge for the oxygen ion.

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NEW PROTON-CONTAINING OXIDES OF TITANIUM, ZIRCONIUM AND HAFNIUM

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I HAVE reported[1] the reduction of ZrO_2 and HfO_2 using 'active' hydrogen produced in a discharge at 2,450 Mc/s, and gave an approximate value for the specific resistivity of the reduced ZrO_2. Since that time further experimental work has been done, and it now appears that these golden oxides, which exhibit metallic conductivity and have low resistivities, contain hydrogen and may be related to the various metal oxide 'bronzes'. The reactions do not occur with the neutral or excited hydrogen atoms produced by lower frequency discharges (50 c/s to 30 Mc/s) although many oxides are readily reduced under such conditions.

The high-frequency generator used was a Mullard fixed tuned (2,450 Mc/s) magnetron, 'Type *JN* 2/2·5*A*', capable of delivering about 2·5 kW of power at anode voltage and current of 4·8 kV and 750 m.amp respectively. The output of the magnetron was coupled by means of a length of air dielectric 70 ohms coaxial line to a short length of water-cooled wave-guide, constructed in a manner similar to that described by Shaw[2].

Power input to the magnetron was readily varied by adjusting the anode voltage by means of a variable auto-transformer fed from a constant-voltage 50 c/s a.c. source. Powers of 100 W–1·0 kW were used.

Materials utilized were: (*a*) TiO_2, ZrO_2 and HfO_2 powders prepared in a highly pure state in the laboratory, either by precipitation, or oxidation of 'iodide' metal turnings in oxygen; (*b*) compacts of these oxides made by pressing the powders at 10,000 lb./in.^{2} and sintering at 1,700° C; (*c*) pieces of ZrO_2 ceramic cut from 'Degussa' brand tubing; (*d*) titanium, zirconium and hafnium metal foil 0·005 in. thick, the latter containing approximately 1 per cent zirconium. Also tested in the apparatus were 'Specpure' ThO_2, CeO_2, Ta_2O_5, Nb_2O_5 and V_2O_5, and high purity aluminium and beryllium oxides in both powder and ceramic form. The hydrogen for the reductions was commercial cylinder gas containing approximately 0·5 per cent nitrogen.

Samples of oxides were placed centrally between the guide walls; powder in silica boats; compacts, ceramic pieces and metal in small supports made from 1/16-in. silica rod.

Pressures of hydrogen between 0·5 mm and 5 mm were used, but normally 2 mm was favoured since the discharge appeared to be most stable at this pressure, although the greatest yield of hydrogen atoms is achieved at about 0·5 mm (ref. 2.) After pumping the apparatus down to a few μ the hydrogen was fed into the working tube via a needle valve to the required pressure and the input to the magnetron was adjusted to about 100 W. If the discharge did not form, a high-frequency vacuum 'leak tester', held near the tube, proved an effective 'trigger'. The power input was then raised slowly to the desired level, which was, in the majority of experiments, about 500 W. Temperatures at the sample, which were measured by means of fine thermocouple wires, varied from 200° C to 1,200° C. depending on the power used, the latter value obtaining for input powers of the order of 1 kW.

The samples of metal foils were first coated with a layer of oxide by passing oxygen through the apparatus at about 1–2 mm pressure, and establishing a discharge with a power of 300–400 W. For treatment times ranging from a few minutes to 0·5 h, in the case of zirconium and hafnium, dense strongly adherent layers of oxide usually grey in colour were formed varying from a few tenthousandths of an inch to 0·0015 in. in thickness. Once the requisite oxide had been formed, hydrogen was substituted for the oxygen, resulting in a thin layer of 'golden oxide' on the previously formed oxide layer. Fig. 1 shows two cross-sectional views of metal-oxide–golden oxide layers, one a taper section, the other at right angles to the plane of the foil. The maximum thickness attainable was about 0·0003 in. For titanium the procedure was similar; but if oxidation was continued for a longer period the surface of the oxide layer became friable, and after hydrogen treatment the golden material could be scraped off quite readily.

In order to obtain the golden zirconium compound in a fully converted state, thin layers of ZrO_2 were deposited on silica plates from an aqueous suspension of finely ground oxide. These were then treated in the apparatus. There was no sign of reaction when the oxides of thorium, beryllium and aluminium were used, even when powers of 1 kW were used. CeO_2 reduced to the mustard-



Fig. 1. Showing from left to right Zr metal, ZrO_2, and 'golden ZrO_2' layers. *a*, × *c.* 75 and at an angle of a few degrees from plane of foil; *b*, × *c.* 560 at right angles to the plane of the foil

coloured CeO_3 : Ta_2O_5, Nb_2O_5 and V_2O_5 to black, electrically conducting lower oxides of metallic appearance, without exhibiting any golden colour.

The oxides of titanium, zirconium and hafnium, either as powders, compacts, ceramic bodies or layers on metal substrates were all readily converted on their surfaces to the golden materials. During hydrogen treatment metals underlying the oxide layers became brittle due to the uptake of hydrogen and some nitrogen (weak lines of ZrN were observed in X-ray examinations of the metal layers in samples of zirconium) and required careful handling. However, there is no evidence to suggest that nitrogen plays any part in the formation of the golden materials, and they certainly do not contain nitride.

It has already been noted that hydrogen atoms produced at relatively low frequencies have no effect on ZrO_2 or HfO_2 although they cause a marked greying of TiO_2. In the 2,450-Mc/s discharge there is a noticeable preferential reaction on surfaces which are at right angles to the direction of the electrostatic field within the guide, although the attack, is not necessarily confined to these surfaces. Reaction also occurs on such surfaces even when the spacing between them is of the order 0·001 in.

The extent of the reaction with time may be measured by noting the change of resistance across a surface. In Fig. 2 typical resistance values shown as ohms across a given surface of a ceramic body are plotted against time. Slight treatment resulted in a grey or black coloration; but the golden form quickly became apparent. Golden layers were produced equally satisfactorily by using lower power (about 100 W) for a longer period or higher power for a shorter period, and temperature did not appear to be an important factor—no differences in the final products could be detected. When zirconium oxide ceramics containing 3–5 per cent CaO as a stabilizer were treated much longer times were necessary for the formation of the layer, but the final result was the same.

Analysis of the materials established the fact that they contain appreciable amounts of hydrogen, the formula of the titanium compound being $H_{0\cdot2}Ti_{1\cdot00}O_{1\cdot3}$ and that of the zirconium $H_{0\cdot2}Zr_{1\cdot00}O_{1\cdot66}$. This indicated that reduction had occurred and that hydrogen was incorporated. The titanium compound was shown by X-ray analysis to be the TiO phase which is stated[3] to exist in the compositional range $TiO_{0\cdot7}$–$TiO_{1\cdot25}$. The specific resistivity, from measurements done on compressed powder was found to be approximately 0·004 ohm cm, and the conductivity was metallic in character, the resistance increasing slightly as the temperature was raised.

The golden zirconium compound has a structure which appears to be essentially that of ZrO_2, but the presence of several additional lines suggests a distortion of the lattice that could be due to the incorporation of hydrogen atoms. It has a specific resistivity of approximately 0·002 ohm cm, metallic conductivity, and at a hot junction between zirconium metal and the golden oxide electron flow is from the former to the latter. The thermo E.M.F. under these conditions is approximately 0·1 μV/deg. C.

All the golden oxides rapidly re-oxidize to white oxides when heated in air or oxygen, and in most chemical properties bear a close resemblance to the dioxides. They are quite stable under normal conditions.

Hydrogen atoms are not energetic enough to reduce ZrO_2 and HfO_2, although they reduce TiO_2. Hence a species having a higher energy than that possessed by a hydrogen atom must be responsible. The preferential reduction which occurs on surfaces at right angles to the electrostatic field suggests charged particles that are orientated to some degree in the field, possibly even accelerated to some extent in it. The most likely species appears to be the H_2^+ ion since it is unlikely that protons would be produced under the conditions obtaining in the wave-guide. It is interesting to examine the thermodynamic relationships of the oxides to hydrogen atoms and H_2^+ ions. We have the following equations to consider[4]:

$$\underset{-204}{TiO_2} + \underset{200}{4H} - Ti + 2H_2 + O_2 \quad \text{Additional energy required} + 4 \text{ kcal} \qquad (1)$$

$$\underset{2X-204}{2TiO_2} + \underset{100}{2H} - \underset{-340}{Ti_2O_3} + H_2 + O_2 \quad ,, \quad ,, \quad ,, \quad -32 \text{ kcal} \qquad (2)$$

$$\underset{-238}{ZrO_2} + \underset{200}{4H} - Zr + 2H_2 + O_2 \quad ,, \quad ,, \quad ,, \quad +38 \text{ kcal} \qquad (3)$$

$$\underset{-238}{ZrO_2} + \underset{714}{2H_2^+} - Zr + 2H_2 + O_2 \quad ,, \quad ,, \quad ,, \quad -476 \text{ kcal} \qquad (4)$$

In atomic hydrogen reactions such as these, water is not a final product, the gaseous products of reduction

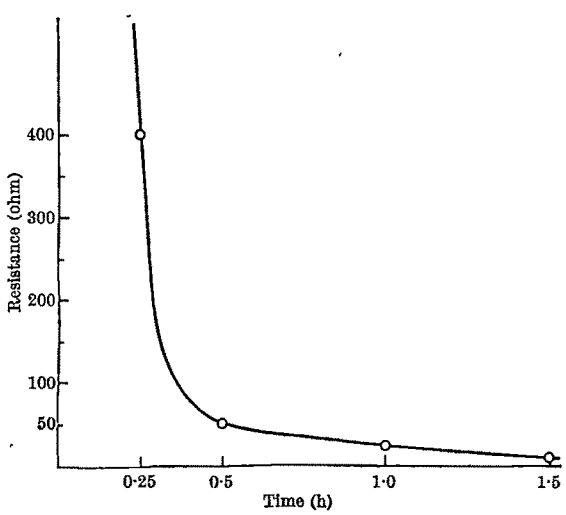


Fig. 2

being hydrogen and oxygen. Equation (1) indicates no reduction of TiO_2. However, reduction is known to occur, but probably not beyond the composition represented by Ti_2O_3. Substituting this compound for titanium metal we have equation (2), which agrees well with observation. For ZrO_2, equation (3) indicates no reduction, which is in fact what is observed. There is no evidence so far to suggest that there are any phases in the Zr—O system analogous to Ti_2O_3, Ti_3O_5, etc., so that equation (3) is probably valid. Equation (4) shows that for H_2^+ ions reduction should readily occur. This is again in agreement with observations. However, the H_2^+ ion carries sufficient energy, according to thermodynamic data, to reduce ZrO_2 to the metal, and also ThO_2, BeO, Al_2O_3, etc., yet these last-named oxides show no sign of being affected. A possible explanation is that at a composition MO_{2-x}, where x may be very small, these oxides exhibit greatly increased stability.

The phases in the Ti—O system have been thoroughly investigated and are now well established. In the present work it would appear that reduction so far as the monoxide phase occurs and that hydrogen enters this lattice as protons, giving rise to electrons which confer a high conductivity in the material.

Simonenko[5] stated that ZrO_2 was reduced in a d.c. arc between tungsten electrodes, in an atmosphere of hydrogen, and that this was due to the effect of ions of molecular and atomic hydrogen. It is not clear to what extent the ZrO_2 was reduced, although the impression is obtained that the claim is for reduction to the metal. If this is so it must be regarded as doubtful, since many other workers have failed to obtain metal even under extreme conditions. Norton and Kingery[6] and Kramers and Smith[7] have shown that the black material obtained when ZrO_2 is fired in vacuum at 2,200° C is a solid-solution of zirconium in ZrO_2, which may contain as much as 15 mole per cent zirconium. X-ray patterns of the solid solutions all conform to ZrO_2. Specimens of these black materials prepared in our laboratory were not electrically conducting. It is probable, however, that the golden zirconium (and hafnium) compounds are analogous to them in so far as the zirconium : oxygen ratio is concerned, but that, as in the case of the titanium compounds, the incorporation of hydrogen into the lattice has the effect of producing electrons, which are available for conduction as in metals. The question of the relationship of these golden compounds with the tungsten oxide and other 'bronzes' (for example, ref. 8) arises. It may well be that these hydrogen-containing, electrically conducting oxides, and in particular those of zirconium and hafnium, are bronzes in which hydrogen atoms replace the usual alkali metal atoms. Electron diffraction investigations at present being carried out should resolve this, and it is hoped to present detailed structures in the near future.

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TEMPERATURE OF SHOCKED ARGON AND METHANE

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RECENTLY an experiment was reported[1] which, it was claimed, showed that the light emitted from within a granular pressing when strongly shocked did not, for the most part, arise from the compression of the interstitial gas. Evidence on this point was obtained by repeating an experiment of Paterson[2] on granular sodium chloride shocked by high explosives. Light emitted by a pressing when the interstitial gas was methane was compared with light emitted when the gas was air. It could easily be estimated, and in fact was demonstrated experimentally, that free-running shocks from high explosives in these two gases have very different brightnesses. Failure to note much difference in brightness between pressings filled with methane and pressings filled with air was cited as evidence that the gases were not responsible for the major part of the very intense light observed.

It may be objected that much higher local pressures exist in pressings than in free-running shocks and that the difference in temperature between these two gases would therefore be expected to disappear in the case of the pressings. Such higher pressures could indeed exist in small interaction regions even though it has been observed that the overall velocity of the shocks through the pressings was lower than through the open gas chambers. Assuming that the conditions in the pressings are in fact severe, we propose to demonstrate that the temperature differential between two such gases as methane and air will remain.

We have performed calculations of the temperatures to be expected for various shock pressures in methane and in argon. These calculations are of interest not only because of the foregoing specific experiment but also as illustrations typical of the behaviour of ionizing and dissociating gases. Argon was not used in the experiment, but perhaps should be in future experiments, since it is a good example of a monatomic low-heat-capacity gas which can be expected to reach very high shock temperatures. Methane provides perhaps the best example, from the point of view of both experiment and calculation, of the effects of high heat capacity and dissociation in producing relatively low shock temperatures. Most gases which can be conveniently used in experiments lie between these two extreme examples.

The initial conditions for the calculations were one atmosphere and 300° K. The usual hydrodynamic equations and the equilibrium relations among the various possible species were solved simultaneously on an IBM 704 computer, using a programme written by R. E. Duff and Wildon Fickett[3]. For argon the species considered were Ar, Ar^+, and e^-. For methane the species were CH_4, C, H_2, H, H^+, C^+, and e^-; in a few calculations several other possible hydrocarbon-fragment species[4] were allowed in the equilibrium with only minor alterations in the results. The individual species were assumed to obey the perfect gas law; but the behaviour expected of perfect gases, such as a density-ratio limit of four for argon, was not in evidence. This was the result of the nature of the enthalpy and free-energy functions used, which included the contributions of internal degrees of freedom with the proper temperature dependence. Allowance was made for the increasing number of states at high energies, for stretching, and anharmonicity where appropriate. The functions used have been given in tables elsewhere[4–7].

The ratio of shock temperature T to initial temperature T_0 for the two gases is plotted in Fig. 1 for a

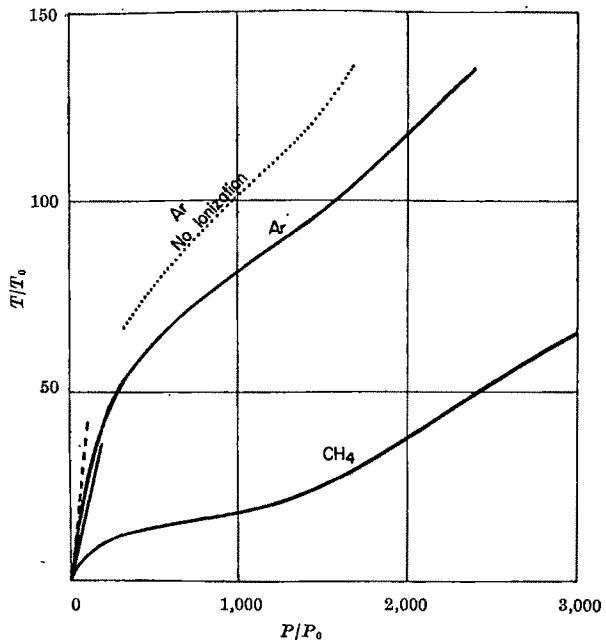


Fig. 1. Temperature ratios across shock fronts in argon and methane as a function of the pressure ratios. Subscript zeros refer to unshocked conditions (P_0 = 1 atm, T_0 = 300° K). The dashed line shows the temperature ratios in argon if the gas were considered to be a perfect gas with constant heat capacity $C_P = 5/2R$. The solid straight line shows the corresponding perfect-gas, no-reaction temperature ratios for methane with a constant value of the heat capacity appropriate to 300° K (with the internal degrees of freedom included). The dotted line indicates the case for argon in which the heat capacity has been allowed to increase as the higher excited states become important, but no ionization is permitted

range of shock pressures. The dashed and solid short segments in Fig. 1 show the temperature which would exist in argon and methane respectively if the gases were considered to be perfect, non-reactive, and to have constant heat capacities. Under these assumptions the heating in both gases is extreme, and the temperatures at a given pressure ratio are approximately proportional to the specific heats.

In contrast to the constant heat-capacity assumption, the more realistic calculations show that the argon temperature is soon restricted by increase of the heat capacity due to excitation and ionization. The temperature calculated when no ionization is allowed but the heat capacity is considered to increase due to excited states is shown by the dotted line in Fig. 1. The degree of ionization in argon for various pressure ratios across incident and reflected shock fronts is shown in Fig. 2, where it may be seen that ionization starts at low pressures and continues throughout the range of shock strengths calculated.

For methane, a similar restriction of the temperature (Fig. 1) results from dissociation and the increase in the heat capacity. In Fig. 3, it will be noted that dissociation occurs at relatively low shock pressures. Consequently, the temperature in this material remains relatively low until quite high pressure ratios are reached. Ionization is appreciable only for pressure ratios above 2,500.

The relation of these calculations to the measurements that have been made can be seen in Fig. 4, where the shock velocity has been plotted against the shock pressure ratio. On this plot the measured velocities of the free-running shocks in argon and methane (measured from the film reproduced in ref. 1) induced by explosion of Comp. *B*-3 are shown by circles on the appropriate curve, and the measured velocity of a shock driven by Comp. *B*-3 through granular sodium chloride at pour density is indicated by a dashed line. This last velocity is practically the same whether argon or methane is used as the interstitial gas. For matching at interfaces between these gases and other materials, the pressure versus particle-velocity plot included in Fig. 4 will be useful.

High-pressure regions, if present, must occur as a result of multiple reflexions within the interstices of a granular pressing. As an indication of how such repeated reflexions might affect the state of argon and methane, Figs. 2, 5, 6 and 7 present a comparison of the conditions reached in the initial shock and in a reflexion against an incompressible wall. No more than one reflexion can be calculated with the present programme. It will be noted that for a given pressure ratio the temperature is always lower if achieved by reflexion than if achieved by one (stronger) shock. The relation between a typical incident

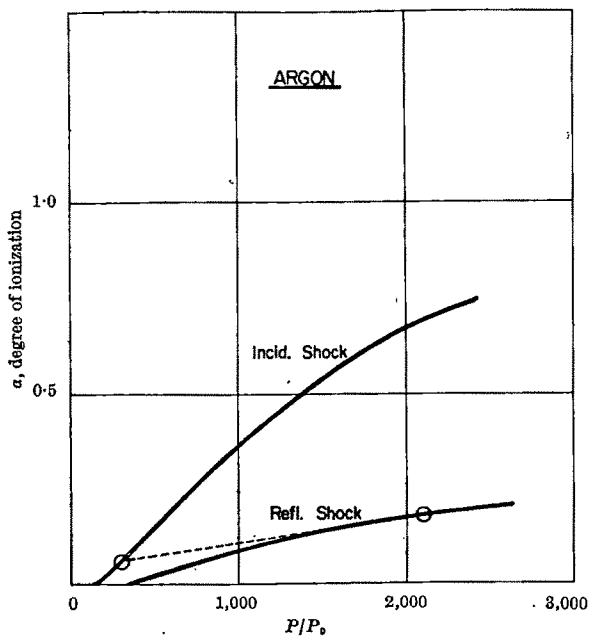


Fig. 2. The degree of ionization α, the number of electrons divided by the initial number of argon atoms, as a function of the pressure ratio. The upper curve is for incident shocks, and the lower curve is for the reflexion of those shocks against a rigid wall. The two circles represent a typical associated pair of incident and reflected shocks

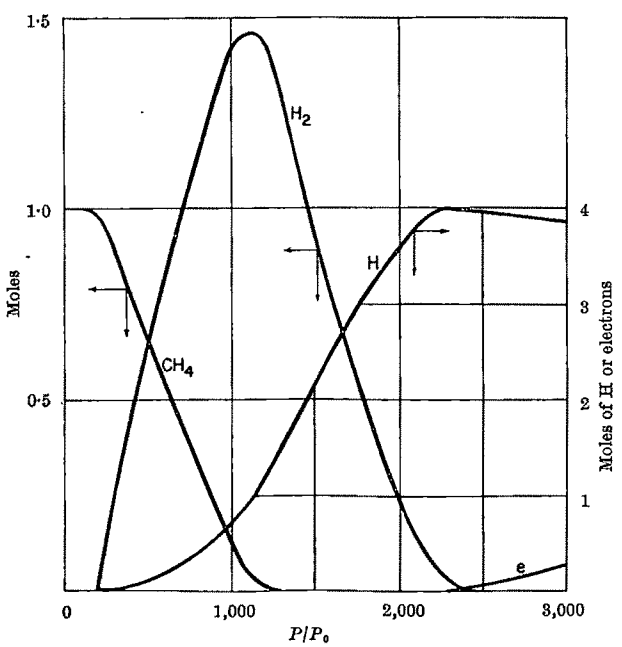


Fig. 3. The amounts of some of the species in shocked methane as functions of the pressure ratio across the incident shock front. The curve marked *e* is the number of moles of electrons; this would reach the number five on the scale at the right when each atom in the gas had lost one electron

shock and its reflexion is shown in Figs. 2, 5, 6 and 7 by the circles on the incident and reflected shock curves.

Attention is directed to the Hugoniot for the incident shock in Fig. 7, which shows that for a pressure ratio of about 500 the density ratio is about 20, whereas for a stronger shock with a pressure ratio of about 2,500 the density ratio is approximately ten. However, on reflexion of even a fairly weak shock a very high density is achieved. This is an example of the complicated effects caused by processes that absorb energy and change

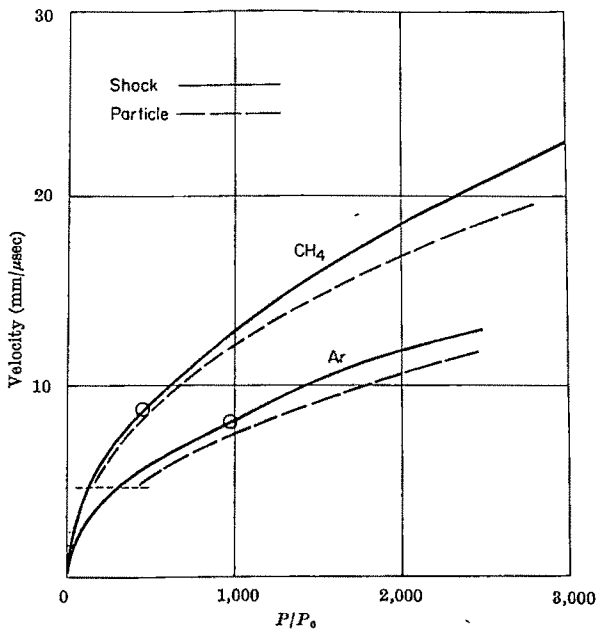


Fig. 4. Shock and particle velocities in argon and methane for various pressure ratios across the fronts. The circles show the velocities of a free-running shock from Comp. *B*-3 in methane and argon, the former measured on the film published by Blackburn and Seely (ref. 1), and the latter specially measured by Gardner. The dashed line represents the velocity caused by Comp. *B*-3 in a sodium chloride pressing. This has about the same value when either gas fills the interstices

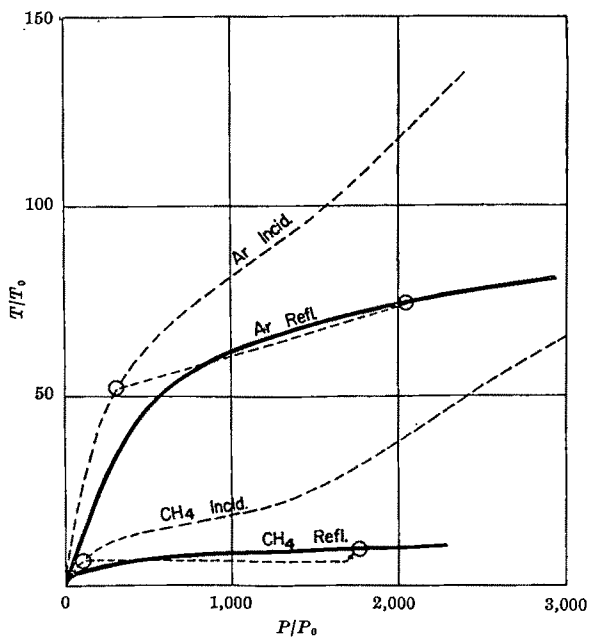


Fig. 5. Comparison of temperature ratios in incident shocks with ratios achieved by a single perfect reflexion for argon and methane. The circles on the incident curves represent values for free-running shocks in the low strength range (4·7 mm/μsec). The circles on the reflected curves indicate the perfect reflexions associated with those initial shocks

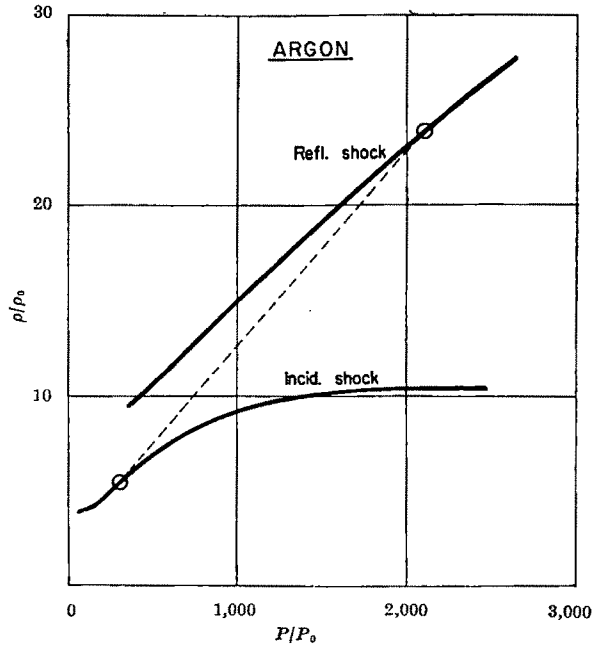


Fig. 6. Comparison of the density ratios of argon in incident shocks with the corresponding density ratios in reflected shocks

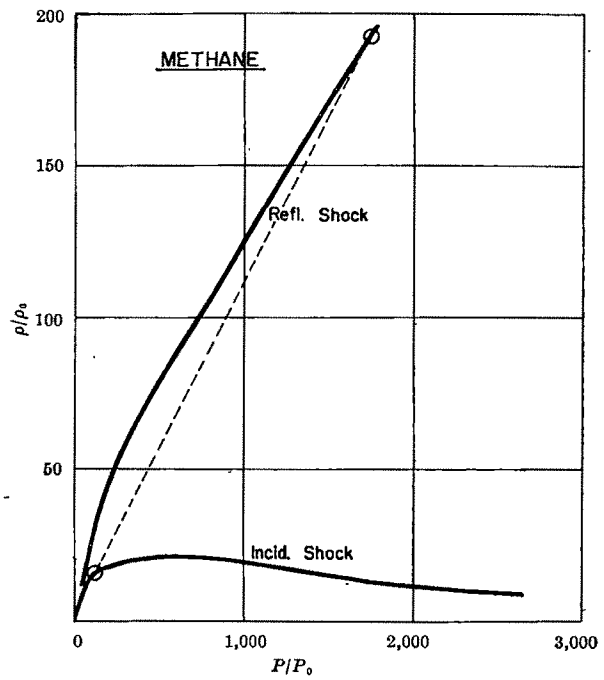


Fig. 7. Comparison of the density ratios of methane in incident shocks with the corresponding density ratios in reflected shocks

the number of particles in a shock. Hydrodynamic treatments which ignore such processes can yield very misleading results. Additional corrections to the present calculations, such as the use of a real-gas equation of state, would be expected to produce relatively minor changes in the results.

These calculations demonstrate that for shock strengths that are nearly the same in argon and methane the shocked argon is always much hotter. At the most severe conditions in the calculations all indications are that this situation will continue to even stronger shocks. The temperature differential is maintained in the first reflexion.

We thank Garry L. Schott for his help in setting up these calculations and Robert L. Mowrer for preparing the

graphs. We thank S. D. Gardner for the measurement of the shock velocity in argon from Comp. *B*-3. This work was carried out under the auspices of the U.S. Atomic Energy Commission.

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LIMITATIONS OF ELECTRONIC INTERPRETATIONS OF CHEMICAL PHENOMENA

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At the present time many widely used chemical texts seek to interpret numerous phenomena in terms of what are known as electronic theories. These views emphasize the transfer (or 'displacement', as in the 'inductive effects' of certain works on organic chemistry) of electrons, and sometimes succeed on such a basis in correlating a range of chemical properties. It seems timely, however, to examine carefully and critically certain serious limitations of such theories.

Much of the present article is concerned with the more plausible of these views, but some preliminary remarks deal with what is probably the crudest (and, fortunately, least credited) of the electronic theories—that which seeks to reduce all chemistry to a ruling tendency of atoms to attain an inert-gas electronic structure. A quantitative examination of the demerits of such a view is indeed instructive. Consider, for example, the formation of sodium chloride from its elements. The sodium atom does not readily lose an electron, as is clear from the large quantity of energy * (the ionization energy) required to bring about such a loss:

$$Na(g) \rightarrow Na^+(g) + e^-(g) \qquad \Delta H^\circ = + 120{\cdot}0 \text{ kcal mole}^{-1}$$

By the relevant thermochemical criterion, the dissociation of hydrogen to atoms is a process which occurs more readily :

$$H_2(g) \rightarrow 2H(g) \qquad \Delta H^\circ = + 104{\cdot}2 \text{ kcal mole}^{-1}$$

Nor does the positive electron affinity (Table 1) of chlorine make electron transfer energetically favoured:

$$Na(g) + Cl(g) \rightarrow Na^+(g) + Cl^-(g) \quad \Delta H^\circ = + 35 \text{ kcal mole}^{-1}$$

and for this process equilibrium lies far over to the left. At 1,000° K the equilibrium constant (equal to 0·110 $e^{-\Delta H^\circ/RT}$, where the factor 0·110 allows for the statistical weights of the species concerned) comes at the most to 7×10^{-9}. At equilibrium in a gas of atoms, only a fraction slightly less than 10^{-4} would be present as ions. In the formation of sodium chloride from its elements, the process actually concerned is (stoichiometrically) :

$$Na(s) + \tfrac{1}{2}Cl_2(g) \rightarrow NaCl(s)$$

and it must be pointed out that the only exothermic step in the similar sequence to which this may be reduced is that involving the lattice energy :

$$Na^+(g) + Cl^-(g) \rightarrow NaCl(s)$$

which has no connexion with inert-gas structures as such.

Properties of the halogens. An approximate generalization exists to the effect that among comparable atomic species the smaller (1) are more strongly bound in molecules (2) more readily acquire an extra electron. The properties of the halogens (Table 1) show that this rule has some force, but that there are unquestionably exceptions to it. The dissociation energy, D(F–F), of molecular fluorine and also the electron affinity of that element are smaller than the corresponding quantities for chlorine, in contrast both to this rule and to trends among the heavier of the halogens.

* Thermochemical data are here given, unless specified otherwise, as standard changes of enthalpy at 25° C.

Table 1. Properties of the Halogens

All quantities are given as ΔH° (kcal mole^{-1}) at 25° C

Ref.		F	Cl	Br	I
2	Ionization energy	403·3	300·3	274·6	242·2
2	Electron affinity	83	87	82	76
2*a*	Electron affinity	81·1	84·9	79·0	72·1
1	$D(X-X)$	37	57·9	46·1	36·1
1	$D(H-X)$	135	103·1	87·4	71·4

Data from Cottrell (ref. 1), Pauling (ref. 2) and Berry *et al.* (ref. 2*a*)

It is interesting to note that there is evidence[3] that the (first) electron affinity of oxygen is less than that of sulphur.

Standard electrode potentials. A quantitative discussion of equilibria involving ions in solution may be based on measurements of standard electrode potentials, that is, of the E.M.F. of a reversible cell one of the electrodes of which consists of a metal M dipping into a solution of its ions at unit activity, and the other consists of hydrogen at unit pressure passing over an inert (platinum) rod dipping into a solution of hydrogen ion at unit activity. The chemical reaction occurring in such a cell is:

$$\frac{z}{2}H_2(g) + M^{z+}(aq) \rightarrow zH^+(aq) + M(s)$$

and the associated standard change of free energy ΔG° and standard electrode potential E° are related by the equation:

$$\Delta G^\circ = - zFE^\circ$$

(where F is the Faraday). Thus the standard electrode potential is directly proportional to the standard change of free energy for the reverse of the reaction written.

The (reverse) reaction may be expressed in terms of a sequence of four processes, of which the first three depend on the identity of the metal concerned.

(1) $M(s) \rightarrow M(g)$ sublimation

(2) $M(g) \rightarrow M^{z+}(g) + ze^-(g)$ ionization

(3) $M^{z+}(g) + zH^+(aq) \rightarrow M^{z+}(aq) + zH^+(g)$ 'solvation'

(4) $zH^+(g) + ze^-(g) \rightarrow \frac{z}{2}H_2(g)$

Changes of enthalpy and free energy for the whole process may be obtained by summing those for these four steps, and contributions to the standard electrode potential thus deduced. Of the three which depend on the metal, only step (2), ionization, involves the transfer of electrons, and, in general, contributions from both sublimation and solvation may be important.

The electrochemical data required for examining these points can be obtained from a compilation by Parsons[4], while the latent heats of sublimation have been taken from Cottrell[1]. It is evident (Fig. 1) that the standard electrode potential is not closely correlated with the contribution to it arising from ionization. Further (Fig. 2), there is no close correlation with the first ionization potential. There is only one even moderately successful correlation

Table 2. An Analysis of Data relating to Standard Electrode Potentials

		Be	Zn	Cu	δ(Cu–Be)	δ_v(Cu–Be)	δ(Cu–Zn)	δ_v(Cu–Zn)
First ionization potential (v)		9·32	9·39	7·72				
Second ionization potential (v)		18·2	18·0	20·2				
Average (v)		13·75	13·7	13·95	+0·2	+0·2	+0·25	+0·25
Latent heat of sublimation* (kcal mole^{-1})		77	31·2	81	+4	+0·1	+50	+1·08
Thermodynamic functions (kcal mole^{-1}) for the process $M^{2+}(g) + 2H^+(aq) \rightarrow M^{2+}(aq) + 2H^+(g)$	ΔG°	?	36·36	23·77	?	?	−12·59	−0·273
	ΔH°	−73	32·84	19·45	+92·5	+2·00	−13·39	
Standard electrode potential (v)		−1·85	−0·763	+0·337	+2·14		+1·100	

$\delta(M–M')$ is the difference between values of a quantity for metals M and M'
$\delta_v(M–M')$ is the corresponding contribution to the standard electrode potential

		Li	Ag	δ(Ag–Li)	δ_v(Ag–Li)
First ionization potential (v)		5·40	7·58	+2·18	+2·18
Latent heat of sublimation* (kcal mole^{-1})		38	68	+30	+1·3$_0$
Thermodynamic functions (kcal mole^{-1}) for the process $M^+(g) + H^+(aq) \rightarrow M^+(aq) + H^+(g)$	ΔG°	138·39	146·02	+7·63	+0·331
	ΔH°	137·67	147·12	+9·45	
Standard electrode potential (v)		−3·045	+0·799	+3·844	

* For sublimation to monatomic vapour.

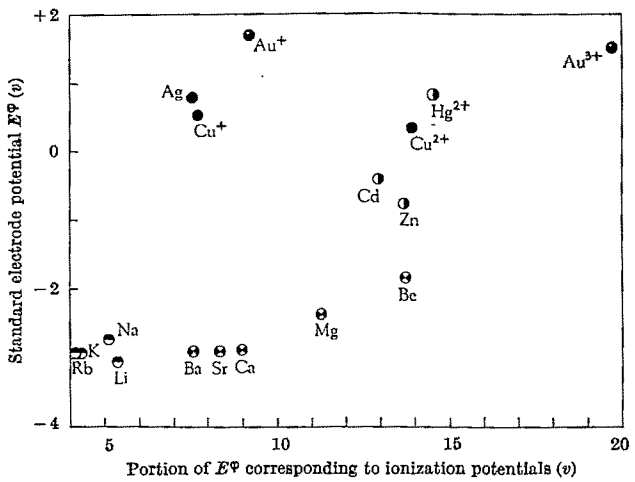


Fig. 1. Comparison of standard electrode potentials with contributions arising from ionization potentials
◓, Alkali metals; ⊖, alkaline earths; ●, coinage metals; ◑, zinc, cadmium, mercury

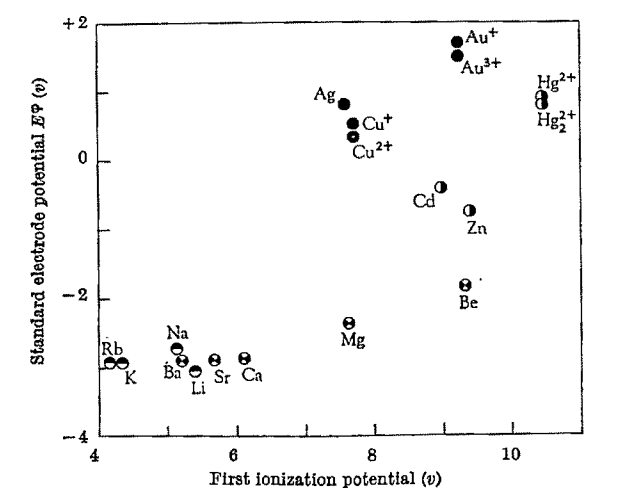


Fig. 2. Comparison of standard electrode potentials with first ionization potentials

with a definite quantity relating to a propensity to transfer electrons, the correlation[5] with the (thermionic or photoelectric) work function (Fig. 3). Even this relation is obviously of limited validity.

A detailed examination of certain pairs of metals (Table 2) confirms that any or all of processes (1)–(3), sublimation, ionization, or solvation, may be decisive for the observed differences of standard electrode potentials*. The difference between copper and beryllium is

* For each process, values of ΔG° should properly be compared rather than values of ΔH°. However, the terms due to changes in entropy are relatively small (for example, about 8 kcal mole^{-1} for sublimation), and do not vary greatly between one metal and another.

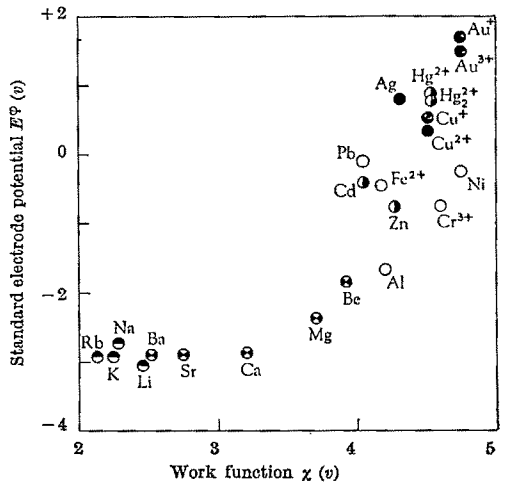


Fig. 3. Comparison of standard electrode potentials with work functions. (Values of the work function are from Rivière (ref. 6) and Landolt-Börnstein (ref. 7))

clearly due almost entirely to differences in the thermochemistry of solvation, while the difference between copper and zinc is mainly due to the large difference in their latent heats. The greatest contribution to the difference between lithium and silver comes from the difference in ionization potentials, but both other factors contribute (solvation making the smallest contribution).

In conclusion, the evidence shows clearly that standard electrode potentials do not reflect very closely any relative propensity of atoms to lose electrons.

Ionization of organic acids. Relative strengths of such acids as chloroacetic and acetic have been correlated with some success on the basis of an assumed 'inductive effect':

$$Cl \longleftarrow CH_2 \longleftarrow CO \longleftarrow O \longleftarrow H$$

reducing the chance of finding an electron between the final O and H, and thus weakening the binding of the proton to the remainder of the molecule. The ionization constants of such acids differ markedly, but among some groups of acids (Table 3) the strengths differ relatively little, and no interpretation in terms of 'inductive effects' is possible.

As is clear from the data[8,9] presented in Table 3, no regular change in acid strength results from substitution of either (1) n-alkyl groups of increasing length; or (2) successive methyl groups, for the α hydrogen atoms of acetic acid. The really significant point[10] about these acids is that their ionization constants at a given temperature are nearly the same.

Certain pairs of acids reverse their relative strength as the temperature changes. Thus, if differences between acetic acid and 2-ethyl butanoic acid were really a consequence of 'inductive effects', the inductive effect of the two ethyl groups would have to be in opposite senses above and below 29° C. Again, relative strengths of acids

Table 3. Data for the Ionization of Substituted Acetic Acids (in Aqueous Solution)

Values of pK_a at 25° C:
(1) CH_3COOH 4·756; CH_3CH_2COOH 4·875; $CH_3CH_2CH_2COOH$ 4·818; $CH_3(CH_2)_3COOH$ 4·845; $CH_3(CH_2)_4COOH$ 4·857
(2) CH_3COOH 4·756; CH_3CH_2COOH 4·875; $(CH_3)_2CHCOOH$ 4·849; $(CH_3)_3CCOOH$ 5·032

Temperatures at which the order of acid strength is inverted (the first member of each pair is the stronger at temperatures below that stated):

$(C_2H_5)_2CHCOOH$, CH_3COOH	29° C
$(CH_3)_2CHCOOH$, CH_3CH_2COOH	46° C
$C_2H_5CH_2COOH$, CH_3CH_2COOH	90° C

Thermodynamic functions for the process:
$HX(aq) + CH_3COO^-(aq) \rightarrow X^-(aq) + CH_3COOH(aq)$

Acid	[Formic]	Propanoic	Lactic	Chloracetic	Bromacetic	[Benzoic]
ΔG°	−1·4	+0·2	−1·2	−2·6	−2·5	−0·75
ΔH°	+0·1	−0·1	−0·1	−1·0	−1·1	+0·5
$-T\Delta S^\circ$	−1·45	+0·3	−1·2	−1·6	−1·4	−1·3

[Data for 25° C: ΔG°, ΔH° and $(-T\Delta S^\circ)$ in kcal mole^{-1}]

Table 4. Data for the Hydrogenation of Alkenes (after Kistiakowsky *et al.* (ref. 12))

Values of ΔH° (kcal mole^{-1}) for hydrogenation to alkanes
$R\,R'\,C = C\,R''\,R''' + H_2 \rightarrow R\,R'\,CH{\cdot}CHR''\,R'''$
in the gaseous phase at 82° C

Simple alkenes		Cyclic compounds	
Ethylene	−32·8	*Cyclo*pentene	−26·9
Propene	−30·1	*Cyclo*hexene	−28·6
But-1-ene	−30·3	*Cyclo*heptene	−26·5
Cis but-2-ene	−28·6	*Cyclo*-octene	−23·5
Trans but-2-ene	−27·6		
2-Methyl propene	−28·4		
2-Methyl but-1-ene	−28·5		
2-Methyl but-2-ene	−26·9		
2,3-Dimethyl but-2-ene	−26·6		

Dienes	
1/2 Buta-1,3-diene	−28·5
1/2 Penta-1,3-diene	−27·05
1/2 Penta-1,4-diene	−30·4
1/2 *Cyclo*pentadiene	−25·4
1/2 *Cyclo*hexa-1,3-diene	−27·7
1/2 *Cyclo*hepta-1,3-diene	−25·65

are sometimes reversed by change of solvent: in aqueous solution *o*-nitrobenzoic acid (pK_a 2·21) is stronger than 3,5-dinitrobenzoic acid (pK_a 2·80), but in ethanolic solution the reverse is the case[11] (respective pK_a's 8·62 and 8·09). Here, too, factors outside the molecule have to be taken into account.

One further important point brought out by the results[8] is that differences in strengths of acids in aqueous solution are in the majority of cases due largely or entirely to differences not in heats of ionization but in entropies of ionization.

Thermochemistry of the hydrogenation of the alkenes. Since accurate and reliable data[12] have been available for the heats of hydrogenation of numerous alkenes, various attempts have been made to explain the variations (Table 4) among the various substances. For example, buta-1,3-diene has often been compared with but-1-ene, and 'resonance energies' deduced. Over-enthusiastic interpreters seem, however, in general to have overlooked the fact that the heat of hydrogenation changes more between but-1-ene and *trans* but-2-ene than between but-1-ene and buta-1,3-diene. There are very considerable variations among compounds which are written with one isolated double bond (ethylene and *cyclo*-octene being the two extremes); and the variations between dienes classed as conjugated and those classed as not conjugated are smaller. In short, comparison with simple alkenes provides no thermochemical evidence for conjugation in dienes, and any discussions of such matters must rest on, for example, data for internuclear distances (cf. Pauling[2]).

We thank Dr. J. L. Copp, Dr. R. Foster, Dr. R. F. Jameson and Dr. E. T. Stewart for valuable discussions on a number of points related to this work.

One of us (P. G. W.) is the Edward A. Deeds Fellow of Queen's College, Dundee.

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GEOLOGY AND GEOCHRONOLOGY OF THE SEYCHELLES ISLANDS AND STRUCTURE OF THE FLOOR OF THE ARABIAN SEA

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THE Seychelles Archipelago in the western Indian Ocean consists of a large number of islands, most of which are coral atolls and sand cays of relatively recent construction. The Mahé-Praslin group of islands, the principal islands of the archipelago, are situated approximately 1,000 miles east of Mombasa, and have been known for some time to be composed largely of granite[1,2]. Recently, however, a comprehensive geological survey of the Seychelles has been made by one of us (B. H. B.)[3], and a number of age determinations of Seychelles rocks have been made by Miller and Mudie[4]. Furthermore, geophysical work is in progress in the Seychelles area as a part of the oceanographic research of the International Indian Ocean Expedition[5]. The Mahé-Praslin group consists of 24 inhabited islands totalling some 75 square miles in area, which rise from the central part of the Seychelles Bank, a submarine bank generally at less than 200 ft. below sea-level, which covers approximately 16,000 square miles, and is the northern extremity of the Mascarene Bank linking the Seychelles, Reunion and Mauritius.

The dominant rock type in the Mahé-Praslin group of islands, excepting Silhouette, is Precambrian hornblende granite which shows very little variation, and which is evidently the core region of a granite batholith. Although Bauer[2] reported blocks of hornfelsic sediments on Silhouette and Ile aux Cerf, these occurrences have not been found during subsequent surveys. No wall rocks are therefore exposed, and the margins of the batholith lie somewhere on the Seychelles Bank beyond the islands.

The Mahé granite is characterized by ubiquitous microperthitic feldspar and a deep green hastingsite amphibole. Some varieties of the granite in Praslin contain biotite in place of hornblende, and local variations in composition range between alaskite and granodiorite. In western Mahé and on some of the eastern islands of the group, granite porphyry and porphyritic granite are developed

Table 1. Results of Potassium–Argon Age Determinations

Material	Mineral	K_2O (%)	Atmos. contam. (%)	Vol. of radiogenic ^{40}Ar (mm^3 N.T.P.) / Weight of sample (g)	Apparent age ± error (m. yr.)
S/26 Hornblende granite, Mahé	Hornblende	0·70	55·4	0·01423	532 ± 46
S/101 Hornblende alaskite, Mahé	Hornblende	1·34	11·6	0·02826	549 ± 48
S/147 Hornblende granite, S. Mahé	Hornblende	1·12	12·5	0·02515	580 ± 50
S/75 Hornblende granite, Central Praslin	Hornblende	1·59	8·7	0·04061	647 ± 55
BT.1*a* Basalt dyke, N.W. Mahé	Total Rock	1·20	4·1	0·03054	645 ± 55
BS.3*a* Epidiorite, N.W. Mahé	Total Rock	1·63	7·1	0·03444	550 ± 48
BS.4 Metabasalt, N.W. Mahé	Total Rock	1·15	3·2	0·02510	566 ± 49
S/460 Syenite, S.W. Silhouette Is.	Altered pyroxene	0·88	79·0	0·00100	34 ± 7
S/460 Syenite, S.W. Silhouette Is.	Altered pyroxene	0·88	67·3	0·00185	62 ± 12
S/460 Syenite, S.W. Silhouette Is.	Altered pyroxene	0·88	71·1	0·00126	43 ± 8
BW.1 Olivine dolerite, Praslin	Total Rock	0·79	32·5	0·00137	52 ± 10
BW.1 Olivine dolerite, Praslin	Total Rock	0·79	45·4	0·00127	48 ± 9

$\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$ $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$

as a gradational facies of the main body. Aplites and small quartz veins are present, though pegmatites are absent. Certain zones of the Mahé granite, at the north-western coasts of the island, contain numerous flow-aligned xenoliths of dioritic and gabbroic composition, and larger partly assimilated rafts of hybrid intermediate plutonic rocks.

The granites display no structural features apart from jointing—they are anorogenic in character. Small shears trending north-east show evidence of slight dextral displacements.

Dolerite and basalt dykes are numerous on all the granitic islands, and occur as north-westerly trending swarms in the granite joint system. Two main varieties of dykes are distinguishable, porphyritic metadolerites and epidiorites of Precambrian age, and olivine alkali-dolerites probably of Tertiary age. The metadolerites are all affected by hydro-thermal alteration to some extent, with the formation of chlorite, the conversion of pyroxene to uralite, and, in extreme cases, saussuritization with the production of uralite, epidote and the breakdown of calcic plagioclase to sodic varieties and quartz. The olivine dolerites, on the other hand, are fresh or only slightly altered, and in most specimens an analcite mesostasis can be detected, indicating an alkaline tendency.

Silhouette Island and its neighbour, Ile du Nord, situated on the western side of the granitic islands, are composed of Tertiary syenite, diorite and microgranite. The greater part of Silhouette is composed of syenite, locally olivine-bearing, enclosing a microgranite stock. The occurrence of annular zones of microsyenite on the eastern side of the Island confirm that the intrusive is in the form of a syenite–microgranite ring complex of a kind common in Africa.

Age measurements were made on the samples listed here using the isotope dilution technique of potassium–argon dating. Potassium oxide contents were measured using a flame photometer, and argon-40 volumes estimated from the isotopic ratios of the argon released by fusion of the rock sample plus a known volume of enriched argon-38 added as internal standard.

Specimens *BT*.1*a*, *BS*.3*a*, *BS*.4 and *BW*.1 were measured using chippings of the whole rock as described by Miller and Mussett[6], whereas the remaining samples were measured on separated minerals. Results are shown in Table 1.

A previous measurement made on a sample of biotite from Mahé granite[4] yielded an age of 654 ± 17 million years. Wasserburg *et al.*[7] have since confirmed this figure by applying the rubidium–strontium method (638 ± 25 and 687 ± 34 million years). The average of the three samples of hornblende (*S*/26, *S*/101, *S*/147) from Mahé granite is somewhat lower (554 million years), due perhaps to slight alteration.

Hornblende from Central Praslin (*S*/75, 647 ± 55 million years) is in good agreement with the potassium–argon determination on Mahé biotite and the rubidium–strontium measurements of Wasserburg *et al.* It is concluded that the true age of the Mahé Island granite is about 650 million years.

Samples of basic rocks from Mahé are somewhat altered, thus the apparent ages they yield can only be taken as minimum values. The close agreement of sample *BT*.1*a* (645 ± 55 million years) with the age of the granite would suggest that they are closely associated genetically, and date from the late Precambrian.

The ages of the Silhouette syenite, though they show variations, average at 46 million years and indicate a late Eocene age for this ring complex. These variations are thought to have arisen from unrepresentative sampling of the altered pyroxene. Measurements on the olivine–alkali basalt dyke from Praslin (averaging at 50 million years) suggest that this phase of dyke injection was associated with the syenite–microgranite complex of Silhouette. This conclusion is supported by the common association of olivine alkali basalts and olivine syenites in other alkaline complexes.

The samples that have been dated are listed here, with localities and mention of the salient petrographic features:

S/26. Hornblende granite: 400 yards north-west of Anse Etoile, north-east Mahé. Patch-perthite and oligoclase feldspar, aggregates of fine deep green hornblende, iron oxide grains, sphene.

S/101. Hornblende alaskite: north-west summit of Trois Frères, Central Mahé. Perthite feldspar, ragged hornblende partially chloritized. Slight limonitic alteration.

S/147. Hornblende granite: south-east end of Anse Bazacar, near Pte. Police, south Mahé. Perthite feldspar, small deep blue-green riebeckite grains, some epidote and magnetite.

S/75. Hornblende granite: Nouvelle Decouverte survey beacon, Central Praslin. Turbid patch-perthite, minor oligoclase, sub-hedral blue-green hornblende.

BT.1*a*. Basalt dyke: west end of Bel Ombre bay, north-west Mahé. Prismatic plagioclase, augite mostly converted to uralite, iron oxide and brown chlorite. Pools of secondary calcite.

BS.3*a*. Epidiorite: 150 yards south of Sunset Café, north-west Mahé. Highly altered rock composed of hornblende, epidote, zoisite, chlorite and an oligoclase–quartz matrix.

BS.4. Metabasalt: 150 yards south of Sunset Café, north-west Mahé. A saussuritized basalt with chlorite in the matrix.

S/460. Porphyritic syenite: Roche Marceline, south-west Silhouette. Perthite feldspar, diopsidic pyroxene prisms altering to uralite–chlorite–magnetite aggregates with apatite.

BW.1. Olivine–dolerite: on Grand-Anse–Baie St. Anne road 100 yards south of the Vallee de Mai sign-post, Praslin. Almost unaltered olivine–augite dolerite. Partial chloritization of olivine and some carbonate in matrix.

Geochronology and Regional Geological Setting

The discovery that the Seychelles granites are of late Precambrian age renders their setting in an oceanic environment a unique geological phenomenon. The

Mahé–Praslin granite is almost certainly a single plutonic mass of anorogenic character, and must have been emplaced in a crust at least 1 mile thick. Seismic refraction work by Gaskell and Swallow[8] shows that granite underlies the coral on the Seychelles Bank and extends to a considerable depth. This crust is represented on the extensive Seychelles Bank, the central part of which is apparently composed of Precambrian granite[5], and is traversed by metadolerite dykes. The extent of this crust of continental type is unknown, but it is not unlikely that the adjacent Fortune and Saya de Malha Banks, which together with the Seychelles Bank form the northern extremity of the Mascarene Ridge, are composed of sedimentary or plutonic rocks. The islands of the southern part of the Mascarene Ridge—Reunion and Mauritius—are, however, relatively recent volcanoes. The Amirante Ridge to the west of the Mahé–Praslin group is covered by coral formations, but is topographically linked to the Seychelles Bank by a ridge some 5,000 ft. above the general level of the ocean floor, and may also be composed of sedimentary and igneous rocks, for it is in direct alignment with the major fault which bounds the eastern side of Madagascar.

It is therefore probable that the Seychelles area contains substantial expanses of continental formations which are partly or wholly of late Precambrian age. The ages obtained from the granites and metadolerites fall within the age-range of the Mozambique Belt of the eastern part of Africa and Madagascar[9]. This observation has led to renewal of the suggestion that the Seychelles may have originated during the fragmentation of Gondwanaland by continental drift[4,10]. The geology of the coastal region of East Africa and Mozambique contains evidence of intermittent down-flexuring of the Mozambique parageosyncline situated along the Mozambique Channel and in the Somali basin, from Karroo (late Carboniferous–Permian) to late Tertiary times[11]. It seems possible that the eastern part of the continent of Africa once extended as far as the line of the Mascarene Ridge, a hypothesis supported by the contrast between the form of the floor of the Arabian Sea and that of the remainder of the Indian Ocean[12]. The East African and Lebombo swells on one side and Madagascar and the Mascarene Ridge on the other may represent the marginal uplifts of this para-geosynclinal zone.

The occurrence of an early Tertiary syenite ring complex with associated alkali dolerite dykes strengthens the connexion with Africa, for similar complexes of about the same age are widespread from Kenya to Nyasaland. Such syenite complexes are typical of continental kratonic areas.

Present-day work by the geophysicists in R.R.S. *Discovery III* during the International Indian Ocean Expedition may be expected to throw much further light on the structure of the floor of the Arabian Sea. In particular, the hypothesis outlined here can be tested by gravity, magnetic and bathymetric traverses across the Mascarene and Carlsberg Ridges, and between the Seychelles and the East African coast. The nature and extent of the fracture on the eastern side of Madagascar and its northward continuation towards the Seychelles can also be investigated, together with the structure and composition of the crust in the Mozambique Channel and in the off-shore region of the East African coasts.

We thank Dr. G. J. Wasserburg *et al.* for informing us of their rubidium–strontium age determination on Mahé granite, prior to publication.

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LAVA TUNNELS ON SUSWA MOUNTAIN, KENYA

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SUSWA mountain (Ol Doinyo Nyukie) is situated centrally in the eastern Rift Valley, some 30 miles west-north-west of Nairobi. It is composed entirely of Quaternary volcanic rocks, mainly trachytes, soda-trachytes and phonolites, together with a variety of pyroclastic material. The section of the Rift Valley including Suswa has yet to be mapped systematically by the Geological Survey of Kenya, but a great deal of information has already been obtained by several Government officers during visits to the area[1]. Suswa is essentially a well-preserved caldera, some seven miles across, in the centre of which is situated a remarkable circular moat more than 700 ft. deep, a quarter to one mile wide and three and a half miles in outside diameter. The inner wall of the moat is formed by a massive cylindrical block of lava two miles across. The highest point of the mountain (7,732 ft.) is on the south side of the moat and on the rim of a large truncated explosion vent, while the surrounding plains lie at altitudes of 5,000–5,500 ft.

Large cave-like or tunnel-like features, leading from deep depressions in the lava surface on the eastern flanks of Suswa, were first reported by Mr. D. Hobden—a Government prospector—during geothermal investigations in 1962. A field party led by Mr. Hobden briefly examined the tunnels later in the same year and, in January 1963, I was present during a further expedition to Suswa when the underground features were investigated in a little more detail. The success of this excursion was largely attributable to the work carried out by Mr. L. Wateridge of the Kenya Veterinary Department, ably assisted by a party from the Royal Staffordshire Regiment.

Recognition of the Suswa tunnels depends on fortuitous roof collapse to produce roughly circular holes or depressions in the lava surface. Aerial photographs show no deep clefts in the lava, such as would be expected from the total collapse of a tunnel roof. At present, holes are confined to an area of less than a square mile immediately outside the original wall of the caldera, the distribution suggesting that two main tunnels or networks diverge eastwards. The only known underground features occur in a series of lava flows which poured eastwards through a breach in the caldera wall. Individual flows, which are evidently 30–60 ft. thick, were extruded rapidly one upon the other and preliminary examination suggests that the dark glassy lava is of phonolitic or trachytic composition. The flows clearly post-date the main caldera subsidence but eruption occurred before formation of the moat, which truncates lavas overlying those bearing the tunnels. These overlying flows partly inundated the caldera floor and poured over the rim to the south and east.

The holes which provide access to the tunnels contain mounds of lava blocks and soil representing the remains

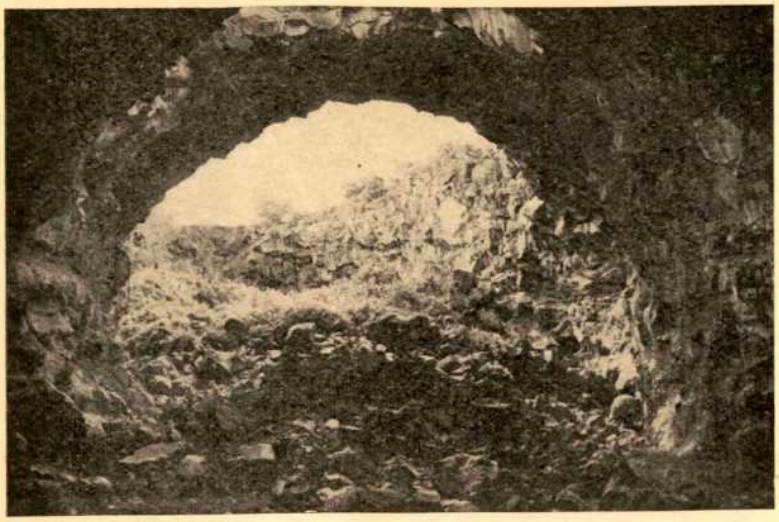


Fig. 1. Entrance to a lava tunnel showing the hole produced by collapse of the roof. The lava blocks in the foreground were derived from recent collapse immediately overhead. (*Photo: L. Wateridge*)

of the collapsed roof (Fig. 1). Smaller accumulations of lava blocks occur at intervals on the floors inside the tunnels and are readily related to early stages of roof collapse immediately overhead. The two tunnels so far investigated clearly represent parts of an originally continuous feature. They are approximately semi-circular in cross-section and attain a maximum height of 20–30 ft. at their entrances. A total length of about 600 yards was examined, one portion extending some 200 yards east of the main hole and the other running westwards. The west tunnel provides a link with a second area of roof collapse and continues for a short distance beyond it before becoming impassable.

Several minor passages were seen entering the main gallery above floor-level and, although at present unexplored, large caverns occur under at least part of the floor. These caverns are locally about 30 ft. deep and undoubtedly represent an earlier tunnel system immediately beneath the one examined. The roofs of the upper tunnels are 20–30 ft. thick and are composed of large irregular lava blocks formed by crude jointing, a feature largely responsible for their ready collapse; in contrast, the lava dividing upper and lower levels is often exceptionally thin. Near the holes produced by roof collapse, soils have been washed into the tunnels to form a smooth flat floor, broken by only occasional outcrops of ropy lava. Away from the tunnel entrances, the floors become more rocky and contain only isolated pockets of soil. A circular hole, some 3 ft. across, occurs on a small updomed portion of the floor of one tunnel, providing a view into a large cavern beneath. The floor at this point is extremely thin, and the bulge and hole give the impression of being explosion features. Minor explosions, caused by the combustion of gases generated from inundated vegetation, have been reported in Hawaii by Wentworth and Macdonald[2]. There, hydrocarbon gases are believed to have accumulated in tunnels in underlying lava and a similar explanation might well apply to the Suswa example.

All the tunnel walls are marked by flow and drip phenomena indicating some mobility of the lava during and after formation of the tubes. These wall features vary in appearance and form; many are stalactitic cones 2–6 in. long with the apices pointing downwards (Fig. 2); others have a fin-like or ropy structure with an approximate vertical orientation. In places, the entire wall crust flowed downwards, arching under at the lower end but retaining a cover of small stalactites.

Distinctive, highly vesicular ropy lava, found along floors and on some walls of the tunnels, is clearly the remnants of the mobile central portion of the flow which evacuated the tube. Some evidently came from overlying flows, entering a cavity beneath through collapsed portions of the roof. Ropy lava which festoons the tunnel walls with flow generally at right angles to stalactitic features represents benches left by subsiding lava in the tube and corresponds to similar occurrences described by Cotton[3]. Locally, aprons of ropy lava are draped over pre-existing ledges on the walls, emphasizing the junctions of individual lava flows and showing that the ropy lava must have been flowing from an upper to a lower tunnel. More convincing evidence of ropy lava flowing into a lower tunnel was seen at one point where the material forming an extremely thin floor apparently cascades into a cavity beneath, the upper tunnel terminating at this locality. Rarely, the ropy lava bears grooves caused by flow against solidified stalactites on the wall. On the other hand, a notable feature of part of one tunnel is a continuous gap about 6 in. wide between the ropy lava forming

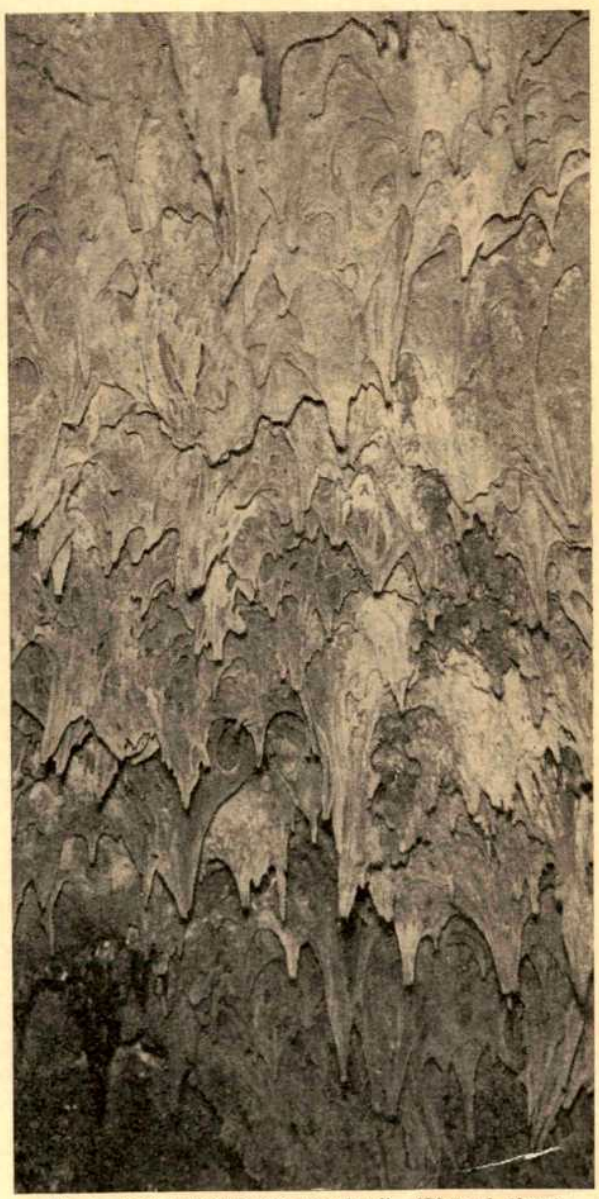


Fig. 2. Lava stalactites on a tunnel wall. (*Photo: J. Glover*)

the floor, and the tunnel walls. The latter continue to bear icicle-shaped stalactites down into the clefts, which are thought to have formed by the inability of the ropy lava to solidify against portions of the walls that had remained hot. The wall stalactites in the gap clearly do not represent 'shark-tooth projections'[2] caused by pulling away of the floor lava from the walls because the surface of the ropy material remains quite smooth. No lava stalagmites were seen in the tunnels examined and is is probably unlikely that any conspicuous ones ever formed judging by the small size of the stalactites. If stalagmites were produced, they have since been obliterated by movement of ropy lava.

The actual formation of lava tubes or tunnels in basaltic lava has been observed in Hawaii[2], where it was found that the process commences with flow in an open channel. A crust quickly forms and, a short distance from the vent, a continuous roof develops and thickens as lava congeals against its under side. Some tubes remain filled; in others the lava drains away to leave open tunnels which range up to 50 ft. in diameter, though only rarely exceeding 20 ft. In Hawaii the main tubes, which may extend for miles, branch into numerous smaller ones, each about 1–3 ft. across, that feed the advancing flow front.

Although the complete pattern of the Suswa tunnels is as yet unknown, it seems remarkable at first sight that they should have developed immediately above each other in successive lava flows. Such an arrangement is readily explained, however, because the dividing lava was thin, allowing the mobile central portion of an overlying flow to drain away through collapsed areas of its floor. Lava flow in open channels, if it ever occurred, probably played only a minor part in the development of these features, and formation of the earliest tunnels probably depended on unusual circumstances, such as extrusion of the lava over patches of hot ground which helped to keep portions of the flow mobile. Steam jets accompanied by small areas of heated ground occur abundantly on Suswa mountain at present. Lava tubes have not been reported from surrounding areas of the Rift Valley where many of the volcanic rocks are phonolites and soda-trachytes similar to those at Suswa. Small tunnels have, however, been noted[4] in Recent basalt flows in the Chyulu range, north-east of Kilimanjaro, and examples described from other parts of the world also appear to have developed primarily in basaltic lavas.

I thank Dr. P. E. Glover, Veterinary Department, Nairobi, for providing the opportunity to examine the tunnels, and also the Commissioner of Mines and Geology, Kenya, for permission to publish this account.

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CONCENTRATIONS OF COMMON LEAD IN SOME ATLANTIC AND MEDITERRANEAN WATERS AND IN SNOW

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KNOWLEDGE of lead in the oceans is important not only to investigations of lead isotope evolution in the Earth's crust, but also to those of oceanic and atmospheric mixing. The concentrations of common lead in sea-waters off southern California have been previously examined[1], and were found to decrease abruptly from surface to deep waters. The high concentrations of lead in surface waters were ascribed to industrial contamination, mainly lead tetraethyl aerosols. The work recorded here is an extension of these investigations to the Atlantic and Mediterranean Oceans. Samples of rural snow were also analysed because the finding of high concentrations of lead in such precipitation would validate the view that the oceans have been contaminated by lead tetraethyl aerosols.

Samples of sea-water were collected by means of the stainless steel device used in previous investigations. The sites of the collecting stations are shown in Fig. 1. Because of the extensive use of lead compounds and paints aboard ship, extreme caution was continuously exercised to prevent contamination. Lead in sea-water was assayed by isotope dilution procedures which have been outlined earlier[1].

Snow was collected from central portions of the strata of three separate falls (aggregating about 20 in.) in an open meadow inside the south entrance of Lassen Volcanic National Park. The site was 6 miles from a lightly travelled State highway at an elevation of about 7,000 ft. The branch road had been closed to motor-cars since the time of the first snow fall. Snow was scooped by a stainless steel shovel into a stainless steel funnel, and then forced into polyethylene bottles with a 'Teflon' ramrod. Again, special precautions were taken to prevent contamination. Plastic coverings were worn over outer clothing to minimize contamination from these articles while operations were carried out in the field. The concentration of lead in snow was determined by isotope dilution, the analytical procedure being similar to that used for sea-water. It was necessary to show that lead was not a constituent of dust, therefore the concentration of silica was also determined. This was achieved by adding pure graphite to melted snow in a 'Teflon' beaker, evaporating to dryness, and measuring silicon in the graphite by emission spectrography.

Results of the analyses of sea-water are listed in Table 1. The average error is ± 30 per cent, which is determined

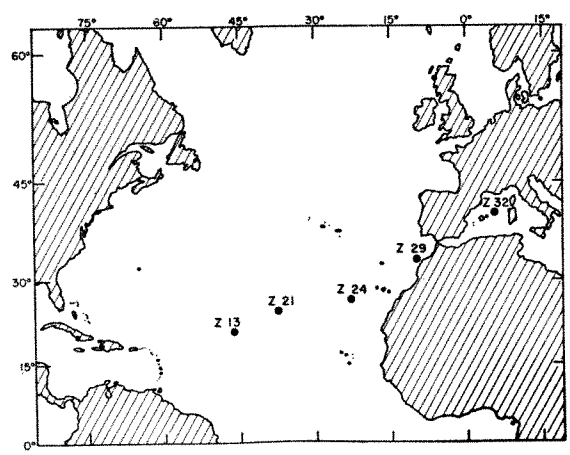


Fig. 1. Location of sampling stations

Table 1. Concentrations of Common Lead in Atlantic and Mediterranean Sea-water

Station No.†	Location	Bottom depth (m)	Uncorrected sample depth (m)	Salinity (‰)	Lead (μg/l.)
Z-13	17° 27·7′ N. 55° 21·6′ W.	3,914	50	n.d.	0·06
			1,000	34·931	0·08
			2,500	34·964	0·02
Z-21	21° 06′ N. 46° 30·5′ W.	5,139	50	n.d.	0·06*
			1,000	35·207	0·08
			3,150	34·939	0·10*
			5,300	34·887	0·02*
Z-24	26° 41·5′ N. 22° 56·1′ W.	4,962	50	n.d.	0·04
			1,000	n.d.	0·06
			3,050	35·016	0·11
Z-27	33° 35·4′ N. 9° 43·2′ W.	4,332	50	n.d.	0·09
			1,000	35·679	0·002
			2,500	35·039	0·09
			4,000	34·898	0·03
Z-32	40° 39′ N. 05° 48′ E.	2,725	50	n.d.	0·20
			1,000	38·419	0·03
			1,850	38·401	0·02
			2,720	38·411	0·02

* These values are averages of two or more determinations.
† The stations were occupied during the last two weeks in July 1962, on the Atlantic leg of the Scripps Institution of Oceanography *Zyphrus* Expedition.

largely by the amount of contamination lead relative to sample lead. Contamination and reproducibility were determined by multiple analyses of 4- and 8-l. samples from station Z-21. These characteristics were then transferred to other determinations, which were single analyses of either 4 or 8 l. of water. Yields were checked colorimetrically, and analyses associated with aberrant yields were rejected. As discussed previously, this procedure measures dissolved lead only and does not refer to particulate lead.

Results of analyses of 0·01 N hydrochloric acid-soluble lead in snow are shown in Fig. 2. The concentration, as determined by the slope of the regression, is about 2 μg lead/kg snow. In terms of the quantities of snow analysed, this is a very large value, and there is no possibility that it can be ascribed to contamination during collection, transportation, or analysis. No attempt was made to control sample homogeneity, so that in retrospect we are certain that varying amounts of the three falls of snow and of sections within the falls were introduced into our different containers. This unfortunate circumstance probably accounts for the scatter of the individual results and for the spurious contamination intercept, since it is quite possible that the lead content of snow will not only vary from fall to fall, but also will vary during a fall. Except for sample inhomogeneity, the concentration

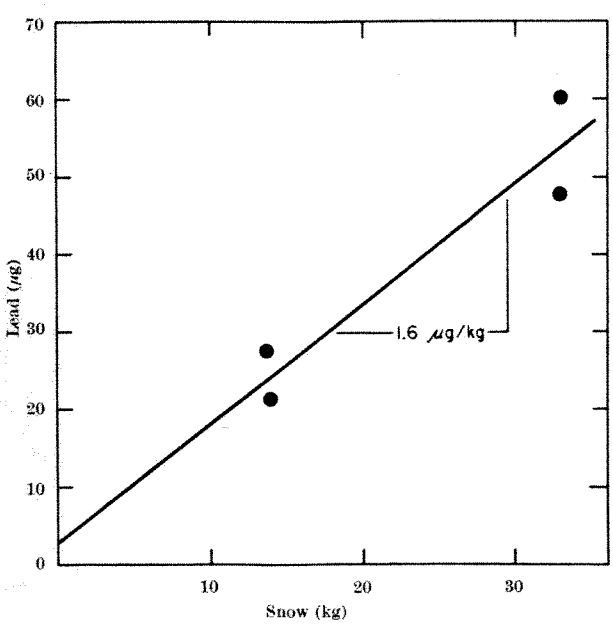


Fig. 2. Concentration of lead in snow. The large scatter in lead values probably results from sample inhomogeneity

analyses of snow should be as accurate as those for sea-water. The isotopic composition of lead from snow was also measured. The values were: $^{206}Pb/^{204}Pb = 18{\cdot}01$; $^{206}Pb/^{207}Pb = 1{\cdot}144$; and $^{206}Pb/^{208}Pb = 0{\cdot}469$. The concentration of total silicon in this snow was 6 μg/kg. This figure is accurate to ± 25 per cent, and can be used as an index of the silicate dust content where silicate dust contains approximately 30 per cent silicon. The average concentration of lead in shale or igneous rock is 15 p.p.m.

In our previous work it was shown that, if the high concentrations of lead observed in a few surface waters of the Pacific were representative of the sea surface of the entire northern hemisphere, the bulk of this lead could be readily accounted for as originating from leaded gasolines. A necessary condition for this point of view is that rain or snow should contain abnormally high concentrations of lead, that is, the concentrations should exceed those which might ordinarily be assigned to silicate dusts. We have found the concentrations of lead and silicon to be about equal in snow collected from a high altitude and 500 miles removed from the Los Angeles complex. In this case, the concentration of lead exceeds that expected from dust alone by a factor of 10^4, which strongly supports the idea of contamination from leaded gasoline exhausts.

Moreover, the concentration of lead measured in snow was found to be 10–100 times larger than those measured in sea-water, so that only a small number of years of such precipitation are required to provide the amount of lead now present in the surface waters of the oceans of the northern hemisphere. The isotopic compositions of lead in surface and deep oceanic waters are not yet known, but the average isotopic compositions of leads precipitated from the oceans at various locations during periods extending throughout the past 10^5 to 10^6 years have been measured[2]. The isotopic compositions of leads in Quaternary pelagic sediments lying within a 1,000 mile-wide strip along the west coast of North America are listed in Table 2.

Table 2. Isotopic Composition of Lead in Pelagic Sediments* and in Snow in the Region of Western North America

Atomic ratio	Range Low	Range High	Mean (of 11 stations)	Snow
206/204	18·63	18·85	18·71	18·01
206/207	1·190	1·203	1·199	1·144
206/208	0·470	0·485	0·483	0·469

* Corrected according to Chow and Patterson (ref. 3).

In this area of the world the composition of a sample of recent snow lead lies far outside the composition-range of oceanic leads deposited during prehistoric times. It seems possible that lead tetraethyl contamination can be used as a tracer in the investigation of oceanic circulation.

The concentration profiles of lead in the Atlantic were found to be different from those in the Mediterranean and the Pacific. This is illustrated in Fig. 3. At the Mediterranean and Pacific stations, lead concentrations are high in surface waters and uniformly low in deeper waters. At the Atlantic stations the concentrations do not follow such a uniform trend with depth, and, in addition, the scatter is confined to considerably smaller values. However, in general, lead concentrations at the Atlantic stations appear to increase from surface to intermediate or deep waters, and then decrease again in very deep or bottom waters. Common lead concentrations in Central North Atlantic surface waters are about 0·05 μg/l. Values in offshore surface waters increase to 0·09 μg/l. near Morocco and to 0·2 μg/l. in the Mediterranean.

There are two different interpretations of these results which can be outlined as follows: (1) the Atlantic stations were thousands of miles from large industrial centres while the Pacific and Mediterranean stations were within a few hundred miles of them, and the two types of concentration profiles might reflect two different degrees of industrial lead contamination, the most natural or least contaminated conditions being represented by the Atlantic profiles; (2) industrial lead contamination, being pervasive but very recent in the oceans, has not appeared in the deep

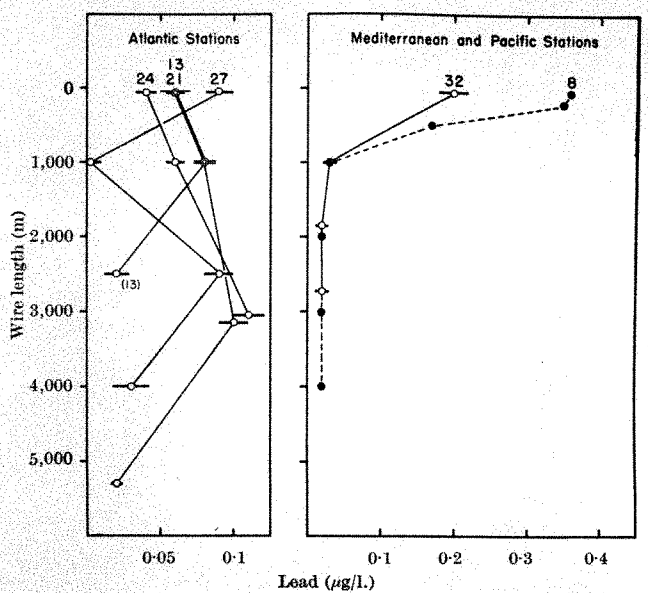


Fig. 3. Lead concentration profiles in the Atlantic and Mediterranean Oceans. Pacific Station (8) is given for comparison (data from ref. 1). The length of the horizontal bar at each point denotes estimated analytical uncertainty. Note the expanded concentration scale for the Atlantic stations

waters of the Pacific and Mediterranean because such waters are centuries old, but it might appear in the deep waters of the Atlantic if such waters are only decades old.

Some investigations suggest that intermediate and deep Atlantic waters may be young. Bowen and Sugihara[4] have found nuclear bomb debris (strontium-90, cerium-144 and promethium-147) to be present in considerable amounts at depths of one thousand metres in the Atlantic. Bowen and Sugihara believed that distribution of strontium-90 shows actual water mass movement and cerium-144 and promethium-147 follow particle settling. It should be pointed out that Miyake *et al.*[5] believed that a high rate of eddy diffusion does account for the bomb debris which they have found at great depth in the Pacific. Broecker[6] reported that he could not find bomb debris at the great depths investigated by Bowen and Sugihara. Perhaps the best arguments for young waters at depth in the Atlantic rest on calculations of current velocities from geostrophic and density effects. Wüst (quoted in ref. 7) estimated minimum velocities of about 500 km/yr. for deep waters originating near the surface at high north latitudes and flowing southward in the North Atlantic. It is this current that could be expected to show high concentrations of common lead at great depths near 20°–30° north latitudes, since it originates at the surface in regions of high precipitation (and lead aerosol contamination) and sinks and flows southward at velocities large enough to convey the effects of contamination occurring within the last decade or so. The 'young water' interpretation of lead concentrations in the Atlantic rests heavily on these latter calculations.

Other investigations suggest that deep Atlantic waters are centuries old, or even older. Koczy[8] found that radium-226 concentrations in the Atlantic doubled from surface to deep waters and he attributed the difference to radioactive decay, since he believes radium-226 in surface waters originates from the sediment-water interface. Koczy estimated turn-over times in the order of one thousand years in the Atlantic. Recently, Koczy (private communication, 1963) calculated a total velocity over the Atlantic of about 5 m/yr. and the turn-over time as 400–600 years. It is possible that the interaction of the biosphere with trace elements such as radium-226 may account for their depletion in surface waters, thus causing over-estimates of turn-over times by this model. Broecker *et al.*[9] observed the depletion of carbon-14 in deep Atlantic waters and interpreted their results so as to yield ages of a few centuries for those waters. It has not yet been shown how these ages can be in error by an order of magnitude. The interpretation of the common lead concentrations in the Atlantic which holds that these are least contaminated by leaded gasoline exhausts depends heavily on the carbon-14 age determinations by Broecker *et al.*[9].

Lead in the surface layers of both the Atlantic and Pacific Oceans may constitute no more than a fourth of all dissolved oceanic lead, so that it can be ignored as an unnecessary complication when the average concentration of common lead in sub-surface Atlantic waters is compared with that in sub-surface Pacific waters. The difference is large enough to merit attention: lead is more concentrated in sub-surface Atlantic waters by a factor of two. This need not be regarded as evidence for rapid 'mixing in' of tetraethyl lead contamination in Atlantic waters. The amount of river run-off entering the Atlantic exceeds that entering the Pacific, and it would be expected that more soluble denudation lead was introduced into the Atlantic before appreciable amounts of lead tetraethyl had been manufactured. In addition, this would be diluted by a smaller volume of sea-water in the Atlantic.

It may be worthwhile to consider whether agricultural stimulation of soluble denudation is responsible for the increase of concentration of lead in deep Atlantic waters. Such effects would appear in waters that are centuries old and might be more pronounced in the Atlantic than in the Pacific. These effects might be small, since it has been estimated[1] that the amounts of additional lead introduced into the oceans as a result of agricultural stimulation are only about one-tenth of the lead introduced to the oceans from lead tetraethyl and other industrial lead chemicals.

The isotopic compositions of dissolved lead in ocean-water have not yet been measured. It is possible that such measurements, when made, will settle some of the issues presented here and provide a better understanding of the nature of oceanic circulation. This should be true because we have found the composition of lead in snow to be very different from the composition of lead which has been deposited on the ocean floors during the past 10^5 years. This is what would be expected if lead in snow was a man-made mixture from a few lead mines instead of a natural mixture from large continental surface areas. It seems very likely that this artificial mixture, added to the oceans during the past three decades, can be used as a tracer for investigating the ocean circulation.

The sea-water samples were collected and spiked by M. Koide and Prof. E. Goldberg of the Scripps Institution of Oceanography. We thank them for their considerable efforts and attention to detail which were required during these operations. Ship time was provided by the University of California, San Diego. C. Bauman of the California Institute of Technology aided in the development of the snow-collecting apparatus. This investigation was supported by American Petroleum Institute project 53 and U.S. Atomic Energy Commission Contract No. *AT*(04–3)–427. Publication has been authorized by the Director, U.S. Geological Survey.

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FOSSIL DINOFLAGELLATES FROM UPPER TRIASSIC SEDIMENTS

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IN the course of a palynological examination of core samples from the Stowell Park borehole put down for H.M. Geological Survey, Dr. W. G. Chaloner encountered fossil dinoflagellates in a preparation from the Upper Triassic (Rhaetic); these were passed to me for description. Jansonius[1] has described and figured three imperfectly known forms in residues from the Permian and Lower Triassic of western Canada as dinoflagellates: two of these, each based on a single specimen, show a clear transverse furrow and afford faint indication of tabulation, the third being treated as a dinoflagellate on grounds of overall morphology alone. The earliest tabulate dinoflagellates previously known were from the Lower Jurassic (Hettangian) of Denmark[2]. The Stowell Park specimens are the first to be described from the Upper Triassic, and the earliest known dinoflagellates with a determinable tabulation.

The sample was from a depth of 2,059 ft. 2 in. in the borehole put down at Stowell Park, Northleach, Gloucestershire (specimen No. *Bj* 6011; location of borehole, Ordnance Survey, 7th Edition, One-Inch Sheet 235, National Grid reference *Sp* 08351173), the stratigraphy of which was described in 1956 [3]. It consisted of greenish calcareous sandstone from a position in the Cotham Beds, 1 ft. above typical marine shales with *Pteria contorta* and 4 ft. below marls with '*Estheria*'. The microfossils here described are contained in slide number *P.S.* 1983, in the collections of H.M. Geological Survey; they are stained with safranin and mounted in glycerine jelly.

More than 120 complete or partial shells, attributable to two new species, were found. Complete shells are relatively few in number; in both species, rupture of the theca after encystment occurred just above the anterior margin of the transverse furrow, the greater part of the epitheca being thrown off; the bulk of the specimens comprise either epithecæ or hypothecæ with transverse furrow. A numerical analysis of 100 incomplete shells produced the following result:

Gonyaulax rhaetica sp. nov. Epithecæ 27.
Hypothecæ with transverse furrow 37.
Gonyaulax chaloneri sp. nov. Epithecæ 10.
Hypothecæ with transverse furrow 26.

The proportionately greater number of hypothecæ probably merely reflects a tendency of the epithecæ, when thrown off, to become so damaged as to be unrecognizable.

This method of archæopyle formation, by breakage immediately anterior to, and not along, the transverse furrow, has not previously been described (cf. ref. 4).

Systematic Description of Species.

CLASS DINOPHYCEAE
Family Gonyaulaceae
Genus *Gonyaulax* Diesing
Gonyaulax rhaetica sp. nov.
Figs. 1 and 2 (left)

Diagnosis. A species of *Gonyaulax* having a spindle-shaped shell, with a cover of coarse granules or very short spines, absent from the furrows. Transverse furrow broad, lævorotatory; longitudinal furrow narrow on the epitheca but broadening considerably as it approaches the antapex. Low ridges outline the following tabulation—four apical plates (1–4′), with plate 1′ elongate and situated in the anterior prolongation of the longitudinal furrow; one anterior intercalary plate (1*a*); six pre-equatorial

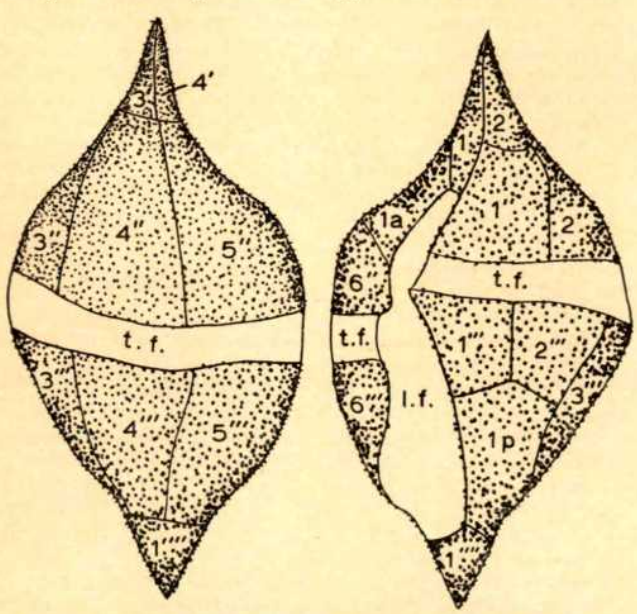


Fig. 1. *Gonyaulax rhaetica* sp. nov. Left, in dorsal view; right, in ventral view. Sketch based on the holotype and numerous paratypes. *t.f.*, transverse furrow: *l.f.*, longitudinal furrow

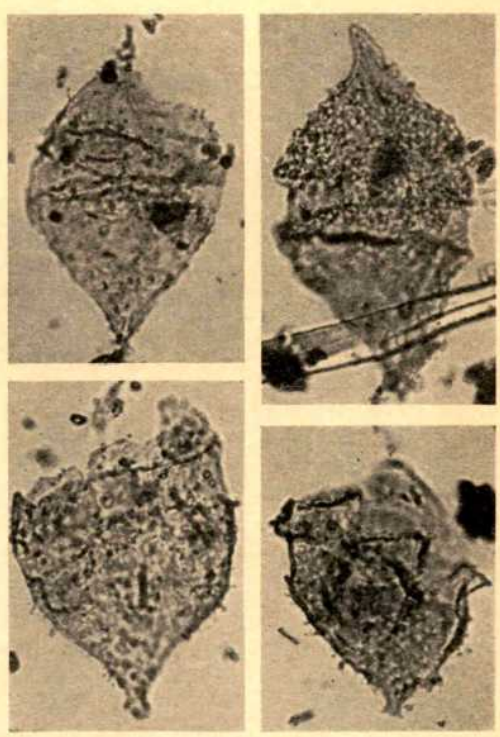


Fig. 2. Top left, *Gonyaulax rhaetica* sp. nov.: the holotype, in dorsal view; bottom left, *G. rhaetica* sp. nov.: a paratype, lacking the epitheca; top right, *Gonyaulax chaloneri* sp. nov.: the holotype, in oblique ventral view; bottom right, *G. chaloneri* sp. nov.: a paratype, lacking the epitheca

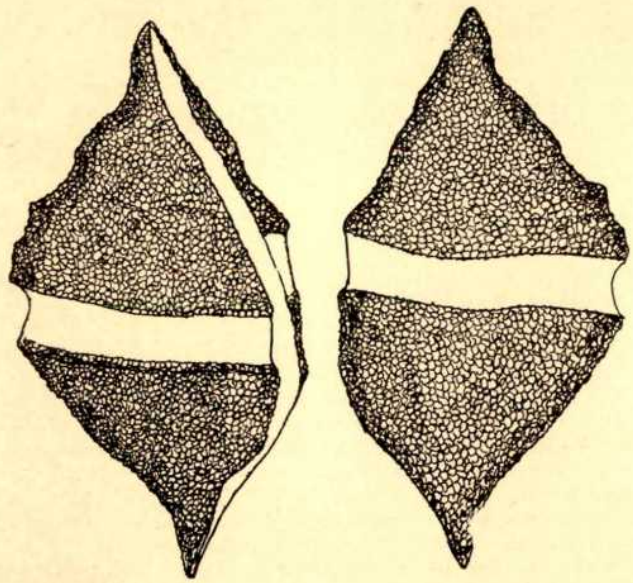


Fig. 3. *Gonyaulax chaloneri* sp. nov. Left, in oblique dorsal view; right, in oblique ventral view. Sketch based on the holotype

plates (1–6″); six post-equatorial plates (1–6‴), plates 1‴ and 2‴ being reduced and quadrate; one posterior intercalary plate (1*p*); and one antapical plate (1⁗).

Holotype. Specimen 98, slide *P.S.* 1983. *Dimensions.* Holotype; length 65μ, breadth 40μ. Estimated range; length 60–90μ (based on complete specimens and on probable original size of incomplete specimens). *Number of Specimens*, 75.

Derivation of Name. Named after the Rhætic Stage of the Triassic, in which this assemblage occurs.

Remarks. Gonyaulax rhaetica differs in its combination of symmetry and tabulation from all other described fossil species.

Gonyaulax chaloneri sp. nov.

Figs. 2 (right) and 3.

Diagnosis. A species of *Gonyaulax* having a spindle-shaped shell with a dense reticulate ornamentation formed by low ridges on the shell surface; this ornament is absent from the furrows. Transverse furrow very broad, lævo-rotatory; longitudinal furrow narrow, extending from apex to antapex. A tabulation is suggested but was not capable of determination, even in approximate terms, in any specimen seen.

Holotype. Specimen 43, slide *P.S.* 1983. *Dimensions.* Holotype; length 75μ, breadth 39μ. Estimated range: length 50–80μ (based on complete specimens and on probable original size of incomplete specimens). *Number of Specimens*, 45.

Derivation of Name. Named in honour of Dr. W. G. Chaloner, Department of Botany, University College, London.

Remarks. Gonyaulax chaloneri differs in its combination of symmetry and shell ornament from all other described fossil species. In absence of a determinable tabulation, its attribution to *Gonyaulax* was made largely on the basis of comparable symmetry and manner of pylome formation to *G. rhaetica*. Species of *Gonyaulax* having a reticulate ornament, but quite different in shell shape, have been described previously, for example, *G. areolata* Sarjeant[5].

I thank the Director of the Geological Survey for permitting description of these forms. I also thank Dr. F. W. Anderson and Dr. W. G. Chaloner for their help and advice.

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QUINACILLIN: A NEW PENICILLIN WITH UNUSUAL PROPERTIES

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Chemistry and Biology Divisions, Research Department, Boots Pure Drug Co., Nottingham

ACYLATION of 6-aminopenicillanic acid (6-APA)[1] has provided a number of clinically effective, semi-synthetic penicillins[2] some of which are resistant to hydrolysis by staphylococcal penicillinase. In the course of a programme concerned mainly with heterocyclic side-chain penicillins, we noted at an early stage that 2-pyridylpenicillin (I) was inactivated more slowly than was benzylpenicillin by staphylococcal penicillinase and was more active against moderately benzylpenicillin-resistant strains of *Staphylococcus aureus* than were the 3-pyridyl-, 4-pyridyl- or phenyl-analogues. Alkyl substituents in the pyridine ring had little effect on the activity but in the case of the 5,6-benzo-derivative, 2-quinolylpenicillin (II), the activity was increased against both sensitive and moderately resistant strains.

Concurrently, efforts had been directed towards the preparation of *bis*-penicillins, particularly from vicinal dicarboxylic acids, on the supposition that the two penicillanic acid moieties might provide mutual steric protection against penicillinase attack. In apparent agreement with this hypothesis, the penicillins prepared from pyridine-2,3-, pyrazine-2,3-, and 5,6-dimethylpyrazine-2,3-dicarboxylic acids by the mixed anhydride method[3], using two equivalents of ethyl chloroformate and of 6-APA, had significant activity against highly resistant strains of *Staphylococcus aureus*. Where the carboxy-groups were non-vicinal, as in pyridine-2,6-, pyridine-2,5-, and pyrazine-2,5-dicarboxylic acids, the derived penicillins were of low activity. It was expected by analogy with (I) and (II) that the penicillins (III) and (IV) derived from quinoline-2,3-dicarboxylic acid and quinoxaline-2,3-dicarboxylic acid, respectively, would show a much enhanced activity and this expectation was fully realized (Table 1).

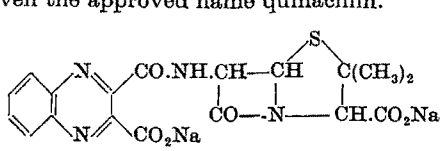
Further investigation into the application of the mixed anhydride method to the preparation of penicillins from vicinal dicarboxylic acids showed that the supposed *bis*-penicillins (III) and (IV) were in fact mixtures of mono-penicillins. In a model experiment, *bis*-triethylammonium phthalate (1 mol) in tetrahydrofuran was reacted with ethyl chloroformate (2 mol), triethylammonium chloride removed, and the filtrate reacted with *cyclo*hexylamine to give *N-cyclo*hexylphthalamic acid[4] with no trace of *N,N′*-di*cyclo*hexylphthalamide[5]. Furthermore, low-temperature (5°) evaporation of the filtrate before treatment with *cyclo*hexylamine afforded only phthalic anhydride and diethyl pyrocarbonate[6]. Similarly, quinoline-2,3- and quinoxaline-2,3-dicarboxylic acids gave the corresponding cyclic anhydrides and diethyl pyrocarbonate. The reaction conditions used in the preparation of (III) and (IV) would therefore be expected to give mixtures of ethoxypenicillin, derived from diethyl pyrocarbonate, and the carboxy-mono-penicillin derived from the cyclic anhydride. Evidence from paper chromatograms (butan-1-ol (4) : ethanol (1) : water (5), Whatman No. 1) supported these conclusions. Paper chromato-

Table 1. ACTIVITY OF SOME HETEROCYCLIC PENICILLINS AGAINST *Staphylococcus aureus*

Penicillin side-chain acid	Penicillin	M.I.C.* μg/ml.		
		Highly penicillin-resistant	Moderately penicillin-resistant	Penicillin-sensitive
Phenylacetic	Benzyl	> 500	33·3	0·01
Pyridine-2-carboxylic	I	500	11·1	0·4
Pyridine-3-carboxylic		> 500	100	1·2
Pyridine-4-carboxylic		500	100	0·4
3-Methylpyridine-2-carboxylic		500	33·3	0·4
6-Methylpyridine-2-carboxylic		500	3·7	0·4
Quinoline-2-carboxylic	II	500	1·2	0·04
Pyridine-2,3-dicarboxylic		11·1	11·1	3·7
Pyrazine-2,3-dicarboxylic		33·3	11·1	1·2
5,6-Dimethylpyrazine-2,3-dicarboxylic		33·3	11·1	3·7
Quinoline-2,3-dicarboxylic	III	0·4	0·4	0·4
Quinoxaline-2,3-dicarboxylic	IV	0·4	0·4	0·4
6,7-Dimethylquinoxaline-2,3-carboxylic†		11·1	3·7	3·7
6,7-Dichloroquinoxaline-2,3-dicarboxylic		33·3	11·1	3·7

* Minimum inhibitory concentrations, tested by serial dilution in peptone, yeast-extract agar; surface-inoculated and incubated for 24 h at 37°.
† Prepared by Dr. N. W. Bristow.

graphy of the crude quinoline-derivative (III) gave a bioautograph with three zones of inhibition, R_F 0·20, 0·45, and 0·60, against a benzylpenicillin-sensitive strain ('Dakin') of *Staphylococcus aureus*. Only the two zones, R_F 0·20 and 0·45, were observed when the test organism was a benzylpenicillin-resistant strain (N.C.T.C. 8452) of *Staphylococcus aureus*. Similarly, the crude quinoxaline-derivative (IV) gave two zones, R_F 0·12 and 0·60, against 'Dakin' but only the one zone, R_F 0·12, against '8452'. The zone of inhibition, R_F 0·60, was attributed to ethoxypenicillin since an authentic sample, prepared from ethyl chloroformate and 6-APA, had the same R_F and was inactive against *Staphylococcus aureus* 8452. (In agar dilution tests, ethoxypenicillin did not inhibit highly-resistant strains of *Staphylococcus aureus* and was active against sensitive strains only at concentrations greater than 10 μg/ml.) Quinoxaline-2,3-dicarboxylic anhydride reacted with 6-APA to give a single product, 3-carboxy-2-quinoxalinylpenicillin, R_F 0·12 ('Dakin' or '8452'), while the unsymmetrical quinoline-2,3-dicarboxylic anhydride gave a mixture of two products, R_F 0·20 and 0·45, presumably the isomeric 2-carboxy-3-quinolyl- and 3-carboxy-2-quinolyl-penicillins. The products from the cyclic anhydrides were highly active and accounted for the activities of the mixtures (III) and (IV) against *Staphylococcus aureus* 8452. The penicillins from both the cyclic anhydrides were of sufficient potential interest to merit clinical assessment. However, 3-carboxy-2-quinoxalinylpenicillin offered considerable advantages because of its accessibility and its freedom from the complication of isomerism presented by the quinoline analogue. The disodium salt (V) was selected as the most suitable form for medical and veterinary use and has been given the approved name quinacillin.



V

Quinacillin is prepared readily by the condensation of quinoxaline-2,3-dicarboxylic anhydride with 6-APA in dimethylformamide and triethylamine[7]. The product separates from acetone as the *bis*-triethylammonium salt monohydrate, m.p. 135°–137° (decomp.), $[\alpha]_D^{20}$ + 142° (c, 0·376 in water) (found: C, 56·3; H, 7·4; N, 13·4; S, 5·1; H_2O, 3·3; $(C_2H_5)_3N$, 31·4. $C_{30}H_{46}N_6O_6S$, H_2O requires C, 56·6; H, 7·55; N, 13·2; S, 5·0; H_2O, 2·8; $(C_2H_5)_3N$, 31·7 per cent). Treatment of an aqueous solution of the *bis*-triethylammonium salt with saturated aqueous sodium acetate gives quinacillin as cream needles, m.p. about 260° (decomp.), usually containing about 9 per cent water after drying *in vacuo* at 40° (found: C, 42·7; H, 4·3; N, 11·3; S, 5·9; H_2O, 9·2. $C_{18}H_{14}N_4Na_2O_6S$ with 9·2 per cent water requires C, 42·6; H, 3·8; N, 11·05; S, 6·3 per cent), λ_{max} 242 (ε, 32,100) and 326 mμ (ε, 7,280), ν_{max} 3,440, 1,759, 1,692, 1,596, 1,524, 1,415, 1,323 and 779 cm^{-1} (potassium bromide disk). Anhydrous quinacillin, obtained by drying at 100°/2 mm, m.p. 261°–262° (decomp.) and $[\alpha]_D^{23}$ + 183·5° (in water) (found: C, 47·0; H, 3·3; N, 12·1. $C_{18}H_{14}N_4Na_2O_6S$ requires C, 46·95; H, 3·0; N, 12·2 per cent), is very hygroscopic. There is little definite evidence for the formation of any true hydrate, and as much as 30 per cent of water may be taken up at 21° depending on the relative humidity. Quinacillin acquires a bright yellow colour on exposure to strong sunlight but is stable at 100° for at least 3 months. It is freely soluble in water and a 25 per cent aqueous solution is stable for 2 months at 0° and has a half-life of 12 days at 37° (hydroxylamine assay). The half-life in solution in 50 per cent ethanolic 0·1 N hydrochloric acid is 290 min (hydroxylamine assay). This very high acid stability is consistent with the presence in quinacillin of an electron-attracting group situated β to the side-chain amide carbonyl group as in ampicillin and other acid-stable penicillins[8]. Quinacillin gives a deep violet chelate with ferrous ions and a red colour with cuprous ions.

For a penicillin, quinacillin has some unusual properties. Thus, antimicrobial activity is highest against *Staphylococcus aureus* and both antimicrobial activity and rate of diffusion in agar are enhanced as the *p*H of the medium is decreased. The induction of staphylococcal penicillinase formation in the one strain tested is surprisingly low.

The bacteriostatic activity of quinacillin is summarized in Table 2. It is clear that this penicillin is more active against *Staphylococcus aureus* than against any other microbial species tested.

Table 2. BACTERIOSTATIC ACTIVITY OF QUINACILLIN
(Serial dilutions in agar* surface-inoculated with 0·01 ml. of appropriate overnight cultures; incubated for 24 h at 37°)

Organism	M.I.C. μg/ml.
Staphylococcus aureus (see Table 3)	0·15–0·62
Streptococcus pyogenes	3·7
Streptococcus (Groups B, C and D. 5 species)	3·7–> 100
Diplococcus pneumoniae	3·7
Corynebacterium (4 species)	3·7–11·1
Sarcina lutea	11·1
Bacillus (6 species)	33·3
Lactobacillus (3 species)	> 100
Bordetella parapertussis	> 100
Neisseria catarrhalis	> 100
Escherichia coli	> 100
Proteus (4 species)	> 100
Salmonella (6 species)	> 100
Shigella (3 species)	> 100
Pseudomonas (2 species)	> 100

* Peptone. yeast-extract, glucose agar, *p*H 7·0, containing 10 per cent v/v ox serum.

The penicillin is not highly bound to serum proteins. Bacteriostatic activity is not depressed by more than one two-fold dilution when citrated human blood is incorporated in nutrient agar at a concentration of 50 per cent (v/v). A similar result is obtained in serial dilution tests against *Staphylococcus aureus* when ox serum at a concentration of 10 or 50 per cent is added to 'Oxoid' nutrient broth. In equilibrium-dialysis experiments with the penicillins at initial concentrations of 1 mg/ml. in ox-serum, about 20 per cent of quinacillin, compared with about 35 per cent of benzylpenicillin, was bound to the non-dialysable fraction.

The activity of quinacillin is enhanced as the *p*H of the medium is lowered over a *p*H range compatible with growth. In agar-dilution tests the minimum inhibitory concentrations (M.I.C.) for 20 strains of *Staphylococcus aureus* fell from 0·62–0·15 μg/ml. at *p*H 7·5, to 0·15–0·03 μg/ml. at *p*H 5·5. Using various nutrient agars at *p*H 7·0, concentrations of quinacillin below 10 μg/ml. could not be assayed by diffusion against a variety of test organisms, presumably because the penicillin diffuses poorly in agar at this *p*H. However, when nutrient agar is buffered at *p*H 5·3, concentrations of quinacillin as low as 0·1 μg/ml. give measurable zones of inhibition when *Staphylococcus aureus* is used as the test organism.

In Table 3 the inhibitory activity of quinacillin against 50 strains of *Staphylococcus aureus* obtained from clinical sources is compared with that of benzylpenicillin.

Table 3. Bacteriostatic Activity of Quinacillin against 50 Strains of *Staphylococcus aureus* from Clinical Sources

	M.I.C.* μg/ml. and No. of strains							
	<0·07	0·07	0·15	0·31	0·62	1·2	50	>100
Quinacillin	0	0	7	26	17	0	0	0
Benzylpenicillin	6	0	0	0	0	1	2	41

* Serial dilution in agar, *p*H 7·0.

The new penicillin is highly inhibitory to both penicillin-sensitive and penicillin-resistant strains of this organism. Replicates[9] from agar-dilution tests and sub-cultures from serial dilution tests in broth indicate that, as with other penicillins, quinacillin is bactericidal to growing cells. The M.I.C. is not significantly altered in serial dilution tests in broth against *Staphylococcus aureus* when the number of penicillinase-producing cells in the inoculum is varied one thousand-fold. This indication of high resistance to staphylococcal penicillinase was confirmed by manometric measurements[10]. The rate of hydrolysis of quinacillin at *p*H 7·0 and 37° by staphylococcal penicillinase was determined using *Staphylococcus aureus* (Parker) cultures which had been induced to form high levels of the enzyme with methicillin (0·5 μg/ml.) in casein hydrolysate medium. Significant hydrolysis of quinacillin was not detected over a period of 4 h when the penicillin (25 μmoles) was incubated with 60 units of enzyme activity (1 unit hydrolyses 1 μmole of benzylpenicillin per hour under these conditions). Using higher concentrations of cell-bound enzyme, preliminary iodometric determinations[11] indicated that the K_m of the penicillin at *p*H 6·5 is about 5 mg/ml. compared with 7 μg/ml. for benzylpenicillin[12] and about 8 mg/ml. for methicillin[12,13]. At high substrate concentration, when the enzyme is saturated, the rate of hydrolysis (V_{max}) of quinacillin is about 5 per cent of that for benzylpenicillin. However, because of the low affinity of quinacillin for penicillinase it is inactivated much more slowly at lower concentrations[12].

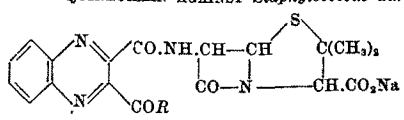
At low concentrations in casein hydrolysate medium quinacillin is a relatively poor inducer of penicillinase synthesis in *Staphylococcus aureus* (Parker) despite the high inhibitory activity of the compound and its resistance to penicillinase. The induction constant[14] (about 5 μg/ml.) is about 25 times greater than that for methicillin in this system. Benzylpenicillin gives low and variable results due to its rapid destruction[12,13].

Quinacillin, like other penicillins, is virtually non-toxic to animals and has proved effective in the treatment of staphylococcal infections when administered parenterally. Although highly acid-stable it is poorly absorbed by the oral route and absorption appears to vary in different species of animals.

Quinoxaline-2,3-dicarboxylic acids disubstituted symmetrically in the benzene ring are not readily accessible. Only two, 6,7-dimethyl- and 6,7-dichloro-quinoxaline-2,3-dicarboxylic acids, were prepared and converted into the corresponding penicillins. Activity (Table 1) was decreased markedly compared with quinacillin.

Several related penicillins were made from half-esters and half-amides (prepared from the anhydride with the appropriate alcohol or amine) of quinoxaline-2,3-dicarboxylic acid. The lower alkyl esters had activity against resistant staphylococci comparable with that of quinacillin but activity decreased with increasing chain length or chain branching. The amides were less active than quinacillin against resistant staphylococci (Table 4).

Table 4. Activity of some Ester and Amide Derivatives of Quinacillin against *Staphylococcus aureus*



	R	M.I.C.* μg/ml.		
		High res.†	Mod. res.†	Sensitive†
Parent Acid	HO–	0·4	0·4	0·4
Esters	CH_3O–	1·2	0·4	0·12
	C_2H_5O–	1·2	0·4	0·4
	$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}O$–	1·2	0·4	0·12
	$(CH_3)_2CH{\cdot}O$–	11·1	1·2	1·2
	$CH_3{\cdot}[CH_2]_2{\cdot}CH_2{\cdot}O$–	3·7	0·4	0·4
	$CH_3{\cdot}[CH_2]_5{\cdot}CH_2{\cdot}O$–	>100	33·3	11·1
	$(C_2H_5)_2N{\cdot}CH_2{\cdot}CH_2{\cdot}O$–	3·7	1·2	0·4
	C_6H_5O–	1·2	0·4	0·4
	$C_6H_{11}O$–	100	11·1	3·7
	$C_6H_5{\cdot}CH_2{\cdot}O$–	1·2	0·4	0·12
Amides	NH_2–	100	3·7	0·4
	$(C_2H_5)_2N$–	100	3·7	1·2
	$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}NH$–	>100	1·2	0·4
	$C_5H_{10}N$–	33	1·2	0·4
	$C_2H_5{\cdot}NH$–	>100	3·7	1·2
	$C_6H_5{\cdot}N(CH_3)$–	11·1	1·2	0·4

* Serial dilution in agar, *p*H 7·0.
† As Table 1.

Replacement of the 3-carboxy-group of quinacillin with hydrogen or with chloro-, hydroxy-, ethoxy-, piperidino- or 1,2,3,4-tetrahydro-1-quinolyl-groups virtually eliminated activity against highly resistant staphylococci. However, activity in the quinoline series was retained when the side-chain carboxy-group was replaced with phenyl. In our experience this behaviour has some parallel in the monosubstituted phenylpenicillin series and only *o*-carboxyphenylpenicillin (and derived side-chain amides)[15] and *o*-biphenylylpenicillin[16] have been described as having useful activity against resistant staphylococci. 3-Carboxy-2-naphthylpenicillin, isosteric with quinacillin, has similar microbiological activity but is unstable to acid (half-life 9 min, at 37° in 50 per cent ethanolic 0·1 N-hydrochloric acid).

Penicillins derived from a number of other vicinal dicarboxylic acids containing a five-membered heterocyclic ring, for example, benzofuran-2,3-dicarboxylic acid, 5-methylisoxazole-3,4-dicarboxylic acid and pyrazole-3,4-dicarboxylic acid, were inactive against resistant staphylococci.

We thank Drs. D. A. Peak and G. Woolfe for their encouragement, and those of our colleagues who gave assistance.

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ACTINOMYCIN D AND VACCINIA VIRUS INFECTION OF HELA CELLS

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INHIBITION of synthesis of DNA by 5-fluorodeoxyuridine[1] or of formation of RNA by actinomycin *D* (C_1)[2] prevents the replication of vaccinia virus in tissue culture cells. It has been reported that, when DNA synthesis is blocked, viral protein formation proceeds[3]. It seemed of interest, then, to examine the effect of RNA suppression by actinomycin on viral protein synthesis.

The procedures utilized for the growth and infection of HeLa *S*3-1 cells with strain *WR* vaccinia virus, the plaque assay for infectious virus[4], and the indirect immunological precipitation of radioactive viral protein[3,5,6] have already been described.

The formation of infectious vaccinia virus in suspension cultures of HeLa *S*3-1 cells is more than 99 per cent inhibited by 0·1γ actinomycin/ml. added 1½ h after infection (Table 1). Furthermore, treatment of cells prior to infection with 2γ actinomycin/ml., a concentration which limits RNA synthesis within 1 h to less than 2 per cent the normal rate, results in an almost complete block in viral protein synthesis (Fig. 4). Conversely, when RNA formation is similarly blocked in uninfected cells, protein synthesis continues at an optimal rate for 5 h followed by a diminished rate for at least another 7 h (Fig. 1). To examine whether renewal of RNA in infected cells is necessary for continued viral protein synthesis, 2γ actinomycin/ml. was added to a suspension culture at 5 h after infection, a time when only viral protein synthesis is proceeding rapidly. Within 1 h after addition of actinomycin, protein synthesis stopped (Fig. 2).

Table 1. ACTINOMYCIN INHIBITION OF INFECTIOUS VIRUS SYNTHESIS

Actinomycin concentration (γ/ml.)	Virus titre (PFU/ml.)
—	3×10^8
0·01	$2{\cdot}8 \times 10^8$
0·1	2×10^3
0·5	$< 10^3$

Actinomycin at the concentrations shown was added 1½ h after infection to replicate suspension cultures containing 2 × 10^5 HeLa cells per ml. Virus titres were determined after 19 h. The control culture contained 2×10^8 PFU/ml. at 3 h after infection.
PFU, plaque-forming units.

It has been reported previously that the synthesis of protein(s) less than 1 h prior to virus maturation is necessary for infectious virus formation[4]. Therefore, the interruption of viral protein synthesis by actinomycin might be expected to interrupt whole virus synthesis within this interval. As seen in Table 2, virus production is blocked within 1 h when actinomycin is added 5 h following infection. The maturation process remains sensitive to the antibiotic throughout the infectious cycle (Fig. 3). Addition of actinomycin at 3½ h after infection, 1 h before the onset of maturation, blocks subsequent infectious virus formation completely. Inhibition also occurs within 1 h when actinomycin is added at 11 h after infection. At this time a full yield of viral DNA[1] and most of the viral protein[3] have been synthesized.

Table 2. INTERRUPTION OF VIRUS MATURATION BY ACTINOMYCIN

Hours after infection	Virus titre (PFU/ml.) Control	Actinomycin
3	$3{\cdot}2 \times 10^4$	$3{\cdot}2 \times 10^4$
5	$1{\cdot}1 \times 10^5$	$1{\cdot}1 \times 10^5$
5½	—	$1{\cdot}5 \times 10^5$
6	—	$2{\cdot}7 \times 10^5$
7	$7{\cdot}6 \times 10^5$	$2{\cdot}7 \times 10^5$
8½	2×10^6	$2{\cdot}4 \times 10^5$
12	4×10^6	$2{\cdot}2 \times 10^5$

At 5 h after infection, the suspension culture was divided, and one of the resulting cultures received 2γ actinomycin/ml. At the indicated times, samples were removed from each and assayed for infectious virus titre.

The formation of cell proteins diminishes following vaccinia virus infection of HeLa cells (Fig. 4). During the first 4 h of infection the rate of protein synthesis is rapid, and viral proteins comprise 30–40 per cent of the total protein synthesized. From the fourth hour on, the total protein synthetic rate parallels that observed for viral protein formation, and after this time in the infectious cycle no net increase in cell protein occurs. The inhibition of cell protein formation by infection is not dependent on the continued synthesis of viral products. Net cell protein synthesis stops at 4 h after infection even when the formation of RNA, viral proteins, and viral DNA[2] has been blocked by treatment of the cells with actinomycin beginning 1 h prior to infection (Fig. 4).

Vaccinia virus infection of HeLa cells leads to a sharply decreased, although continued, rate of incorporation of

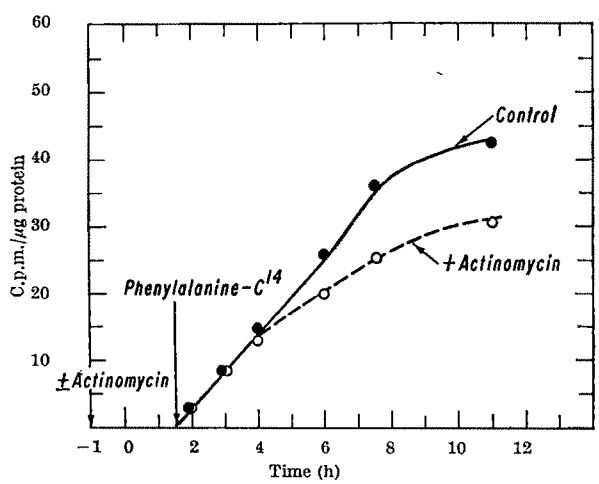


Fig. 1. Continued protein synthesis in uninfected cells in the presence of actinomycin. Actinomycin, 2γ/ml., was added to one of two suspension cultures of 2×10^5 cells/ml. After 1 h at 37° C, the cells were treated as described in the legend to Fig. 4, but without the addition of virus. Following phenylalanine-^{14}C addition (0·01 mM, 2 μc./μM), samples were removed, extracted twice with cold 5 per cent trichloroacetic acid, defatted and hydrolysed with 1 N potassium hydroxide. An aliquot of the hydrolysate was counted and another used to determine total protein[5]

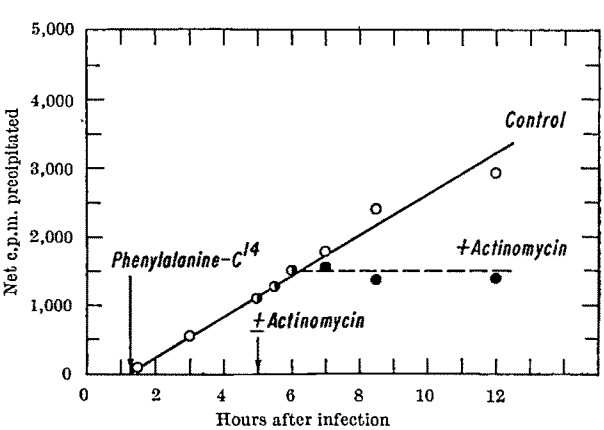


Fig. 2. Inhibition of viral protein synthesis by actinomycin. A suspension culture received phenylalanine-^{14}C at 1½ h after infection. At 5 h the culture was divided and 2γ actinomycin/ml. added to one of the resulting cultures. The incubation was continued, and samples removed from each at the indicated times were assayed for viral protein by immunological precipitation

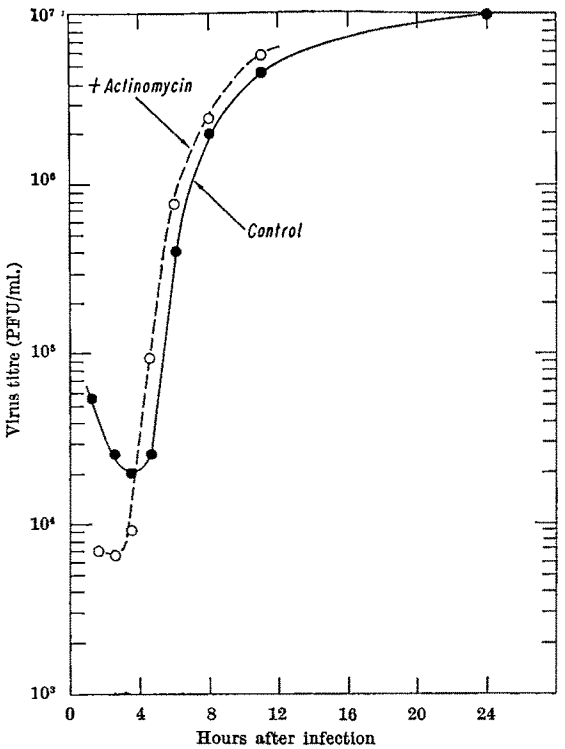


Fig. 3. Inhibition of infectious virus formation by actinomycin. A series of replicate cultures of infected cells was established at a cell concentration of 2×10^5/ml. One culture was sampled at increasing intervals after infection to obtain the infectious virus growth curve which is designated 'control'. At the indicated time after infection, one culture of the series received 0·5γ actinomycin/ml. At 24 h after infection, all inhibited cultures were assayed for infectious virus. The curve labelled 'actinomycin' represents the 24-h virus yields obtained when actinomycin was added at the times indicated by the position of the points on the curve

uridine into acid-insoluble material (Table 3)[7]. The observed reduction in total RNA synthesis may reflect a decrease in cell RNA formation together with an increase in the synthesis of virus-related RNA. Both the base composition and site of synthesis of the RNA made by vaccinia-infected cells are of interest in view of the cytoplasmic formation of both viral DNA and proteins[8]. A decrease in cell RNA synthesis would not by itself explain the cessation of cell protein synthesis in vaccinia-infected

Table 3. Reduction in RNA Synthesis after Vaccinia Infection

Hours after infection	Total CPM incorporated	% of maximum
Uninfected	2,310	100
2	2,110	91
4	1,400	61
7	730	32

At the indicated times after infection, one of a series of replicate suspension cultures each containing 100 ml. of cells at a concentration of 2×10^5/ml. received 2×10^{-5} M uridine-^{14}C (specific activity = 0·56 μc./μM). After 30 min the culture was chilled and the amount of uridine-^{14}C incorporated into RNA was determined[10].

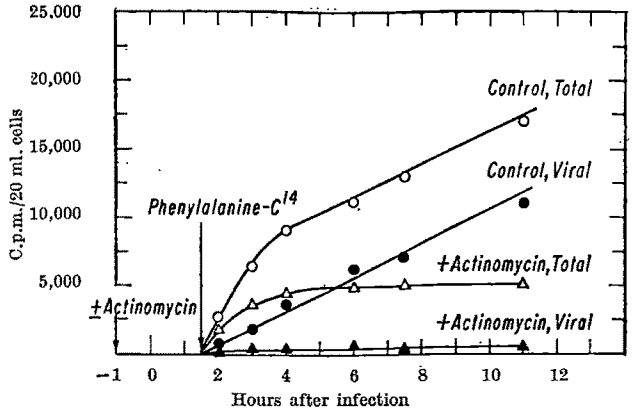


Fig. 4. Inhibition of cell protein synthesis following virus infection. One of two suspension cultures containing 2×10^5 cells/ml. was treated with 2γ/ml. actinomycin for 1 h at 37°. Each was then concentrated ten-fold, infected with an input multiplicity of 10–20 PFU/cell, washed twice in phenylalanine-deficient Eagle's medium[11] containing 20 per cent dialysed human serum, and resuspended to 2×10^5 cells/ml. in deficient medium plus 5 per cent dialysed horse serum. Actinomycin was present throughout. At 1½ h after infection, 0·01 mM phenylalanine-^{14}C (specific activity = 2 μc./μM) was added and the incubation continued at 37° C. Samples were removed at time-intervals and centrifuged. Total radioactivity incorporated into protein was determined following cold 5 per cent trichloroacetic acid extraction and solubilization in 1 N potassium hydroxide of an aliquot of each sample. Viral protein was measured by immunological precipitation of a second aliquot

cells, since protein synthesis in uninfected cells continues for 12 h or longer when RNA formation has been 98 per cent inhibited by actinomycin (Fig. 1). The finding that synthesis of cell protein decreases even when viral products are not formed in infected cells suggests that the infecting virus inhibits the ability of the RNA synthesized prior to infection to support cell protein formation. In the presence of the antibiotic only the inhibition of cell protein synthesis may occur. In its absence, the synthesis of virus-related RNA could proceed and might then direct the synthesis of viral proteins.

The foregoing observations suggest that a vaccinia virus DNA-directed RNA is synthesized in infected HeLa cells and that its synthesis throughout infection is necessary for continued viral protein and infectious virus production. Information now being sought concerning the cellular site of synthesis and chemical nature of this RNA may provide a better understanding of the processes by which virus re-directs the metabolism of cells.

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A POSSIBLE CASE OF GENETIC ASSIMILATION OF BEHAVIOUR

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Clutterbuck and Beardmore[1] recently reported that if *Drosophila melanogaster* is reared on a medium adulterated with a substance to which they are aversive, such as peppermint oil, then the behaviour of the flies may alter so that they become less aversive to the adulterant. These authors found that flies reared on peppermint-flavoured medium for six generations became less averse to that substance, both measured by the relative numbers of eggs laid on it compared with the number laid on a normal medium, and also in terms of the number of flies

found on peppermint food rather than on normal food in a choice situation.

The method of testing which they used is not sensitive, owing to the great discrepancy in the number of eggs laid by different pairs of flies. We have found that in single pair matings the number of eggs laid may vary from zero to more than 100 in flies randomly picked from the same population. Hence their egg-laying results could be due to abnormal fertility in one or two flies, which could more than counterbalance a low egg-laying score on normal food by the majority. Further, their food choice test was performed over periods of 1 h and 24 h, and it is likely that with the volatile oils which they used for adulterants there would have been a considerable spread of the adulterant throughout the apparatus in that time. Their results are, however, suggestive, and we have repeated their experiment with a different design.

A stock of wild-type *Drosophila melanogaster* (Pacific) strain was kindly provided by the Department of Zoology, University of Edinburgh. These were divided into the groups shown in Table 1.

Table 1

AN	Flies averse to peppermint reared on normal food
NAN	Flies non-averse to peppermint reared on normal food
UN	Unselected flies reared on normal food
AP	Flies averse to peppermint reared on peppermint food
NAP	Flies non-averse to peppermint reared on peppermint food
UP	Unselected flies reared on peppermint food

From this Table is will be seen that there was artificial selection for aversion or non-aversion to the adulterant, and natural selection by the presence or absence of adulterant in the food. Artificial selection was carried out in the apparatus shown in Fig. 1. Flies were placed in the chamber *P* and the door *D* opened. After 2 min, *D* was closed. If aversive flies were being selected those which remained in *P* were removed, the remainder replaced in *P*, and the process repeated. This was done 5 times. The remaining flies were used as the 'aversive' (*A*) stock. If non-aversive flies were required, the flies remaining in *P* were retained each time. Each artificially selected line was therefore composed of those members of the population which remained after a 5-fold cumulative screening for the character in question.

Of such a group, mating was between 10 males and 10 females, all virgin, and approximately 4 days old.

Testing of the characteristics of the offspring was carried out in the same apparatus. 50 males and 50 females, in groups of 10 and approximately 2 days old were placed in *P*, and the number remaining in *P* after 2 min was counted. The summed scores are represented as percentage of the population showing aversion (percentage of flies which had passed beyond *D* in 2 min).

The results of artificial selection alone, natural selection alone, and the two in combination are shown in Figs. 2 and 3.

AN tends to show an overall rise over 14 generations. *NAN* shows a slight continuous upward drift. *UN* retains its original level, representing the relatively stable characteristics of the wild-type population. None of these results is particularly striking. The effects of artificial selection alone do not appear violent.

The behaviour of the graph *UP* does not seem to suggest that natural selection alone has much effect:

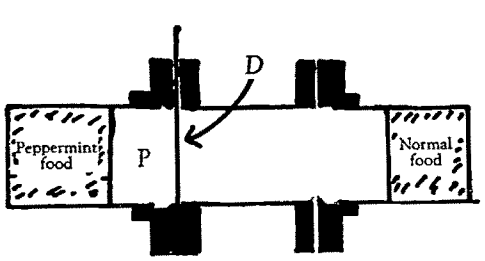


Fig. 1. Longitudinal section through testing and screening apparatus

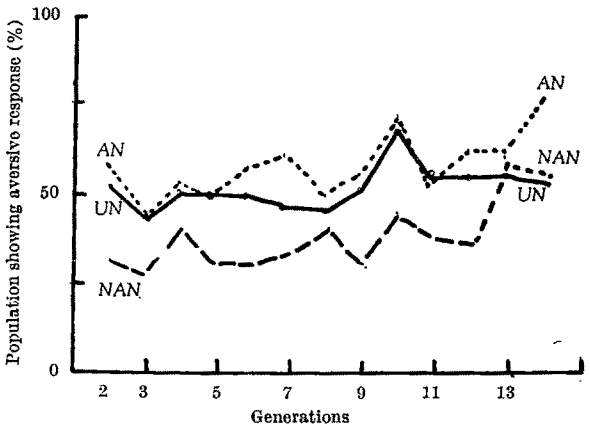


Fig. 2. Percentage of the population showing aversion at each generation. Flies reared on normal food

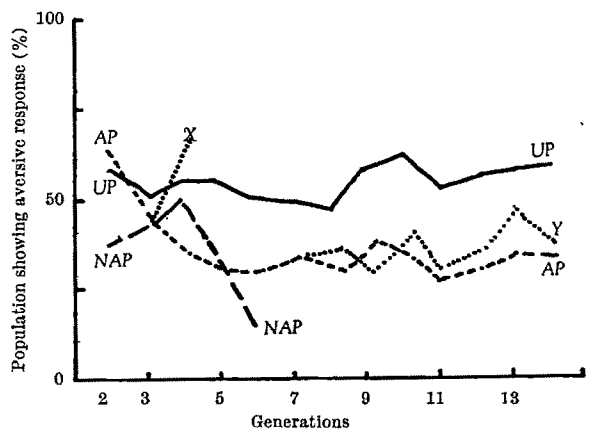


Fig. 3. Percentage of the population showing aversion at each generation. Flies reared on peppermint food

this line remains relatively stable over 14 generations at a level slightly below *UN*.

For the two lines which were subjected both to artificial and natural selection, however, the results are striking. *AP* and *NAP* both become non-aversive, though selected artificially in opposite senses. This result is not surprising for *NAP*. The data are only plotted up to F_6 since beyond this point the line had to be released from artificial selection owing to the very small numbers surviving. It appears to be extremely unfit, and afterwards had to be frequently released from artificial selection to maintain it. Beyond F_6 therefore the data for *NAP* are not reliable.

The most interesting line is *AP*. This line was selected for aversion in each generation, but becomes non-aversive when reared for several generations (approximately the same number over which Clutterbuck and Beardmore observed the effect) on peppermint. Neither artificial selection alone nor natural selection alone produced this effect (*NAN* and *UP*).

A point should be made about the method of testing. The method used means that the aversive response is partly confounded by the activity level of the flies. More active flies are more likely (other things being equal) to go beyond *D* in 2 min than less active flies. But since testing procedure was identical for all the lines the result is not artefactual and due simply to differences in activity-level in the original population. If the effect is due to activity rather than aversion, then we have altered activity by the experimental conditions. But one or other or both has been altered, which is what we are interested in.

A possible explanation might proceed along the following lines. Two processes seem to be occurring at once. If we release line *AP* from the adulterated medium at F_3 (shown by the dotted line in Fig. 3), it returns to its original

level of aversion (point X in Fig. 3). If, however, we wait until F_7 to release them, the progeny do not return to their original level (dotted line ending in point Y in Fig. 3). Moreover, the $AP(Y)$ line does not return to its original level even after a further 8 generations. The aversive character of the flies seems to have been reduced more or less permanently.

We suggest that the short-term effect should be called habituation, and that it is probably a within-one-generation effect. It seems not unlikely that flies which have spent their whole life from egg to adult in a peppermint-scented medium should habituate to it so that when tested as adults they would not be so aversive to it as wild-type flies tested when adults. On the other hand, the relatively stable, long-term effect bears a similarity, we would tentatively suggest, to genetic assimilation of a character which does not have a threshold, as was discussed for structural characters by Waddington[2]. If so, we may proceed with the explanation as follows.

Consider Fig. 4. A 5-fold screening is a rather intensive method of artificial selection, which may be expected to select only the extreme tails of the distribution marked A and NA respectively. In the total population there is a great deal of genetic variability, but comparatively little in the extremes of the distribution. Let us assume that the effect of the adulterated environment is to produce 'canalization' as described by Waddington. Then for the total population, the effect of canalization may well be small in each generation compared with the spread of genetic variability, so that the range of the latter offsets the effect of the former in such a degree that the overall characteristics of the population do not change noticeably. When we select for non-aversion and breed on peppermint (NAP) the two effects reinforce each other and the fall in aversion is precipitous and carries the population into a region where it is unfit; and in the case where we select for aversion but breed on peppermint, the following might occur. The variability is so reduced by the severe artificial selection that the effect of canalization is large with respect to it. Hence, although the artificial selection is in the opposite sense, the shift produced by canalization is not offset by the inherent variability, and the characteristics of the population slowly shift. What variability there is, however, tends to return the population toward the normal population, and so the line does not enter the unfit region which NAP enters, and instead, becomes stabilized at a comparatively low level of aversion.

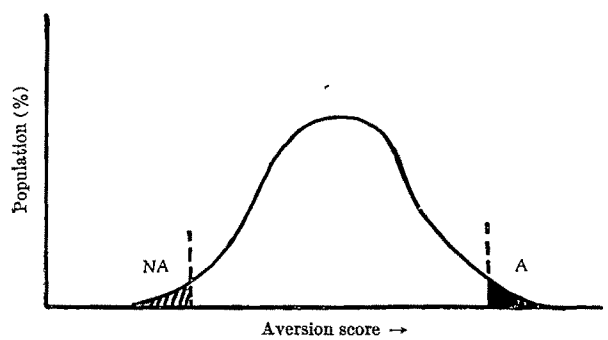


Fig. 4. Regions of population from which breeding is occurring on the basis of 5-fold screening selection

Such an explanation is amenable to experimental verification. It should follow that the rate of change of the characteristics of the population should be a function of the amount of variability in the breeding population. Thus by breeding from more or fewer pairs of flies a series of differently sloping curves should be obtained, all tending, but at a different rate, towards the non-aversive end of the distribution. We have begun experiments on this hypothesis, using an improved form of testing procedure.

We thank the Medical Research Council for financial assistance which made the work possible through the tenure of a research assistantship by one of us (K. C.) at the University of Sheffield.

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CARBON DIOXIDE: A FACTOR INFLUENCING CELL DIVISION

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A HIGH concentration of carbon dioxide promotes mesocotyl growth in etiolated oat seedlings and reduces that of the coleoptile and leaves[1-4]. To explain these growth variations it has been supposed that carbon dioxide promotes cell division in the mesocotyl rather than the content of growth hormone[5]. This speculative hypothesis has now been tested by conducting a 2 × 2 factorially arranged experiment in which 5 per cent carbon dioxide in the air, for 3 days from the time of planting the seed, was combined with exposure of the seedlings to dim red light (1 ft.-candle) for 5 min on the third day, after treatment with carbon dioxide had been terminated. From the second to the seventh day of growth, samples of seedlings were measured and a plant having lengths nearest to the mean values obtained was selected from each sample, embedded in the usual way (either whole or after cutting into a series of 10-mm segments) and sectioned. Cell sizes and numbers were recorded for each mm of the entire length of both mesocotyls and coleoptiles by counting 5 rows of cortical cells and then calculating the mean cell length from the mean number of cells/mm. Fig. 1, which is self-explanatory, shows a typical set of data for a 10-mm segment of mesocotyl.

In Figs. 2*A* and *B* appear respectively the mean lengths of the mesocotyls plotted against time, and the mean

Carbon dioxide: fourth day

Mesocotyl segment	Columns A	B	C	D	E	Mean No.	Mean length
1st mm	23·0	23·0	22·0	22·0	25·5	23·50	0·043
2nd ,,	13·5	15·5	13·0	16·5	13·0	14·30	0·070
3rd ,,	10·0	5·7	8·3	8·5	7·2	7·94	0·126
4th ,,	7·5	6·0	6·3	6·5	6·2	6·50	0·154
5th ,,	4·5	4·3	4·7	4·0	5·0	4·50	0·222
6th ,,	3·0	3·5	5·0	4·0	5·0	4·10	0·244
7th ,,	3·0	2·5	3·3	4·0	3·7	3·30	0·303
8th ,,	4·0	3·0	2·7	3·0	3·7	3·28	0·305
9th ,,	3·7	3·0	2·7	2·5	2·9	2·96	0·338
10th ,,	3·5	3·3	2·5	3·0	2·5	2·96	0·338

Fig. 1. Technique used for cell counts and for determining the distribution of cell size with increase in distance from the apex of the segment

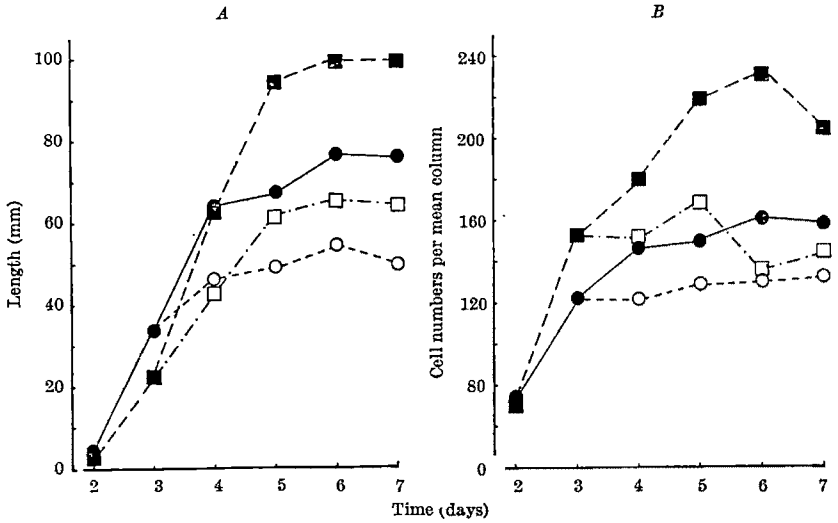


Fig. 2. The influence of carbon dioxide and light on length and cell numbers in the mesocotyl. *A*, mean length; *B*, mean number of cells in a single column, on successive days □, Carbon dioxide and light; ■, carbon dioxide; ○, light; ●, untreated

number of cells in a single column of the mesocotyl of a plant of average size. Length and cell numbers are clearly affected similarly by the treatments. The number of cells in the mesocotyls of untreated plants reached a maximum by the fourth day and afterwards remained more or less constant (Fig. 2*B*, solid circles). The mean mesocotyl lengths (Fig. 2*A*, solid circles) increased rapidly to the fourth day while cell division was proceeding; thereafter growth almost ceased. Since no further new cells were formed, this slight increase was due to the elongation of the cells which were immature on the fourth day. Carbon dioxide (Fig. 2*A*, solid squares) reduced mesocotyl growth up to the third day but the rapid elongation afterwards shown resulted in a greater final length. This elongation can be associated with rapid cell division beginning after the second day (Fig. 2*B*, solid squares) so that more cells were present on the third day, and mitoses continued until about the fifth day when the maximum number was reached. After exposure to light (Fig. 2*A*, open circles) the mesocotyls increased only slowly in length and they were finally some 25 mm shorter than untreated mesocotyls; cell division was inhibited (Fig. 2*B*, open circles). When both treatments were given (Fig. 2*A*, open squares) some capacity to elongate was retained and the final length was mid-way between that of untreated mesocotyls and those which had only been illuminated. Pre-treatment with carbon dioxide did not protect the meristem from the inhibitory effect of light (Fig. 2*B*, open squares). The cell numbers of the sixth and seventh days' samples were much lower than in the earlier ones, but at least they confirm that mitosis had ceased.

The distribution of cell size along the length of the mesocotyl on the fourth (*A* and *B*) and seventh (*C* and *D*) days is displayed in Fig. 3. In plants of all treatments the cells at the base of the mesocotyl were 0·2–0·3 mm long. With increasing distance from the base the cells rapidly attained a mature length with a mean value of 0·5 mm. In plants grown completely in darkness, cells of this size occupied most of the length of the mesocotyl. Near the coleoptilar node, in the case of the untreated plants on the fourth day (Fig. 3*A*) cell-length decreased rapidly over a distance of 5 mm to a minimum of 0·066 mm, whereas in the carbon dioxide treated plants a size of 0·043 mm was reached over a distance of 10 mm (Fig. 3*B*). These two features—more and smaller immature cells—support the observation (Fig. 2*B*, solid squares) that carbon dioxide maintained meristematic activity.

At the time of illumination none of the cells in the mesocotyl was mature. The tissue consequently comprised a sequence of cells, of increasing maturity from apex to base, whose capacity for elongation after return to darkness was determined by age at time of exposure. Thus, cells near the endosperm greater than about 0·13 mm were unaffected by light and elongated as much by the fourth day as the cells in the non-illuminated plants. Cells smaller than this, farther from the base, did not elongate as much as the cells in the corresponding positions of mesocotyls which had not been illuminated (Fig. 3*A*, compare open triangles and solid circles between 30 and 40 mm). The same is true for plants treated with carbon dioxide (Fig. 3*B*, between 20 and 35 mm). The cessation of cell division after illumination is indicated by the size of the topmost cells in the exposed mesocotyls as compared with those which remained in darkness; 0·13 mm and 0·066 mm in Fig. 3*A*, and 0·09 mm and 0·043 mm in Fig. 3*B*—approximately twice as long in both instances.

The measurements for the seventh-day samples (Fig. 3*C* and *D*) show that in darkness carbon dioxide did not affect the mean length which the cells finally attained (solid circles). Also, by this time most of the smallest

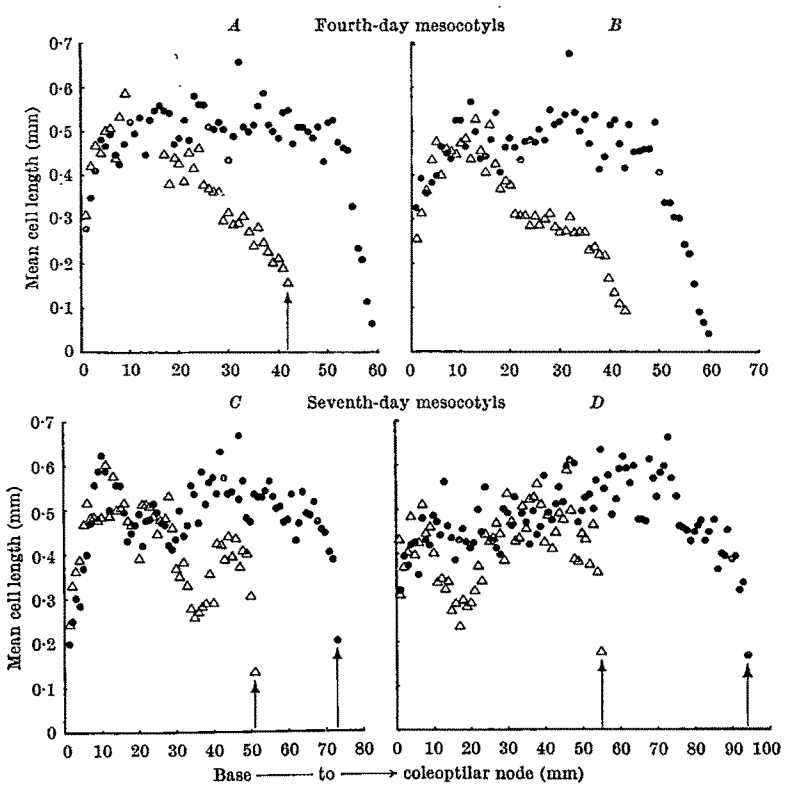


Fig. 3. Variation in cell size with increasing distance from the base of the mesocotyl to the node, indicated throughout by the arrows
Fourth day: *A* } without CO_2 *B* } with CO_2
Seventh day: *C* } without CO_2 *D* } with CO_2
●, Darkness; △, light on the third day

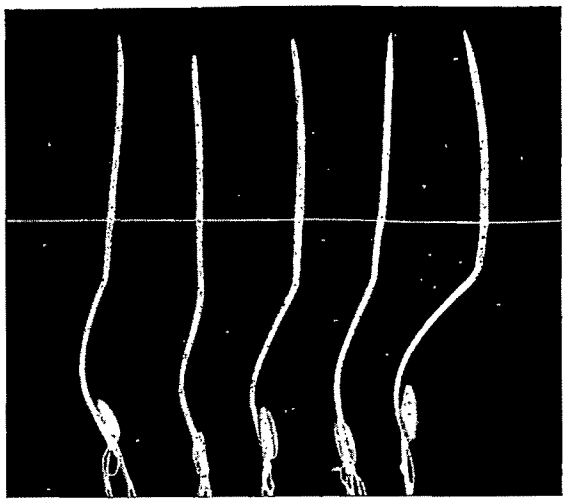


Fig. 4. Appearance of plants 24 h after having been exposed to dim red light for 5 min. (Reproduced from Mer (ref. 5) by permission of the Editor and Publishers of *J. Exp. Bot.*)

which remain small occur at the junction of the curved and straight parts of the mesocotyl (Fig. 4).

Growth of the coleoptiles is depicted in Fig. 5*A*; carbon dioxide depressed growth while exposure to light induced a transient growth promotion whether or no the plants had been given carbon dioxide. These treatment differences cannot be explained by effects on mitosis since cell numbers were not greatly influenced (Fig. 5*B*). However, coleoptile-length and mean cell-size are almost exactly correlated (Fig. 5, *A* and *C*). There are therefore two fundamental differences in the responses of mesocotyl and coleoptile cells to these treatments. First, coleoptile cells are changed in length by the different treatments whereas it is primarily cell division that is affected in the mesocotyl, and secondly, light promotes cell elongation in the coleoptile and depresses it in the mesocotyl. The only suggestion which can at present be advanced to account for the different effects of carbon dioxide on cell division in the mesocotyl and coleoptile is that it will influence mitosis only in a compact meristem such as that found at the node of the mesocotyl. In the coleoptile there is no clearly delimited meristematic region but individual mitoses occur sporadically throughout this structure.

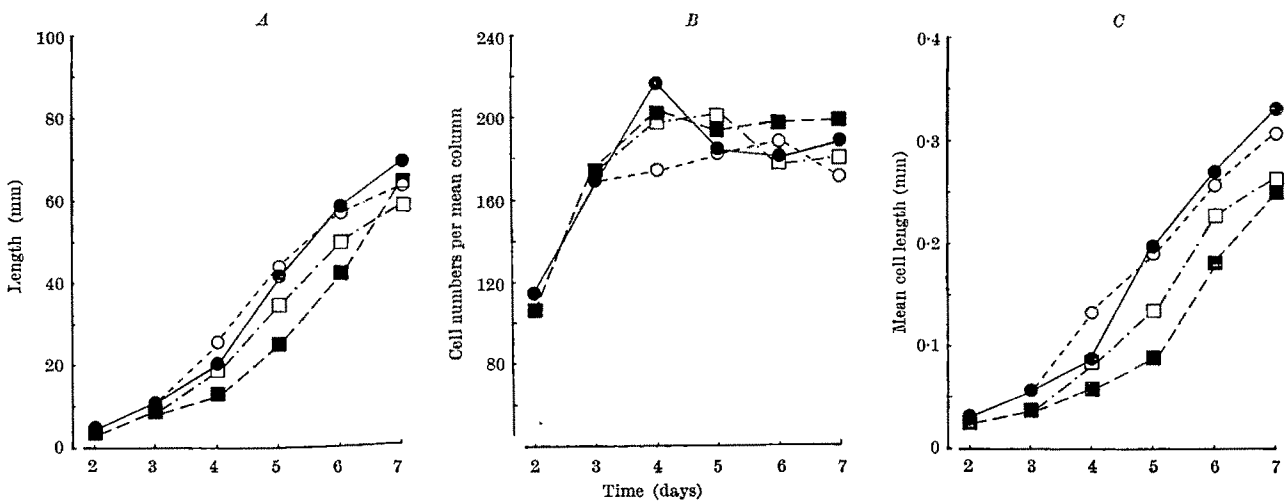


Fig. 5. The influence of carbon dioxide and light on length (*A*), cell numbers (*B*), and cell size (*C*) of the coleoptile on successive days □, Carbon dioxide and light; ■, carbon dioxide; ○, light; ●, untreated

cells in the mesocotyl had elongated further. Without carbon dioxide, the final length of the uppermost cells in the illuminated plants was slightly less than the mean value of 0·5 mm reached by the cells in the unexposed mesocotyls (Fig. 3*C*). In the plants treated with carbon dioxide (Fig. 3*D*) the topmost cells were equal in length, (between 30 and 50 mm). Situated between 35 and 40 mm (Fig. 3*C*) and between 15–20 mm (Fig. 3*D*) from the base, there appeared groups of short cells which apparently were so very sensitive to dim red light that only slight further growth was possible, to about 0·26 mm.

The growth and appearance of the mesocotyls of illuminated plants can now be accounted for in terms of cell division and elongation. The low growth rate of the mesocotyls (Fig. 2*A*, open circles between third and fourth days) can be ascribed to the cessation of mitosis and the inhibition of further elongation of some of the cells. The appearance of the plants on the fourth day is shown in Fig. 4; the white line indicates the position of the node. The lower part of the mesocotyl is characteristically curved and so it appears in the subsequent daily samples. This region contains the cells whose ability to elongate was unaffected by light. The short straight part includes the cells which did not elongate quite so much; the capacity for growth retained by the plants treated with carbon dioxide (Fig. 2*A*, open squares) is due to the large number of cells in this region which can elongate fully (Fig. 3*D*, open triangles). In both instances the cells

Two conditions thus seem to be required for the maintenance of cell division in the meristem: a high concentration of carbon dioxide and darkness. These circumstances normally prevail because the apical meristem and encircling leaves are enclosed within the coleoptile, and respiratory carbon dioxide will consequently be accumulated therein to keep the internal concentration at a high level. The nodal meristem is immediately proximal to the main apex and this group of small, tightly compacted cells will be bathed in respiratory carbon dioxide. In daylight the development of chlorophyll will screen the apical growing point from the inhibitory red radiation, but the nodal meristem is bare, contains no chlorophyll, and so is quite unprotected.

Recently, Sorokin reported that carbon dioxide at a concentration as low as 1 per cent inhibited cell division in *Chlorella*[6]. On this observation was based the suggestion that in multicellular organisms accumulation of carbon dioxide might inhibit mitosis and so could function as a controller of cell division. The present findings show that such a generalization is not possible since in the oat plant stimulation of cell division by carbon dioxide occurs in the meristem of the mesocotyl while the 'diffuse' mitoses occurring in the coleoptile are unaffected.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Solar Flux Fluctuations at 225 Mc/s and Noise-level of Atmospherics at 27 Kc/s

THE correlation between the behaviour of the ionosphere and solar activity has been investigated by many authors[1]. Nevertheless this research is being continued especially in connexion with the undisturbed D layer.

For this purpose the Wolf relative number R is usually considered as an index of solar activity, and the absorption of the D layer during the processes of ionospheric reflexion as an index of the ionospheric effect[2,3]. In our work, two different parameters have been used instead: the level of the solar flux at metric wave-lengths and the average noise-level of atmospherics at very low frequency. Thus we have compared the average antenna temperature at 225 Mc/s produced by the Sun during its meridian transit (excluding bursts) and the median of the hourly values of atmospheric noise recorded at 27 kc/s.

Both values were recorded at the same place (Arcetri Observatory): the solar results expressed in deg. K were obtained with a two-element (Yagi) interferometer spaced at 38 wave-lengths; the atmospheric results were obtained from a conventional receiver (passband 0·500 kc/s) fed by a loop antenna and are expressed in μV/m.

Our recordings cover the period June 1–November 30, 1962, with one gap of 10 days: Fig. 1 shows the values of the solar flux at noon (F) compared with the median of the hourly values of atmospheric noise obtained during the day (D) and separately during the night (N). It appears that maxima of F almost always coincide with maxima of atmospheric noise. The agreement is more evident for the nightly values N. The vice versa is not true possibly because of rather near and concentrated atmospheric sources.

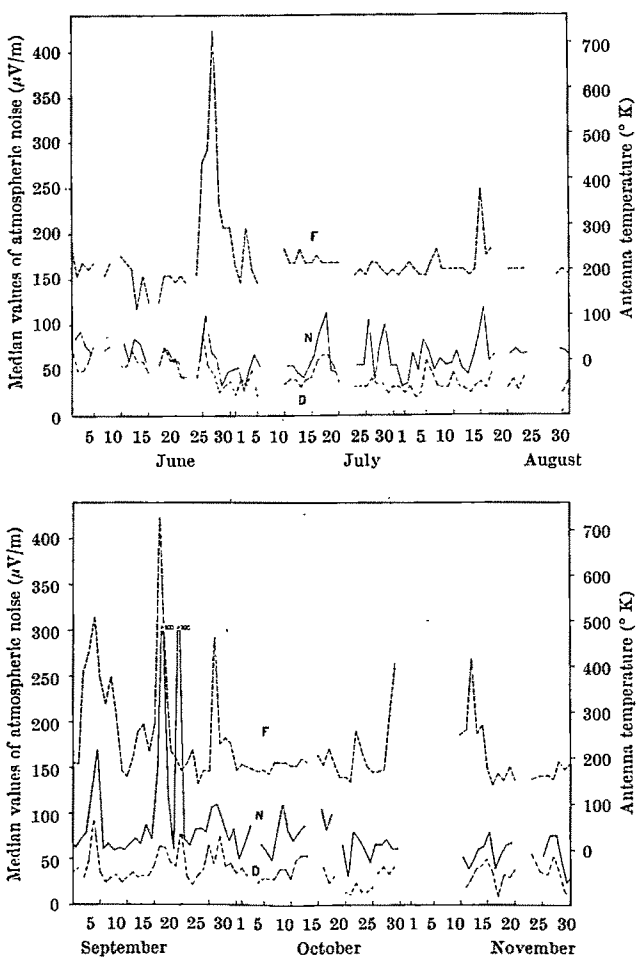


Fig. 1. Daily values of the solar flux at 225 Mc/s (F) at noon. Daily median intensity of atmospheric radio noise at 27 kc/s during the days (D) and the nights (N)

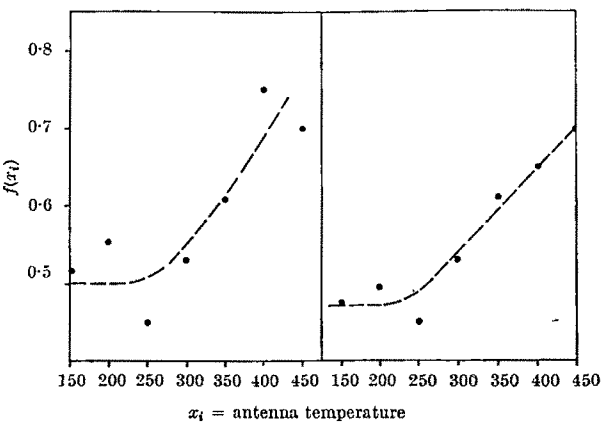


Fig. 2. $f(x_i)$ for the day (left) and for the night (right)

We then computed the correlation coefficients between F and D (same day) and between F and N (following night); the results are not very significant.

Considering the large number of parameters conditioning the atmospheric noise, the low value of these coefficients are not surprising; so we looked for a correlation restricted only to those days in which the solar flux was reaching a certain level.

Since our values for the antenna temperature are at a minimum of about 150° K we counted the coincidences between the F values higher than 150° K and the D and N values higher than their monthly median value.

With $N(x_i)$ as the number of the F values higher than the threshold x_i and $n(x_i)$ the number of the observed coincidences, we computed the ratio:

$$f(x_i) = n(x_i)/N(x_i)$$

for values of x_i increasing from 150° K to 450° K, 50° K by 50° K. If the coincidences are at random, $f(x)$ must be obviously 0·5.

The graphs of Fig. 2 show the behaviour of $f(x)$, as a function of x. It is interesting that increases of $f(x_i)$ occur for the nightly values as well as for the daily ones.

In order to check this result we repeated the same experiment comparing F with the value of D of the following day: no matter what the x_i value was, $f(x_i)$ was statistically ranging around 0·5 and not increased at all with the value of x_i.

We have again repeated the foregoing calculus associating D and N with the daily Wolf number (R), given by the Freiburg[4] maps.

As for F (Fig. 2), also for the Wolf numbers we have found a threshold ($R \simeq 40$) beyond which an increase, though weaker, of $f(R_i)$ with R_i can be seen.

We may therefore say that: (1) The correlation between the solar flux and the atmospheric noise-level refers to the same day and the following night. (2) There seems to be no relation between the two phenomena when $F < 250°$ K; our average antenna temperature for quiet Sun is around 200° K. It will be noted that Nicolet and Aikin[5] in

1960 found by theoretical consideration the existence of a 'threshold' of this kind in the *D*-region ionization.

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GEOPHYSICS

Terrestrial Heat Flow in Japan

Since 1957 a survey of terrestrial heat flow has been conducted in Japan by this Institute[1,2]. As a result of this, heat-flow values have so far been determined at 39 localities in the Japanese Islands. In addition to these, 12 sea measurements have been made in the sea around Japan under a co-operative programme with the Japan Meteorological Agency on board the R.V. *Ryofu Maru*[3]. The results (Fig. 1) seem to indicate a definite pattern of heat-flow distribution in the area.

Estimation of heat flux has been made from combination of the geothermal gradient and the thermal conductivity of rocks in which the thermal gradient was measured. Of the total of 39 land stations, 26 are metal mines, 4 coal mines, 2 oil fields, 3 natural gas fields, 1 railway tunnel, and 3 are sites of other types (Fig. 1).

The geothermal gradient was obtained from measurement in deep boreholes or in short boreholes at various levels in mines. At most of the land stations, the maximum depth of temperature measurement exceeded 300 m. At 24 stations, it was more than 500 m. Thermal conductivity of rocks was measured by the divided-bar method[4]. The apparatus was calibrated with such standard substances as fused silica, crystalline quartz, dense flint glass and borosilicate crown glass, of which thermal conductivities were determined by E. H. Ratcliffe of the National Physical Laboratory, England[5].

Geothermal gradient in the sea bottom has been obtained by a thermogradmeter developed by us[6], and the thermal conductivity of sediments recovered from the bottom was measured either by the divided-bar method or the needle-probe method[7].

Errors accompanying the heat-flow values were estimated from the probable error in the thermal gradient and the degree of ambiguity in assessing the *in situ* thermal conductivity of the strata from the thermal conductivities of individual samples. In most cases, the amount of the errors is about 10 per cent.

As can be seen in Fig. 1, there were two measurements which gave very high heat flow, that is, one at Toyoha Mine in south-western Hokkaido ($> 5 \times 10^{-6}$ cal/cm² sec) and the other at Mt. Kusatsu-Shirane ($10{\cdot}8 \times 10^{-6}$ cal/cm² sec). Both of these high values were found to be due to very local geothermal anomalies. Excluding these two anomalous values, the average value of the 37 heat-flow values on land turns out to be $1{\cdot}55 \times 10^{-6}$ cal/cm² sec, and the average of the 12 sea values $1{\cdot}48 \times 10^{-6}$ cal/cm² sec. Since the land stations are distributed fairly uniformly over the Japanese Islands, the foregoing figure may be regarded as the representative heat flux of the whole area. This value is not much different from the average continental heat flux ($1{\cdot}2$–$1{\cdot}4 \times 10^{-6}$ cal/cm² sec). However, the distribution of heat flow indicated in Fig. 1 seems to have some features which are possibly characteristic of the thermal state in the crust and the upper mantle of an Island arc orogenic zone.

The features to be noted may be as follows. (1) There exists a region of high heat flow on the inner side of the Japanese arc, where the heat flow value exceeds $2{\cdot}00 \times 10^{-6}$ cal/cm² sec. There are indications that the high heat-flow region branches off southward in the central part of the Honshu Island along the Fossa Magna. This may extend farther south to the Izu-Mariana arc. High value ($2{\cdot}54 \times 10^{-6}$ cal/cm² sec) at Konomai in north-eastern Hokkaido may be an indication of existence of similar high heat flow zone in the Kuril arc. It should be noted that these high heat-flow regions coincided with those of Cainozoic volcanic activity of the area[8,9]. Although there are some high heat flow values in the Pacific, the data are still too meagre to define any area of high heat flow. (2) A region of low heat flow ($< 1{\cdot}00 \times 10^{-6}$ cal/cm² sec) exists on the Pacific Ocean side of the north-eastern Honshu. This tendency is confirmed by a sea measurement ($0{\cdot}27 \times 10^{-6}$ cal/cm² sec). As marked by an oval with cross-hatches this low heat-flow area seems to be bounded in the east by the Japan Trench. Although measurements are too few to draw any general conclusion, the bottom of the trench itself so far showed rather normal heat flow ($1{\cdot}14$ and $1{\cdot}39 \times 10^{-6}$ cal/cm² sec).

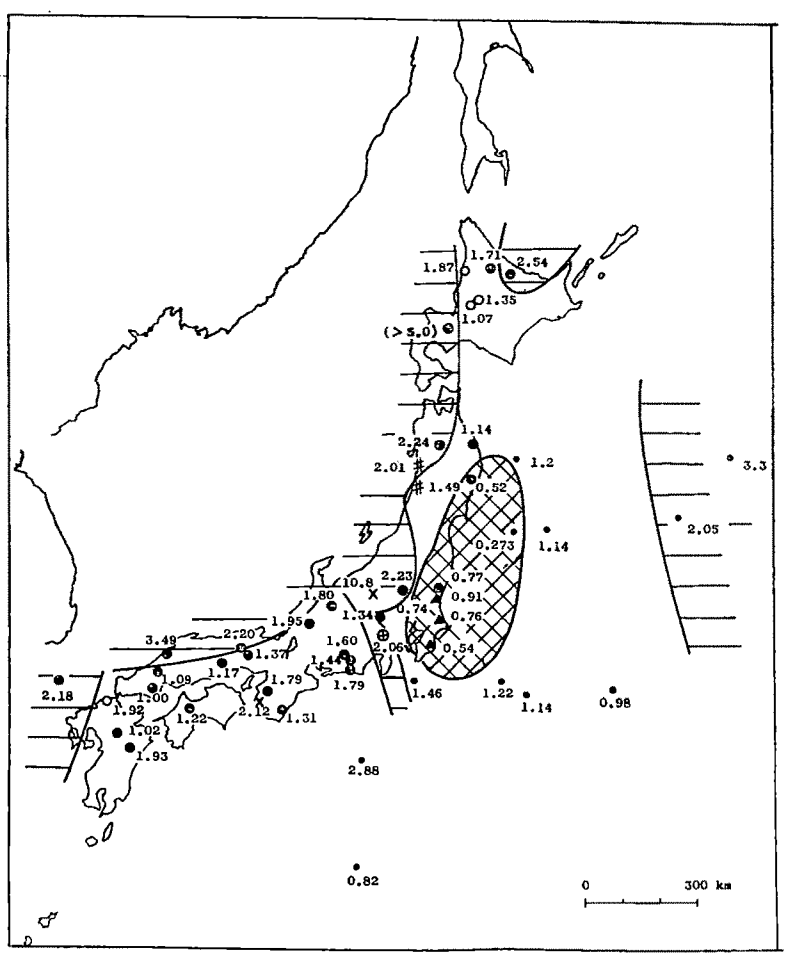


Fig. 1. Distribution of heat flow in and around Japan. Unit: 10^{-6} cal/cm² sec. ●, metal mine; ○, coal mine; #, oil field; ▲, gas field; ⊕, railway tunnel; ×, other type; •, sea station. One sea station out of the map is omitted

(3) With the exception of measurements in these two regions, almost all the land heat flow values are between 1 and 2×10^{-6} cal/cm² sec. The zones of intense Mesozoic orogeny in Japan occupy most of these non-high heat-flow regions[10].

Obviously the distribution of the heat flow obtained here should be examined in relation to many other geological and geophysical factors of the Japanese Islands and their environment. Description of each heat flow station[2] and some discussion on the results will be published elsewhere.

We thank Prof. T. Rikitake of the Earthquake Research Institute and many people at heat-flow stations for their assistance.

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Reverse and Normal Remanent Magnetization in Hydrothermal Veins

AN attempt has been made to apply the measurements of the direction of natural remanent magnetization to the age determinations of epigenetic hydrothermal mineralization. The investigation of materials from Czechoslovakian hydrothermal deposits has brought positive results which have changed some of the previous opinions concerning metallogenetic problems in Czechoslovakia[1]. The work was based on several scores of hydrothermal deposits from four metallogenic areas in the West Carpathians and in the Czech Massif. As a comparative geochronological scale for age-determinations the polar wandering curve computed in the way published by Creer *et al.*[2] was used. In the hydrothermal mineral deposits of the Carpathian System the parameters introduced by Blackett *et al.*[3], particularly the so-called divergence and inclination difference of remanent magnetization, were also used.

The fundamental condition for a correct age interpretation of the products of hydrothermal mineralization is that they reproduce the primary direction of the remanent magnetization. It is thus necessary to eliminate the influence of post-mineralization tectonics[4] and to prove the magnetic stability of the hydrothermal aggregates under investigation. Stability in d.c. magnetic field (up to 56 œ) and especially in a.c. magnetic fields (up to 900 œ) was shown by various hydrothermal minerals, for example, by baryte, fluorite, magnesite, quartz, calcite, which were finely pigmented by hæmatite. A high stability and homogeneous magnetization distinguished the so-called cinopels, hæmatite–quartz aggregates, where hæmatite occurs in a fine dispersed form isolated in a quartz ground-mass.

Laboratory stability tests cannot yet serve as a proof of palæomagnetic stability, especially with products of hydrothermal mineralizations where influence of later hydrothermal processes may occur. I have endeavoured to find in each metallogenic area hydrothermal aggregates with normal and with reverse magnetization differing within the limits of the confidence circles by 180°. Such aggregates have been found in three metallogenic areas, both in the West Carpathians within the same general hydrothermal mineralization, in the Czech Massif, and finally in the Krušné Hory (*Erzgebirge*) in the *Geschieber* vein.

The last case is the most interesting one from the geophysical point of view. In Jáchymov the *Geschieber* vein is developed with a vertically banded structure. The outer bands are constituted by cinopels which are penetrated by a vein of earthy hæmatite. The individual

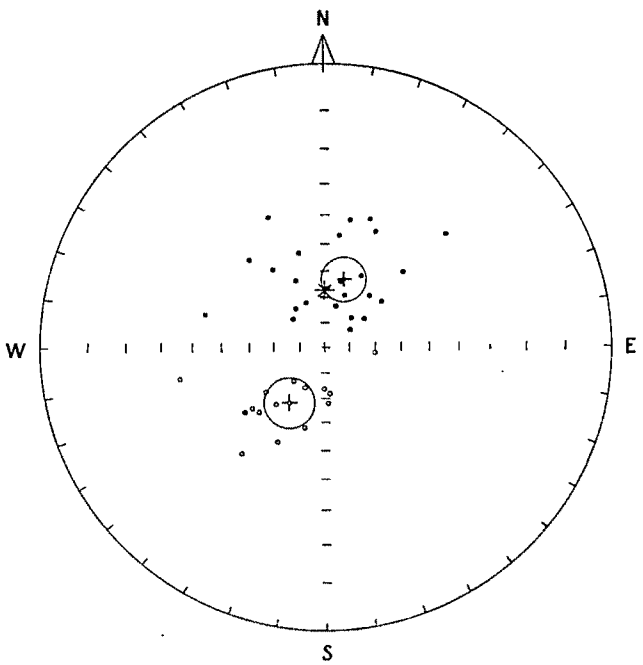


Fig. 1. Stereographic projection of the vectors of palæomagnetically stable remanent magnetization from the *Geschieber* vein, mine 'Svornost', locality Jáchymov, Krušné Hory region, Czechoslovakia. The full circle denotes the projection of the magnetization vector into the lower hemisphere; the empty circle denotes the projection into the upper hemisphere. Around the mean directions marked by a cross traversing a full or an empty circle are drawn the confidence circles. The asterisk marks the direction of the present-day theoretical dipole field

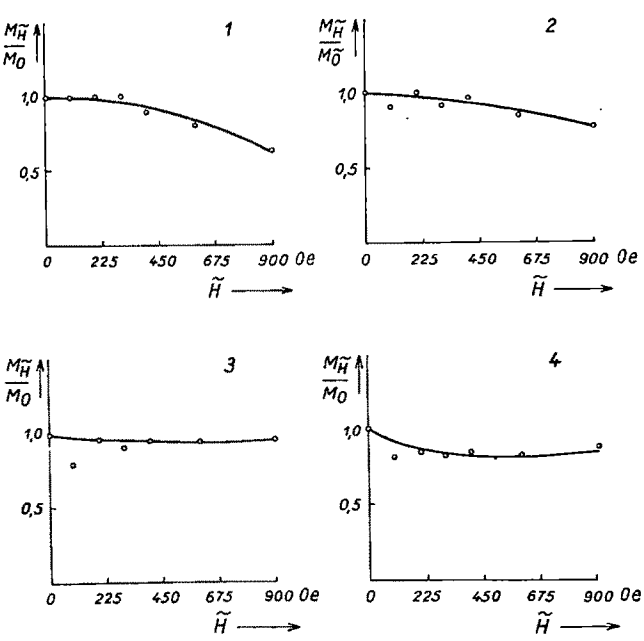


Fig. 2. A.c. demagnetization of samples from the *Geschieber* vein, in Jáchymov. The symbol $M_{\tilde{H}}$ indicates the magnitude of magnetic moment of sample demagnetized by field $\tilde{H}$ and the symbol M_0 magnetic moment of non-demagnetized sample

Table 1. Mean Directions of Remanent Magnetization of Nine Normally (Nor) and of Nine Reversely (Rev) Magnetized Samples

Peak value of a.c. demagnetizing field (œ)	Declination (deg.)		Inclination (deg.)		α (deg.)		k		R	
	Nor	Rev	Nor	Rev	Nor	Rev	Nor	Rev	Nor	Rev
0	17	205	64	−66	10·5	12	24·24	19·51	8·67	8·59
200	17	206	65	−66	10·5	11·5	25·81	21·05	8·69	8·62
400	18	208	64	−67	10·5	12	25·81	19·05	8·69	8·58
600	18	212	65	−64	10·5	14	25·00	15·09	8·68	8·47

veinlets (bands) evidently represent products of different mineralization acts belonging to the same general hydrothermal mineralization. The veinlet of earthy hæmatite showed reverse remanent magnetization with respect to the normal magnetization of the cinopels (Fig. 1). Nine normally and nine reversely magnetized samples were separated from the collection and subjected to the demagnetization in alternating magnetic field. The tests proved a high stability of samples in the alternating field, as it is seen that data given in Table 1 (Fig. 2) illustrate demagnetization graphs of four representative samples, of normally[1,2] and oppositely[3,4] magnetized ones. The foregoing phenomenon indicates that products of different mineralization acts belonging to different periods of one and the same general hydrothermal mineralization reveal remanent magnetizations differing mutually by 180° in their directions. This phenomenon is analogous to that which has been found many times in lavas and sediments, and which in the majority of cases has been interpreted as being due to the reversal of the geomagnetic field in the geological past.

The *Geschieber* vein was formerly considered by most authors to be of Permian age, but the palæomagnetic examinations suggest the Neoid (most probably the Tertiary) age of the hæmatite-containing assemblages in this vein. As it has been palæomagnetically found also in other deposits of the Krušné Hory region, much of the mineralization in this classical area is much later than generally thought.

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PHYSICS

Cæsium-137 in Air and Precipitation in Norway during October 1957

In an earlier communication[1] we reported a relatively heavy deposition of cæsium-137 at Bergen, Norway, during the period September–October 1957. We suggested that this might have been due chiefly to the Windscale accident, meaning that about 5 mc./km² of the measured 7·4 could not easily be accounted for by nuclear weapons tests, as the deposition in neighbouring periods was about 2 mc./km² or less.

Booker and Chamberlain[2] found it unlikely that a cæsium-137 deposition of 7 mc./km² at Bergen, 800 km from Windscale, was due to the accident, considering that the 20 mc./km² isopleth lay at an average distance of about 30 km from Windscale. We wish to re-affirm our suggestion by presenting more results concerning radioactivity in air and precipitation in Norway.

Measurements of cæsium-137 in ground-level air carried out at the Norwegian Defence Research Establishment give the following mean values for October 1957:

Sola	(58·9° N., 5·6° E.)	0·039 pc./m³	
Bergen	(60·4° N., 5·4° E.)	0·016 ,,	
Finse	(60·6° N., 7·5° E.)	0·007 ,,	(1,200 m above sea-level)
Kjeller	(60·0° N., 11·1° E.)	0·017 ,,	(ashed filters)
Gardermoen	(60·2° N., 11·1° E.)	0·022 ,,	
Værnes	(63·5° N., 11·0° E.)	0·006 ,,	
Tromsö	(69·7° N., 19·0° E.)	0·004 ,,	
Vadsö	(70·1° N., 29·7° E.)	0·002 ,,	

The filters from Sola, Finse, Gardermoen, Værnes and Tromsö covering the period October 9–17 were also measured at the Atomic Energy Research Establishment, Harwell. The results were[3]:

All filters, October 9–15, iodine-131, nil.
Sola, October 15–16, iodine-131, 2·03 pc./m³, cæsium-137, 0·75 pc./m³.
Sola, October 16–17, iodine-131, 0·54 pc./m³ (cæsium-137 not given).
Gardermoen, October 15–16, iodine-131, nil.
Gardermoen, October 16–17, iodine-131, 0·66 pc./m³, cæsium-137, 0·23 pc./m³
Finse, Værnes, Tromsö, October 15–17, iodine-131, nil.

The cæsium-137 collected at Sola during October 15–16 accounts for 62 per cent of the mean value for October. The amount collected during October 16–17 accounts for another 17 per cent, using the same iodine-131 to cæsium-137 ratio.

The cæsium-137 collected at Gardermoen during October 16–17 accounts for 34 per cent of the mean value for October.

Apparently the fact cannot be excluded that the filters from Værnes and even Tromsö also contained significant amounts of iodine-131 and cæsium-137 during October 17–18 or 18–19.

The cæsium-137 activity in ground-level air at Chilton (51·5° N. 1° W.) has been reported by Peirson *et al.*[4]. The mean value for October 1957 is 0·0062 pc./kg, that is, 0·008 pc./m³. It is interesting to note that this is about 1/5 of the activity at Sola.

The deposition and activity of cæsium-137 in precipitation in Norway during October 1957 were:

Kjevik	(58·2° N., 8·1° E.)	1·1 mc./km²	10·6 pc./l.
Sola		2·1 ,,	9·5 ,,
Bergen		6·5 ,,	11·7 ,,
Finse		0·31 ,,	2·2 ,,
Kjeller		0·36 ,,	6·1 ,,
Gardermoen		0·56 ,,	7·6 ,,
Værnes		0·61 ,,	4·3 ,,
Bodö	(67·3° N., 14·4° E.)	0·97 ,,	3·2 ,,
Bardufoss	(69·1° N., 18·5° E.)	0·13 ,,	1·6 ,,
Tromsö		0·13 ,,	3·2 ,,
Vadsö		0·18 ,,	3·5 ,,

Of the 6·5 mc. cæsium-137/km² deposited at Bergen during October 1957, 5·0 mc./km², or 77 per cent, was deposited during the period October 13–27, corresponding to 21 pc./l. precipitation. 1·2 mc./km² was deposited during October 1–13 and 0·29 mc./km² during October 27–31.

The activity of cæsium-137 in precipitation at Milford Haven (51·5° N. 5° W.) and Chilton has also been reported by Peirson *et al.*[4]. The results for October are:

Chilton	5·2 pc./l. in 50 mm precipitation, that is, 0·26 mc./km²
Milford Haven	8·4 pc./l. in 149 mm precipitation, that is, 1·25 mc./km²

It appears that activity of cæsium-137 in precipitation and corresponding deposition was less at Milford Haven and Chilton than at Bergen.

A possible explanation is that the cæsium-137 deposited at Bergen came from nuclear weapons tests. However, the cæsium-137 to strontium-90 ratio makes this unlikely. The strontium-90 deposition at Bergen has been measured at the Division of Chemistry of this establishment[5]. The cæsium-137 to strontium-90 ratio is 18 for the period October 13–27. This is about 10 times higher than expected in fall-out from nuclear bombs, and suggests that up to 90 per cent or 4·5 mc./km² of the cæsium-137 deposition at Bergen during the period October 13–27 was due to the Windscale accident, the strontium-90 release at which has been given as less than 1 per cent of the cæsium-137 release[6]. The cæsium-137 to strontium-90 ratio is 1·7 for the period October 1–13 and 1·9 for the period October 27–31.

The weather situation in the area between Windscale and Norway, October 10–17, 1957, has been examined by Storebö[7]. The main feature during the first four days after

October 10 was a centre of high pressure moving from the west over England and spreading out over central Europe. At the same time small changes in the high caused backward and forward air movements over England and the North Sea. During the evening of October 14 a south-westerly wind field over England and the North Sea was initiated by a centre of low pressure moving towards the British Isles from the west. This wind field grew successively over the Scandinavian Peninsula and forced the lower air layers in the former high to move to north-north-east. Thus radioactivity from Windscale reached south-west Norway during October 15–16. In this weather situation, little rain fell while the cloud from Windscale passed over England[8]. In Norway, on the other hand, the passage of the cloud was accompanied by heavy rain in several places. It is concluded, therefore, that the meteorological conditions caused a heavier deposition of cæsium-137 from the accident at some places in Norway than at others closer to Windscale.

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Some Effects of Electron Irradiation on the Young's Modulus of Graphite

Following detailed investigations[1,2] of the changes in the thermal and electrical properties of pure pile graphite after electron irradiation at liquid nitrogen temperature, a similar study has been made of changes in the Young's modulus of this material.

The specimens were in the form of rods 3 mm square and 50 mm long, and Young's modulus was measured using a cantilever technique with the specimen supported at both ends and the load applied at its centre. The central displacement was measured using a transducer.

A preliminary examination was made of the Young's modulus as a function of specimen temperature between 80° K and 400° K. It was found that Young's modulus increased as the temperature was lowered, but that both the magnitude and temperature dependence of this quantity varied from specimen to specimen. It has been pointed out by Simmons[3] that the elastic property involved is the sheer modulus of the crystallites.

The specimens were immersed in liquid nitrogen, and irradiated by 4 MeV electrons using a similar technique to that described for the earlier work[1,2]. The beam current incident on the specimen was 10–20 μamp, and irradiation periods of 1–8 h were used. The actual dose the sample received was monitored in terms of its electrical resistance, and changes of 3–37 per cent were recorded. During the irradiations and subsequent transfer to the measuring and annealing apparatus the specimen temperature was kept below 80° K. The annealing was carried out using the pulse technique described in the previous papers[1,2], that is, the specimen was heated rapidly to the annealing temperature, which was maintained for 1 min, and then rapidly cooled. The specimen temperature was measured by thermocouples inserted in it, and the Young's modulus measurements were all made at a standard temperature of 80° K.

In all the specimens measured there was no initial change in Young's modulus following the irradiation. On annealing, Young's modulus remained constant until a temperature of about 200° K had been reached. Above this temperature there was a sharp inverse annealing peak with a maximum about 270° K. Fig. 1 shows the Young's modulus as a function of annealing temperature for specimens which had different irradiation doses.

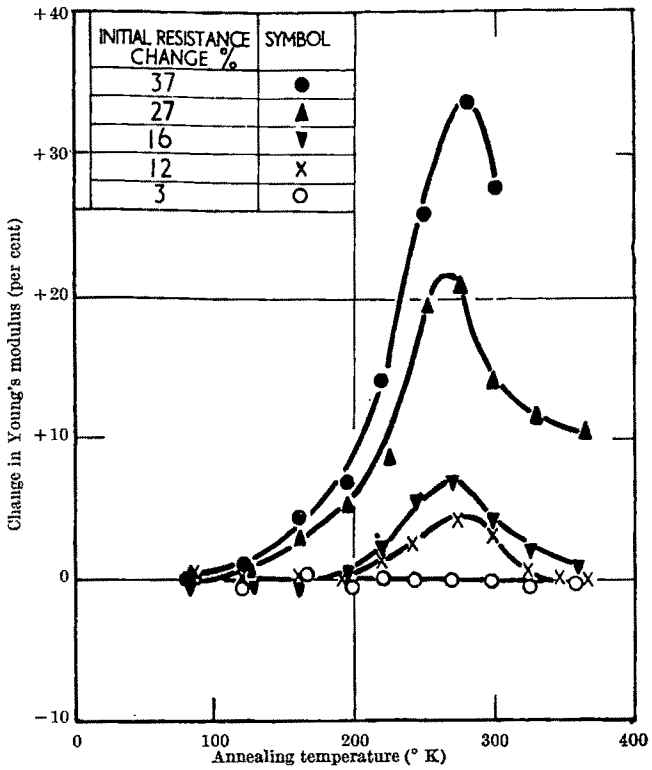


Fig. 1. Change in Young's modulus of graphite as a function of annealing temperature

The height of the peak is found to vary as the square of the initial resistance change. As the irradiation dose is increased the anomalous rise sets in at a lower temperature and the peak develops a plateau on the high-temperature side. It coincides exactly with the inverse annealing peak observed in the annealing of thermal resistivity.

Pile graphite is composed of small crystallites, and single interstitials are only mutually accessible if they lie between the same pair of basal planes in the same crystallite. Then for small numbers of mutually accessible interstitials the chance of di-interstitial formation increases as the square of the dose. Thus the anomalous Young's modulus peak may be associated with the formation of di-interstitials. The plateau seen in the higher dose specimens arises because of the greater probability of forming larger complex groups such as 4's as the dose is increased.

The fact that single interstitials cause no change in Young's modulus, and pairs have such a marked effect suggest that the linking bond of a di-interstitial may serve as a coupling between neighbouring basal planes. This implies that the explanation of the inverse annealing of thermal resistivity in terms of Rayleigh scattering may not be adequate and that it is the increased coupling between neighbouring planes on di-interstitial formation that is largely responsible.

Noting that the anomalous peak in Young's modulus is removed by annealing to 290° K and applying a 2-dimensional diffusion equation, we find that the activation energy of motion of a di-interstitial is 0·9 eV, in agreement with results given previously[2]. The build up of the peak is also consistent with the previously estimated value of 0·5 eV for the activation energy of motion of a single interstitial. It is not consistent with the lower values inferred more indirectly by Schweitzer[4], or those calculated purely theoretically by Iwata *et al.*[5].

The relative insensitivity of Young's modulus to single defects and its high sensitivity to complex groups makes it a useful property for the study of their formation in the graphite lattice.

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Use of Complex Ions in the Efficiency Tracing Method for the Standardization of Pure Beta-emitting Nuclides

The absolute standardization of pure β-emitters by 4πβ-counting involves errors due to uncertainties in self-absorption. This difficulty may be overcome by the use of the efficiency-tracing technique described by Campion *et al.*[1]. The technique involves the inclusion of a suitable β-γ-emitting nuclide with the pure β-emitter in the deposited source and the use of β-γ-coincidence counting to determine the efficiency of detection of the betas arising from the β-γ or 'tracer' nuclide. Knowledge of the β-efficiency for the tracer in a series of sources may then be used to determine the absolute activity of the pure β-emitter.

Choice of a suitable tracer is limited by certain physical requirements. Ideally the spectral shapes of the tracer and the pure β-emitter should be identical. The β-end-point energies of the two nuclides should be close to one another. The decay scheme of the tracer should be simple, if possible with a single β-branch, and there should be little internal conversion.

Nuclides fulfilling the requirements for a simple decay scheme which also have maximum β-energies of less than 1 MeV are scandium-46, cobalt-60, bromine-82, niobium-95, cerium-141, lutecium-177, gold-198 and mercury-203.

The choice of a tracer is further restricted by certain chemical considerations. To ensure that the corrections are reliable it is essential that the tracer and the pure β-emitter are deposited uniformly throughout the volume of the source; any separation of the two nuclides will introduce errors. In practice, as ionic solutions are usually employed, the best results will be obtained with a tracer ion opposite in charge to the pure β-emitting ion. This encourages the formation of a deposit in which the two ions are evenly distributed, and in which, in favourable cases, they are incorporated in a common lattice as a clearly defined compound.

For the determination of anions such as $^{35}SO_4^{=}$ there is a wide choice of tracer cations, but for the measurement of cations the selection is very much more limited. Of the tracers mentioned here, only bromine-82 normally occurs as an anion. In order to rectify this position we suggest the use of anionic complexes of suitable nuclides as tracers for the measurement of pure β-emitting cations. Complexes of this kind can be readily formed by interaction of the cation and a suitable ligand. One such ligand is the cyanide ion, which will combine with cobalt, gold or mercury to give very stable anionic complexes.

Using a solution containing potassium cobalticyanide prepared in this way from a cobalt nitrate solution labelled with cobalt-60 we have applied the technique to the standardization of calcium-45 and promethium-147. Full details of these measurements will be published elsewhere.

There is no reason why the technique should be limited to cyanide complexes and it seems probable that suitable complexes can be prepared for each of the tracer nuclides listed above. Although the number of pure β-emitting cations which require tracer measurements of this kind is small, the wider choice of tracer offered by this technique should be of value in improving the accuracy of the standardizations.

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CHEMISTRY

Energy of Formation of D-Defects in Ice

Much of the literature dealing with the dielectric properties of ice[1] has used Bjerrum's concepts of D- and L-defects[2]. A pair of D- and L-defects is formed (Fig. 1) when a water molecule in ice rotates through 120° around one of its O–H . . . O axes, thus leaving one pair of neighbour O . . . O atoms with no intervening hydrogen atom (L-defect), and another pair of neighbours O–H . . . H–O with two hydrogens (D-defect). A subsequent similar rotation of the appropriate molecule adjacent to the L-defect separates these two defects.

Recently, Dunitz[3] has suggested that the repulsive interaction of the two hydrogen atoms in a D-defect is so great that its formation is unlikely. He proposes an alternative to the D-defect, called an X-defect, in which repulsive interactions of hydrogens are reduced. We wish to point out that if changes in bond-lengths and -angles in the neighbourhood of a D-defect are considered, then the H . . . H repulsion energy in this defect will be considerably less than that calculated by Dunitz. These changes, which we shall refer to as a 'relaxation' of the lattice, occur with relative ease owing to the relatively weak bonding in the neighbouring H . . . O bonds.

If no relaxation occurs in a D-defect, then the two hydrogen atoms are at a separation of 0·78 Å (O_1-H_1 . . . H_2-O_2, Fig. 2). We have confirmed the result of Dunitz that their repulsive interaction energy in this case is about 60 kcal mole^{-1}.

Let us now allow relaxation to occur: O_1 moves a distance Δr to O'_1, and O_2 a similar distance in the opposite direction to O'_2. In addition the length of the bond O_1-H_1 is compressed from a to a''. If we assume that O_3 is fixed, then the O_3-H_3 . . . O_1 angle is reduced

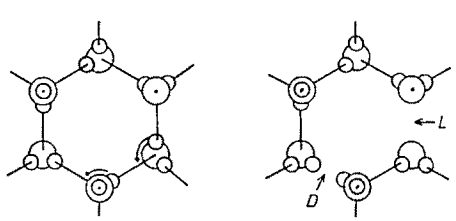


Fig. 1. Formation of a pair of D- and L-defects in ice

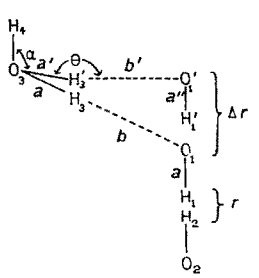


Fig. 2. Changes in bond-lengths and -angles in the neighbourhood of a D-defect shown schematically. The actual lengths and angles are given in the text

from 180° to θ, and the $H_4-O_3-H_3$ angle from 109·47° to α. The $O_3-H_3\ldots O_1$ distance is reduced from $(a+b)$, which is 2·76 Å, to $(a'+b')$.

Similar relaxations occur in the other adjacent molecules.

We now seek the values of a', a'', b', θ, α which minimize the total energy. This total energy comprises $H'_1\ldots H'_2$ repulsion, twice the $H'_1-O'_1$ compression energy, and six times the various $H_4-O_3-H'_3\ldots O'_1$ bending and compression energies. For the H . . . H repulsion we have used Hendrickson's semi-empirical[4] potential:

$$V(r)=10{,}000\ \exp(-4{\cdot}6r)-49{\cdot}2r^{-6}$$

where V is in kcal mole^{-1} and r is in Å. The other force constants are taken to be[6]: k(O−H compression) = 8·07 derived from spectroscopic data (ref. 5), $k(O_3-H_3\ldots O_1$ compression) = 0·155, $k(O_3-H_3\ldots O_1$ bending) = 0·085, $k(H_4-O_3-H_3$ bending) = 0·56, all in units of 10^5 dynes cm^{-1}. The resulting minimum energy is 5·19 kcal per mole of D-defect, and it occurs when $r=1{\cdot}80$ Å, $\Delta r=0{\cdot}50$ Å, $a-a''=0{\cdot}01$ Å, $a'=0{\cdot}99$ Å, $b'=1{\cdot}66$ Å, $\theta=169{\cdot}75°$, $\alpha=105{\cdot}47°$. If we now also allow O_3 and the five other first-neighbour oxygen atoms to O_1 and O_2 to relax, this energy will be even lower.

Since relaxation has the effect of reducing the sum of the repulsive and strain energies in a D-defect from about 60 kcal to about 5 kcal it is clear that the existence of such defects cannot be ruled out.

A more detailed comparison of the total energies of formation of D- and X-defects is being prepared.

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Decomposition of Alkali Metal Nitrates by Light Ions of Kilovolt Energy

When alkali metal nitrates are bombarded by positive ions of energy in the range 1–15 keV a decomposition occurs, the main features of which are represented by the stoichiometric equation:

$$NO_3^- \rightarrow NO_2^- + \tfrac{1}{2}O_2 \qquad (1)$$

Using a fairly uniform ion beam the yield of nitrite, y, is related to the dose, x, by the equation:

$$y=y_s(1-e^{-bx}) \qquad (2)$$

where y_s is the saturation yield and depends on the alkali metal and b is a constant. This is shown in Fig. 1 for the nitrates of potassium and rubidium using the narrow beam (½ in. diam.) and may be deduced for the others since the yields produced by a non-uniform beam are a constant fraction of those from potassium nitrate when a similar dose is given, as shown in Table 1. D^+ ions of 2 keV were used in these experiments and from the initial slopes $G(NO_2^-)$ may be calculated as 0·6, 1·8, 2·0, 1·5 and 1·4 for the nitrates of lithium, sodium, potassium, rubidium and cæsium. At both high and low temperatures there are observed increases in yield, which follow different types of dependence on dose, as shown in Fig. 2.

In a recent paper[1], a model for this decomposition was used which (it seems to me) is inadequate in certain important respects, and the purpose of this communication is to present an alternative interpretation of the reaction processes which is qualitatively consistent with the

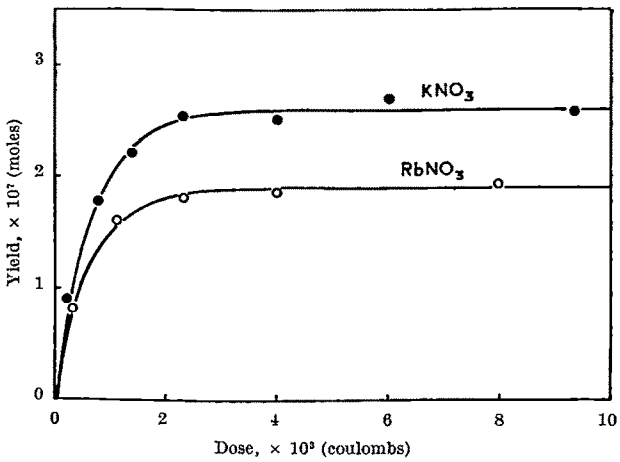


Fig. 1. Nitrite yield from potassium nitrate (●) and rubidium nitrate (○) as a function of the dose of 2 keV D^+ ions

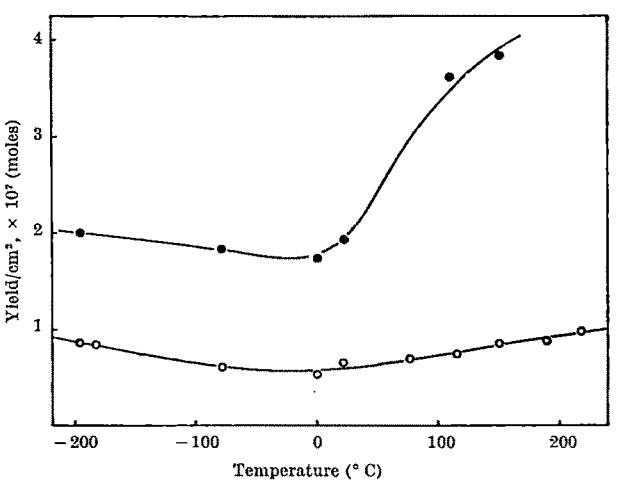


Fig. 2. Variation with temperature of the yields of nitrite/cm² at doses of 0·23 × 10^{-3} (○) and 3·0 × 10^{-3} (●) coulombs/cm² of 2 keV D^+ ions

experimental results and with revised assessments of the displacements produced by atomic collisions.

From equation (2) the increase in yield, δy, produced by an increment of dose, δx, is given by:

$$\delta y=b(y_s-y)\delta x \qquad (3)$$

which is explicable if the effect of an ion striking a surface zone of nitrates is to produce an extent of conversion to nitrite and oxygen which undergoes no net change from subsequent bombardment by another ion. On this model the constant b is a measure of the mean effective cross-section of the ion (calculated as 3·1 Å² from Fig. 1 for 2 keV D^+ ions), and the saturation yields, which fluctuate by a factor of about three over the nitrates used, depend partly on the depth to which chemical effects occur but chiefly on the degree of reaction achieved within an individual bombarded zone. There is evidence, cited previously[1], that complete decomposition of alkali metal

Table 1. Relative Yields from the Wide Beam (1 in. diam.)

	Dose (coulombs × 10^3)	Yield (moles × 10^7)	Yield / Yield from KNO_3	
$LiNO_3$	1·5	0·97	0·26	0·30 ± 0·03
	6·6	2·24	0·32	
	25·2	2·61	0·31	
	31·5	2·32	0·28	
$NaNO_3$	5·4	5·85	0·91	0·88 ± 0·04
	27·1	7·46	0·88	
	41·6	7·07	0·85	
$CsNO_3$	2·1	3·0	0·67	0·68 ± 0·02
	32·4	5·65	0·67	
	47·2	5·94	0·70	

nitrates cannot be achieved by prolonged radiolytic or thermal treatment.

In calculating yields of displaced oxygen atoms by a collision theory model, considerably higher values would be obtained if it were assumed, as seems reasonable, that all fast displaced atoms of sufficient kinetic energy may cause decomposition of the nitrate group. Since energetic collisions with nitrite ions may be expected to cause their decomposition, the evidence that little further degradation occurred on prolonged bombardment[1] indicates that reactions must occur by which the nitrite ion is re-formed, which seems probable since the fragments produced by collision processes will be in close proximity. Other reactions of primary products are also to be expected and the observed yields can only be correlated with those to be computed on the above assumption if a process such as:

$$NO_2^- + O \rightarrow NO_3^- \qquad (4)$$

occurs to a significant extent. It would seem probable that the differing extents of decomposition produced in the alkali metal nitrates is determined mainly by the relative importance of reaction (4), since only slight differences in yield are predicted from the calculations.

A quantitative treatment of the reactions of the primary species would require knowledge of the initial distributions, which might be estimated by considering probable collision sequences, and of the rate constants and diffusion coefficients applicable in the track where there is excess vibrational energy due to that lost in non-disruptive collisions. Since in a relatively open lattice the probability of a recombination reaction will be reduced, such calculations might be expected to predict the largest yields from potassium nitrate. However, the relation between yield and crystal volume per molecule is scarcely likely to be a direct proportionality so that formal agreement is unlikely with the expressions obtained[1] using the empirical factor $\theta = V(MNO_3)/V(KNO_3)$. This parameter, which was introduced on the premise that the Bohr equations for atomic collisions might not apply in a tightly bound lattice, is in any event unsatisfactory since it uses potassium nitrate as an arbitrary standard.

The high temperature increase in the yield has been correlated with the occurrence of secondary electron emission. The low temperature enhancement, which is most marked at low doses and thus corresponds to an increase chiefly in b, may be due to a diminished extent of reaction (4) in the track periphery on account of the rapid cooling.

It may be noted from Table 2 (ref. 1) that in all cases higher values of $G(NO_2^-)$ are obtained from D^+ ions and α-particles than from X-rays or γ-rays. The difference is greatest in the case of those nitrates which, having little 'free space' within the lattice, are the most stable towards X- or γ-radiolysis, so Hochenadel[2] suggested that local heating in the α-particle tracks promotes the escape of fragments from the cage, especially where this is retarded by a closely-bound lattice. The similarities in the G values from α-particles and D^+ ions, which probably reflect similar consequences of thermal effects, is not surprising in view of the comparability of the linear energy transfer of α-particles (34 eV/amp) with that of the fast displaced atoms, of which O atoms of linear energy transfer around 10 eV/amp are the most numerous.

Some recent work[3] on the velocity distribution of sputtered metal atoms provides evidence for the generation of thermal spikes by ion bombardment. For example, it was estimated that 4 per cent of the gold atoms sputtered by 43 keV argon ions are evaporated from heated spikes, the temperature of which is deduced to be about 1,750° K.

Regarding the apparent dose-rate effects reported in Fig. 4 (ref. 1), the increase in the yield from a constant small dose with increasing ion current shown in the upper curve may be attributed to space charge spreading since the variations could be predicted by applying the charts of Thonemann[4] and the dose-yield curve of Fig. 1 to a beam of bell-shaped cross-section, taking the initial half-width as ¼ in. on the basis of source and lens geometry. As only a very slight effect is to be expected under the conditions listed for the lower curve, where the beam area is reduced and the mean dose per unit area increased, each by a factor of 4, it is rather surprising that such results should be obtained if the current is varied by a means which cannot otherwise affect the beam intensity profile and so an alternative explanation is required.

I thank S. N. Brown, who performed some additional experiments.

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Height Equivalent to a Theoretical Plate of Tubular Gas Chromatographic Columns lined with a Porous Layer

In a former publication[1] the following expression was derived for the height equivalent to a theoretical plate (HETP), h, of a tubular column of radius r:

$$h = 2\frac{D_g}{v} + \frac{1+6k+11k^2}{24(1+k)^2}\frac{r^2v}{D_g} + \frac{k^3}{6(1+k)^2}\frac{r^2v}{K^2D_l} \qquad (1)$$

where: v = average carrier gas velocity; D_g = molecular diffusion coefficient of component in the gas phase; D_l = molecular diffusion coefficient of component in the liquid phase; k = capacity ratio of liquid over gas phase; r = radius of tubular column; K = partition coefficient.

When the ratio of the last two terms, which is of the order of $\frac{D_g}{K^2D_l}$, is high, it becomes beneficial to disperse the retaining liquid film in a porous layer, of thickness αr, in order to make the time of diffusion in the liquid phase negligibly small.

This porous layer acts as a fixed phase for the inert component as well, and the HETP of such a lined column can be calculated as follows under the assumption that the layer is sufficiently porous, and the size of the average interstices sufficiently larger than the mean free path, so that the molecular diffusion constant within that layer for inert molecules is practically equal to D_g.

Let r designate the inner radius of the porous layer, that is, the radius of the unobstructed gas path. For α small, the HETP of inert components can be calculated by inserting a virtual capacity ratio of value:

$$k_{v_0} = 2\alpha$$

and by making $K = 1$ and $D_l = D_g$ in the above formula.

Since the total relative column capacity has been increased from unity to $1 + k_{v_0} = 1 + 2\alpha$ for inert components, the total relative column capacity should be increased from $1+k$ to $(1+2\alpha)\cdot(1+k)$ for retained components, so that the virtual capacity ratio for these should be:

$$k_v = (1+2\alpha)k + 2\alpha$$

For the partition coefficient K, we should now use the virtual coefficient:

$$K_v = \frac{k_v}{2\alpha} = 1 + k + \frac{k}{2\alpha}$$

while the liquid diffusion term, D_l, should be replaced by D_l/K_v.

Making these substitutions in (1), and neglecting terms in powers of α higher than unity, we obtain for the HETP of a porous tubular column:

$$h = 2\frac{D_g}{v} + \frac{1}{24}\left[\frac{1+6k+11k^2}{(1+k)^2} + 8\alpha + \frac{16k\alpha}{(1+k)^2}\right]\frac{r^2v}{D_g} \quad (2)$$

This formula indicates, for example, that for $k = 3$, and for values of α as high as 0·1, that is, for tubular columns in which the porous layer cross-section equals one-fifth of the unobstructed cross-section, the term within the bracket is increased from 7·4 to 8·5, that is, the dynamic diffusion term is 15 per cent larger than it would be in an ideal tubular column in which the diffusion time within the liquid layer would be negligible. This means also that the performance index achievable in such a column would be 15 per cent higher than theoretically possible in an ideal column.

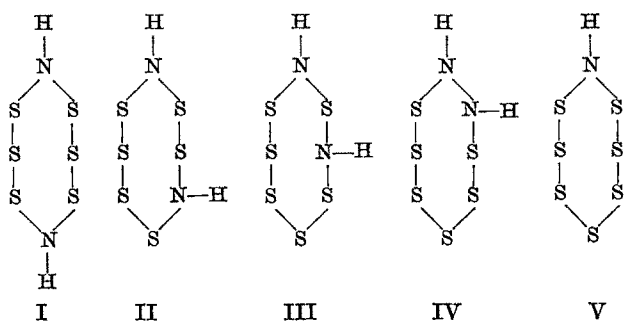
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Isomers of Hexasulphur Diimide

WEISS has reported[1] that he had obtained a little of a new compound $S_6(NH)_2$, hexasulphur diimide, from the products of the reaction between ammonia and disulphur dichloride[2]. He later assigned it the structure (I) on the evidence of an X-ray analysis[3].



I II III IV V

From the products of the same reaction, in formdimethylamide solution[2], I have now isolated no fewer than three compounds of the formula $S_6(NH)_2$, which I believe to be the isomers (I), (II) and (III). For their preparation, 15 g of the crude reaction product (consisting mainly of sulphur and heptasulphur imide) were subjected to chromatography on a silica-gel column 40 cm × 8 cm, eluting with carbon disulphide (8 l.). The sulphur came off first, followed by heptasulphur imide and then by the three hexasulphur diimides, which were cleanly separated from the heptasulphur imide but only partly separated from each other. Pure specimens of the isomers were obtained by two recrystallizations, from carbon disulphide, of appropriate combinations of chromatographic fractions The separations were monitored by infra-red absorption spectroscopy. The diimides all crystallized well as colourless rhombs with a tendency to needle habit.

Some data on the separation and properties of the diimides, identified as *A*, *B* and *C*, are given in Table 1.

The argument for the structures assigned is as follows. *A* gives an infra-red absorption spectrum which differs from that of S_7NH, structure (V), only in being twice as intense and in showing a slightly shifted S—N frequency. *B* shows a split S—N frequency, and *C* a greater split. Therefore the interaction between the two imino groups, and their proximity, must increase from *A* through *B* to *C*. Structure (IV) I exclude because it is difficult to see how it could be formed by the reactants. These assignments are, of course, tentative and require confirmation.

My compound *A* is almost certainly that described by Weiss.

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Application of Atomic Absorption Spectroscopy to the Determination of Cobalt in Steel, Alloy Steel and Nickel

THE determination of cobalt at low concentration has become important in relation to materials associated with atomic energy uses. Specifications for steels for such purposes require 0·02 per cent maximum and in some cases, 0·01 per cent maximum cobalt.

Because of this the British Standards Institute has recently produced a method—BS1121: Part 42: 1961—suited to the range up to 0·04 per cent cobalt for which a reproducibility of ± 0·001 per cent up to 0·01 per cent and ± 0·002 per cent up to 0·04 per cent cobalt is claimed. To obtain the desired precision, a rather involved and longthy method is required, including a zinc oxide separation, precipitation of cobalt with α-nitroso β-naphthol, and re-solution of cobalt with final photometric determination using nitroso *R* salt.

Table 1

	A	*B*	*C*	S_7NH
Probable structure	I	II	III	
IR spectrum (Note 1): N—H region	2·99μ, *0·12*	3·00μ, *0·12*	2·99μ, *0·11*	3·01μ, *0·061*
IR spectrum (Note 1): S—N region	12·28μ, *0·22*	12·21μ, *0·21*; 12·82μ, *0·04*	11·97μ, *0·16*; 12·88μ, *0·13*	12·40μ, *0·117*
Analysis %: N (calc. 12·6)	13·1	13·2	12·9	
Analysis %: H (calc. 0·91)	0·7	0·8	0·8	
Analysis %: S (calc. 86·5)	88·0	87·7	88·7	
Mol. wt. (Note 2) (calc. 222)	200	240	200	
M.p.	155° (dec) (darkening from 130°)	133°	130° (dec) (darkening from 120°)	113·5°
Solubility in carbon disulphide at 18° (molal)	0·023	0·15	0·038	0·57
R_F values (Note 3)	0·27–*0·24*–0·19	0·38–*0·34*–0·27	0·26–*0·22*–0·18	0·72–*0·58*–0·47
Total quantity (g) present in 15 g crude starting material	0·29	0·53	0·29	4·27
Quantity (g) isolated pure	0·12	0·14	0·08	

Notes. (1) All prominent bands between 2·5μ and 15μ are listed. Wave-lengths are correct to ±0·02μ. Figures in italics are optical densities, at band maxima, of 0·023 molal solutions in carbon disulphide; a 0·2-mm cell was used.
(2) Approximate values only, by cryoscopy in tertiary butanol (semi-micro).
(3) Outer limits of band, and band maximum (latter in italics).

In view of the quoted atomic absorption detection limit of 0·2 p.p.m. for cobalt by Allan[1] and Gatehouse and Willis[2], the determination of cobalt by this approach was investigated. Equipment used was that described by Box and Walsh[3]. The cobalt lamp was a commercial hollow cathode supplied by Ransley Instruments. Allan[4] stated the strongest line in absorption is 2407·2 Å and this was used in the work. The monochromator is required to resolve this line from another strong line at 2411·6 and a weak line at 2404·2 Å.

Using 2 g of sample dissolved in hydrochloric acid and oxidized with nitric acid similarly to the BS1121: Part 42 solution procedure, diluting to 50 ml. and using a 10-cm slightly lean air-acetylene flame absorption path, a sensitivity of 0·001 per cent cobalt was obtained and results on plain carbon, low alloy, stainless and silicon steels from the range of B.C.S., N.B.S. standard samples and a number of other samples were within the Part 42 agreement tolerance against certificate values and/or results obtained on the same samples by Part 42.

At the low wave-length there was some absorption by the high iron concentration and calibration series required to be based on cobalt-free iron with added cobalt.

Using the BS1121: Part 30: 1954 solution treatment where 0·5 g of sample is dissolved in 20 ml. phosphoric–sulphuric acid mixture, oxidized with nitric acid and diluted to 100 ml., tungsten was retained in solution and satisfactory results were obtained on a wide range of low- and high-alloy steels, high-speed steels and pure nickel, results generally agreeing with certificate results and/or those obtained on the same sample by Part 30 within the tolerance reproducibility limits of Part 30 (a direct photometric method using nitroso *R* salt).

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Thin-layer Chromatography of Some Food Colours on Silica Gel

Thin-layer chromatography, first devised by Stahl[1], is a very useful technique for the separation and identification of organic compounds on a micro-scale. Among its many advantages is its speed, requiring at the most about 90 min to complete one chromatogram. As yet, we are aware of only one report in the literature describing its use for water-soluble dyestuffs. Wollenweber[2] has described chromatographic systems for dyestuffs on the West German permitted list of food colours using cellulose powder as the thin layer. Here we describe the use of silica gel as an adsorbent for the thin layer chromatography of sulphonated dyestuffs.

Paper-chromatography of water-soluble dyes is quite well known[3]. A large number of solvent systems have been devised for their separation. Generally these contain large amounts of water. In our experience thin layers of silica gel often flake off the glass supporting plate with solvent systems containing more than 10 per cent by volume of water. We have therefore devised solvents which will separate the dyes examined and which contain minimal amounts of water.

The dyes examined were among those present on the list of permitted food colours for New South Wales. They are listed in Table 1 with their Colour Index number and their R_{mo} values for each satisfactory solvent. R_{mo} is defined as:

$$\frac{R_F \text{ of dye}}{R_F \text{ of methyl orange}}$$

This was found to give consistent values in contrast to R_F values which were unreliable, varying greatly with only small differences in composition of any of the solvents used.

The chromatograms, in general, required no more than 30 min for completion and gave excellent separations. Trial mixtures were well resolved. Only indigo carmine gave poor results, either streaking, fading, or not moving from the starting line. This does not happen on paper chromatograms of this dye in similar solvents. In fact our sample of this dye splits into three components. Erythrosine showed at least two components in solvents *B*, *D*, and *E*, and three in solvent *C*. Other dyes revealed as mixtures were chlorazol pink *Y*, chocolate brown *FB*, and brown *FK*. Chlorazol pink *Y* may be a mixture of isomeric sulphonic acids[4], chocolate brown *FB* is a mixture of dyes resulting from the coupling of morin and maclurin with diazotized sulphanilic acid, while brown *FK* is a *bis*-azo dye derived from the coupling of *m*-phenylenediamine with diazotized sulphanilic acid. The separation of these substances into several components is, therefore, only to be expected and confirms the usefulness of the thin-layer technique. Another interesting point is the separation of the isomeric pairs scarlet *GN* and ponceau *SX*, and yellow *FCF* and orange *GGN*, which are easily distinguished.

This work forms part of a programme supported by the National Health and Medical Research Council of Australia, the Council of the Australian Food Technologists' Associations and grant *EF*-258 from the U.S. National Institutes of Health. We thank Prof. S. E.

Table 1. R_{mo} Values of Water-soluble Dyes

Dye	Colour Index 1956 No.	Solvent *A*	Solvent *B*	Solvent *C*	Solvent *D*	Solvent *E*
Methyl orange	13025	1·00	1·00	1·00	1·00	1·00
Erythrosine	45430	1·00	0·80, 1·00	0·00, 0·55, 0·95	0·76, 1·00	1·0, 1·45
Amaranth	16185	0·70	0·50	0·08	0·06	0·00
Ponceau *SX*	14700	0·77	0·65	0·44	0·29	0·70
Scarlet *GN*	14815	0·86	0·80	0·65	0·44	0·50
Carmoisine	14720	0·70	0·70	0·49	0·34	0·40
Brilliant scarlet	16255	0·70	0·25	0·09	0·04	0·09
Chlorazol pink *Y*	14780	0·70, 0·89	0·43, 0·90	0·10, 0·74, 0·84	0·47*	0·50*
Fast yellow	13135	0·80	0·60	0·40	0·32	0·20
Yellow *FCF*	15985	0·85	0·65	0·37	0·34	0·20
Orange *GGN*	15980	0·85	0·70	0·39	0·34	0·35
Tartrazine	19140	0·70	0·25	0·05	0·10	0·00
Indigo carmine	73015	0·70	—†	—†	0·00	0·00
Chocolate brown *FB*	—	0·00, 0·70	0·00†	0·00	0·00	0·00
Brown *FK*	—	0·77	0·50, 0·80	0·30, 0·60, 0·80	0·49, 0·80	0·20, 0·50
Black *BN*	28440	0·70	0·25	0·00	0·00	0·00

Solvent *A*, ethanol/concentrated ammonia (9 : 1); solvent *B*, acetoacetic ester/methanol/concentrated ammonia (5 : 2 : 1); solvent *C*, acetoacetic ester/pyridine/concentrated ammonia (5 : 2 : 1); solvent *D*, *n*-amyl alcohol/ethanol/concentrated ammonia (10 : 10 : 1); solvent *E*, ethyl acetate/pyridine/water (7 : 3 : 1).
* Streaked. † Faded.

Wright of this Department for his interest and encouragement.

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Crystal Structures of *Bis*-(Salicylaldehydato)-copper(II) and *Bis*-(8-Hydroxyquinolinato)-copper(II)

Anhydrous *bis*(salicylaldehydato)copper(II) and *bis*(8-hydroxyquinolinato)copper(II) were obtained by precipitation of the complexes from aqueous solution and recrystallization from water-free chloroform and chlorobenzene respectively. Both compounds formed bronze-green crystals. Their structures have been determined from single-crystal X-ray photographs using copper $K\alpha$-radiation. Both compounds were found to crystallize in the monoclinic space group $P2_1/c$, details being as follows:

Bis(salicylaldehydato)copper(II) had unit cell dimensions: $a = 8{\cdot}77$, $b = 6{\cdot}21$, $c = 12{\cdot}33$ Å, $\beta = 117{\cdot}4°$. The structure was solved by three-dimensional Fourier and Patterson methods and refined by the diagonal least-squares technique. There are two centro-symmetric $(C_7H_5O_2)_2Cu$ groups in the unit cell, the metal ion being co-ordinated to the oxygen atoms in a square planar configuration with O—Cu—O angle within the chelate rings 93·0° (e.s.d. 0·4°). The Cu—O distances are 1·87[5] and 1·93[2] Å for the phenolic and aldehydic oxygen atoms respectively (e.s.d. 0·017 Å in each case), and the next nearest atoms to the copper are aromatic carbon atoms from neighbouring molecules at a distance of 3·24 Å above and below the molecular plane.

The observed structure of *bis*(salicylaldehydato)copper-(II) confirms Stackelberg's conclusion from unit cell measurements alone[1] that the molecule is square planar. The distance of 3·24 Å to the nearest neighbours of the copper atom above and below the molecular plane excludes any intermolecular interaction which could be interpreted as giving the copper atom a co-ordination number of six, such as is found in *bis*(acetylacetonato)-copper(II)[2]; *bis*(salicylaldehydato)copper thus adds to the still small group of copper(II) compounds in which the metal atom has square planar stereochemistry.

Bis(8-hydroxyquinolinato)copper(II) had unit cell dimensions: $a = 10{\cdot}90$, $b = 8{\cdot}61$, $c = 18{\cdot}16$ Å, $\beta = 102{\cdot}0°$. Approximate co-ordinates of the metal atoms were obtained from the three-dimensional Patterson function and the rest of the structure solved by Fourier syntheses; refinement was carried out by the differential Fourier method. There are four $(C_9H_6ON)_2Cu$ groups in the unit cell, forming two dimeric units, one oxygen atom of each $(C_9H_6ON)_2Cu$ group completing a square pyramidal arrangement about the copper atom of another. Each copper atom is thus surrounded by two nitrogen atoms at 1·94[5] Å and two oxygen atoms at 2·00 Å very nearly in a plane and a third oxygen atom at the apex of the square pyramid at 2·85 Å; the Cu—Cu distance in the dimeric unit is 3·49 Å (all distances e.s.d. 0·03 Å) (Fig. 1).

Bridged bimolecular structures with tetragonal pyramidal 5 : co-ordination of the metal atoms have been observed in several other copper(II) complexes. Thus, in *bis*(dimethylglyoximato)copper(II)[3] two planar molecules are linked by co-ordination of one of the oxygen atoms of each to the copper atom of the other, giving an intermolecular Cu—O distance of 2·43 Å, and in dimethylglyoxime-dichloro-copper(II) there is a similar chlorine bridge between the copper atoms of a pair of molecules[4].

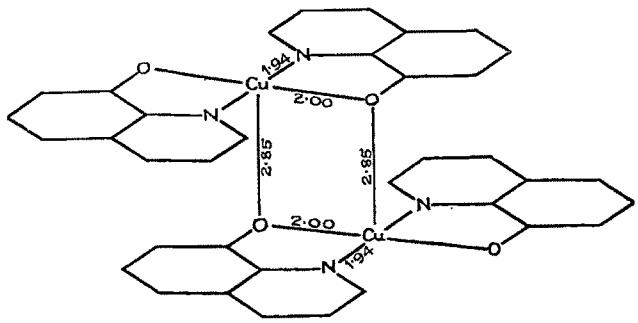


Fig. 1

In disalycilidine-ethylenediamine-copper(II)[5] dimerization occurs through one of the oxygen atoms, the remainder of the molecule being distorted to allow a Cu—O distance of 2·41 Å in the bridge.

In *bis*(8-hydroxyquinolinato)copper(II) only slight distortion is observed and the bridging Cu—O distance of 2·85 Å is correspondingly much longer, though still too short to be considered non-bonding. Observations of the spectra of this complex support the interpretation of this distance as representing a weak co-ordinate bond, the visible region, solid-state, reflectance spectrum being shifted about 70 mμ to longer wave-lengths as compared with chloroform solutions; whereas the reflectance and solution spectra for *bis*(salicylaldehydato)copper(II) are virtually the same. A similar shift to longer wave-length has been observed in 5 : co-ordinated adducts of *bis*-(acetylacetonato)copper(II) and related compounds[6].

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A Synthetic Cyclitol Glycoside

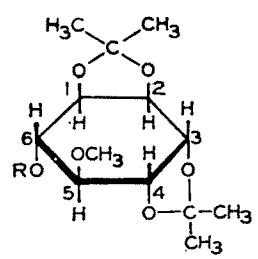
The synthesis of cyclitol glycosides is of interest because of the natural occurrence of these substances as such (galactinol) and as structural moieties of several antibiotics and phospholipids[1,2]. In this laboratory, attempts have been made to couple activated forms of glucose with protected derivatives[3] of *myo*-inosamine-2 and, more recently, (+)-pinitol. The former efforts were unsuccessful; but the latter provided a glucoside (V) of pinitol in fair yield.

The initial condensation was effected by the Koenigs–Knorr method. Diacetono-(+)-pinitol, which has been shown to be 1,2 : 3,4-di-*O*-isopropylidene-5-*O*-methyl-D-inositol (I)[4] (the numbering used here is that of Fletcher, Anderson and Lardy, reviewed in ref. 1), was prepared by the method of Anderson[5]. It was dissolved in alcohol-free chloroform and the solution was stirred for 1 h with dry silver oxide and anhydrous calcium sulphate ('Drierite'), then tetra-*O*-acetyl-α-D-glycopyranosyl bromide and iodine were added. Stirring was continued for 24 h, the reaction vessel being protected from light. Inorganic salts were removed by filtration on a 'Celite' pad, which was washed with chloroform, and the filtrate and washings were concentrated to a syrup under vacuum. The crystalline condensation product was obtained by dissolving the syrup in a minimal volume of hot methanol, adding water to

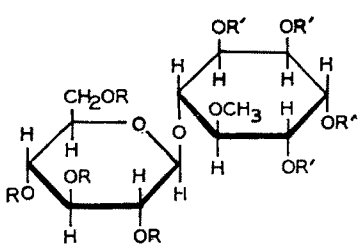
turbidity, and cooling. After recrystallization the compound melted at 167°–168°, and had $[\alpha]_D^{20}$ −3° (c=7, $CHCl_3$). Found: C, 53·02; H, 6·64. Required for $C_{27}H_{40}O_{15}$: C, 53·64; H, 6·67.

It must be assumed that condensation occurred at the only free hydroxyl in I, and it may be further assumed that steric inversion took place at the anomeric carbon of glucose, as usual, to give a β-glucosidic linkage. The condensation product is therefore formulated as 1,2 : 3,4-di-*O*-isopropylidene-5-*O*-methyl-6-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-D-inositol (II). The best yield of II (56 per cent of the theoretical from diacetonepinitol) was obtained with a reaction mixture of the following composition, per gram of diacetonepinitol : chloroform, 50 ml.; silver oxide, 1·2 g; 'Drierite', 20 g; tetraacetylglucosyl bromide, 2 g; and iodine, 0·2 g.

For removal of the isopropylidene groups, II was heated in 25 vol. of 80 per cent (v/v) aqueous acetic acid at 100° (thermostat) for 18 h. The resulting compound, which must be 5-*O*-methyl-6-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-D-inositol (III), was a syrup. On treatment with acetic anhydride in pyridine at room temperature it furnished, in essentially quantitative yield, the octaacetyl derivative of V, 1,2,3,4-tetra-*O*-acetyl-5-*O*-methyl-6-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-D-inositol (IV), needle-like prisms from aqueous methanol, m.p. 135–136°, $[\alpha]_D^{25}$ −31° (c=1·9, $CHCl_3$). Found: C, 49·89; H, 5·79; CH_3O, 4·60. Required for $C_{28}H_{37}O_{18}(CH_3O)$: C, 50·29; H, 5·82; CH_3O, 4·48.



I, R = H; II, R = tetra-*O*-acetyl-β-D-glucopyranosyl;



III, R = CH_3CO, R' = H; IV, R = R' = CH_3CO; V, R = R' = H

The octaacetate was deacetylated by refluxing it in 36 volumes of 0·2 N methanolic sodium methoxide for 2 h, after which the methanol was replaced by water, and sodium ions were removed by passage of the solution over 'Amberlite *IRC*-50 (H^+)'. Evaporation of the deionized solution gave syrupy pinitol β-D-glucoside (6-*O*-β-D-glucopyranosyl-5-*O*-methyl-D-inositol, V). Freeze-dried glucoside dissolved in 2 volumes of methanol is precipitated by 8 volumes of *n*-butanol as an amorphous solid melting from 125–140°, but the compound has so far not been crystallized. The specific rotation, $[\alpha]_D^{25}$, in water is +35° (c= 0·8, established by quantitative determination of the glucose in a hydrolysed aliquot).

The glucoside does not react with Somogyi's copper reagent or with glucose oxidase; but yields glucose-positive solutions on hydrolysis with boiling 2 N sulphuric acid (8 h). A portion of the hydrolysate from which glucose was removed by chlorite oxidation[6] and subsequent deionization showed, when chromatographed on Whatman No. 1 paper with acetone/water 9 : 1 (v/v), a single silver-reducing spot with the same mobility as authentic (+)-pinitol. The observed value for the specific rotation of the glucoside supports the assignment of the β-configuration to the glucosidic carbon. The figure is somewhat higher than those observed for simple alkyl β-D-glucopyranosides (−34 to −41°), owing to the positive contribution of the pinitol moiety (free pinitol, $[\alpha]_D$ +65°), but much lower than would be predicted for an α-glucoside of (+)-pinitol.

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Reaction of Di-isobutyl Aluminium Hydride with Stannic Chloride

General methods for preparation of organo–tin compounds of divalent tin have been reviewed by Gilman *et al.*[1]. The most usual methods include reaction of stannous chloride with a Grignard reagent or lithium alkyl and reduction of a dialkyl tin IV dichloride with sodium amalgam. In addition, reaction of sodium triethoxylalumino hydride with organo–tin IV halides in certain circumstances leads to formation of divalent organo–tin compounds[2].

Reaction of stannic chloride with a reagent which combines alkylating as well as reducing action on tin IV halides does not appear to have been investigated. Dialkyl aluminium hydrides fulfil this requirement and the interaction of di-isobutyl aluminium hydride with stannic chloride has been studied. Gilman *et al.*[1] mention the reaction of triethylaluminium with stannic chloride as leading to diethyl tin II, but this arises from a mistake in the *American Chemical Abstracts*[3]; the original patent specification[4] deals with the synthesis of hydrocarbon tin IV compounds. In the investigation recorded here, a solution of di-isobutyl aluminium hydride (4·0 g) in *cyclo*hexane (50 ml.) was added dropwise to a solution of stannic chloride (16·0 g) in *cyclo*hexane (200 ml.). The reaction mixture was maintained under a stream of nitrogen, stirred rapidly and cooled in an ice bath. On completion of the reaction a biscuit-coloured solid settled in the reaction vessel. The reaction mixture was filtered under nitrogen and the residue washed with *cyclo*hexane (50 ml.) followed by carbon tetrachloride (50 ml.) to remove excess stannic chloride or organo–tin IV chlorides formed during the reaction. The residue (6·0 g) was pumped at diminished pressure to remove solvent and extracted with chloroform (100 ml.). The resulting chloroform solution varied in colour from amber to red while the white residue (2·0 g) consisted substantially of stannous chloride. After filtration under nitrogen, chloroform was removed by vacuum distillation at 0·1 mm pressure. When most of the solvent was removed the temperature was raised to 100° C and the product (4·0 g) obtained as a red to amber friable mass[5], sparingly soluble in hydrocarbon solvents, slightly soluble in carbon tetrachloride, readily soluble in chloroform. On heating, the product charred at 220° C and decomposed in acid solution, immediately reducing silver nitrate solution. Careful oxidation with dilute nitric acid, followed by titration with silver nitrate, revealed ionic chloride present in 1 : 1 ratio with tin. However, even after two days' pumping, several samples retained a more or less definite amount of chloroform. $(4(C_4H_9)SnCl)\ CHCl_3$: requires C, 21·15; Cl, 25·76; H, 3·84 per cent. Found on three sample preparations: C, 20·16, 21·00, 21·08; H, 4·0, 4·1, 4·0; Cl, 25·82, 25·73, 26·0 per cent.

In an attempt to prepare $(C_4H_9)_2Sn$ the amount of di-isobutyl aluminium hydride added was increased to 8·0 g, but a black unresolved mixture was obtained.

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BIOCHEMISTRY

Production of a Cephalosporinase by *Pseudomonas pyocyanea*

Crude preparations of penicillinase from *Bacillus cereus* N.R.R.L. 569, unlike the purified penicillinase from this organism or crude penicillinase from *Staphylococcus aureus* R_1, have been shown to catalyse the hydrolysis of the β-lactam ring of cephalosporin *C* and other derivatives of 7-aminocephalosporanic acid at a significant rate. The enzyme responsible for this hydrolysis was termed a cephalosporinase[1,2]. The maximum rate of hydrolysis (V_{max}) of cephalosporin *C* obtained with a crude enzyme from *B. cereus* was only 5 per cent of that of benzylpenicillin, but this value increased to 33 per cent after selective inactivation of penicillinase in aqueous solution at 60° C. Subsequent studies were made with a crude enzyme from *Bacillus subtilis* N.C.T.C. 6346, induced with cephalosporin *C* and freed from the cells by the action of lysozyme[3]. With this enzyme preparation V_{max} for cephalosporin *C* was about 10 per cent that for benzylpenicillin, but attempts to separate a cephalosporinase from a penicillinase, or selectively inactivate a penicillinase, were unsuccessful.

We have found that a strain of *Pseudomonas pyocyanea* (N.C.T.C. 8230) produces an enzyme which shows a much greater value of V_{max} with cephalosporin *C* or 7-phenacetamidocephalosporanic acid as substrate than with penicillin *N* (which has the same D-α-aminoadipoyl side-chain as cephalosporin *C*) or benzylpenicillin. When *Ps. pyocyanea* was grown in nutrient 'Oxoid' broth the penicillinase or cephalosporinase activity of the culture fluid or whole culture was too small to be detected by the manometric method. Furthermore, no activity was detected after a culture had been subjected to ultrasonic disintegration (60 W at 0° for 2–15 min) or freeze-pressing in an 'X-press'[4]. No detectable amount of enzyme was induced by concentrations of benzylpenicillin sufficient to cause maximum induction of penicillinase in *B. cereus* (1 μg/ml.) or by concentrations of 2 : 6-dimethoxyphenylpenicillin (1 μg/ml.) or cephalosporin *C* (10 μg/ml.), which showed a strong inducing activity in *Staphylococcus aureus* R_1[2]. However, after very high concentrations of benzylpenicillin (10 mg/ml.) or cephalosporin *C* (10 mg/ml.) had been added to a growing culture of the organism, considerable enzyme activity appeared in the culture fluid. A significant but smaller amount of enzyme appeared after the addition of 7-phenacetamidocephalosporanic acid (5 mg/ml.). In some, but not in all, of these experiments the cells underwent partial lysis.

With the crude enzyme, V_{max} at *p*H 7·0 was more than ten times as great for cephalosporin *C* and 7-phenacetamidocephalosporanic acid as for penicillin *N* and benzylpenicillin. But with all these substrates the Michaelis constants were very much higher than those for penicillin *N* or benzylpenicillin with penicillinase from *Staph. aureus* or *B. cereus*. Characteristic changes in ultra-violet absorption spectra, which accompanied the hydrolysis of cephalosporin *C* and deacetylcephalosporin *C* lactone, indicated that the enzyme involved was a β-lactamase.

2 : 6-Dimethoxyphenylpenicillin (methicillin) proved to be relatively highly resistant to the enzyme and a powerful inhibitor of the action of the enzyme on cephalosporin *C*, 7-phenacetamidocephalosporanic acid and benzylpenicillin. Similarly, 3-*o*-chlorophenyl-5-methyl-4-isoxazolylpenicillin (orbenin) was highly resistant to hydrolysis. This compound, and to a lesser extent 6-aminopenicillanic acid and 7-aminocephalosporanic acid, also inhibited the hydrolysis of cephalosporin *C*. α-Aminobenzylpenicillin (ampicillin) was hydrolysed at a rate which was about 15 per cent of that with benzylpenicillin in the concentration used (1 mg/ml.).

The behaviour of 2 : 6-dimethoxyphenylpenicillin to the enzyme from *Ps. pyocyanea* is in striking contrast with its failure to inhibit the hydrolysis of penicillin *N* or benzylpenicillin by staphylococcal penicillinase[2,5], but is analogous with its inhibitory action on a penicillinase from *Klebsiella aerogenes*[6]. The effect of the 2 : 6-dimethoxybenzoyl side-chain on the enzyme from *Ps. pyocyanea* is paralleled in the behaviour of 7-(2 : 6-dimethoxybenzamido) cephalosporanic acid, which is also resistant to hydrolysis by this enzyme. Whether the crude enzyme consists of more than one β-lactamase remains undetermined.

An enzyme produced by a strain of *Enterobacter cloacae* has recently been reported to be highly active against cephalosporin *C* but to have little, if any, activity against benzylpenicillin[7]. It appears that enzymes able to degrade cephalosporin *C* have not been found in *Klebsiella*, but are distributed within a number of other genera of the Enterobacteriaceae and that the species most resistant to the antibiotic are among those which produce these enzymes[7]. However, we have no evidence which would suggest that the production of penicillinases or cephalosporinases is primarily responsible for the high resistance of *Pseudomonas pyocyanea* to any of the penicillins or cephalosporins tested. Methicillin and orbenin, which were relatively insensitive to the enzyme from *Ps. pyocyanea*, and ampicillin, which was hydrolysed at a relatively low rate, showed no significant activity against this organism.

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Phosphoprotein as an Intermediate in Cerebral Microsomal Adenosine-triphosphatase

Microsomal and cell membrane fractions from cerebral cortex contain a powerful ATP-splitting system, activated by Mg^{2+}, Na^+ and K^+ and inhibited by the cardiac glycoside ouabain[1–6]. The nature of the ATP-splitting reaction is not clear; but recently it has been reported that a phosphorylated intermediate is involved in the splitting of ATP by guinea pig kidney cortex[13], human erythrocyte membranes[14] and the electric organ of the electric eel[15]. This note provides evidence that phosphoprotein from cerebral cortex becomes labelled during the splitting of $AT^{32}P$ by the ATPase, and that the time-course of uptake of labelled phosphate from ATP into the phosphoprotein fraction and into inorganic orthophosphate is indicative of the phosphoprotein being a precursor of inorganic phosphate.

In principle, the experiment consisted of the brief incubation of $AT^{32}P$ with a brain enzyme preparation in

media of various compositions. The reaction was stopped with trichloroacetic acid (TCA), and the specific activity of the inorganic phosphate, and of the various phosphate fractions of the acid-insoluble residue, determined. In order to provide sufficient material for analysis of the acid-insoluble residue, the enzyme preparation consisted not solely of microsomes but of a post-mitochondrial supernatant, including the microsomes, prepared by dispersing guinea pig cerebral cortex in 4 volumes of ice-cold 0·32 M sucrose, buffered at *p*H 7·4 with 10 mM *tris*-hydrochloric acid, with a 'Teflon'-glass homogenizer, and centrifuging once at $10{,}000g_{av.}$ for 15 min.[4]. The supernatant so obtained was aged at −18° for at least four days prior to use[3]. According to Deul and McIlwain[3] such a preparation contains 40 per cent of the total MgKNaATPase activity of brain cortex, 82 per cent of the activity present in the post-mitochondrial supernatant being microsomal in origin. 2·5 ml. of this preparation was incubated at 37° in a medium containing 100 mM *tris*-hydrochloric acid, *p*H 7·4, 100 mM sodium chloride, 30 mM potassium chloride, 200 μM magnesium chloride and 50 μM EDTA. After pre-incubation for 5 min 0·5 μmole of β,γ-labelled $AT^{32}P$ (ref. 8), containing 2–5 × 10^5 counts/min, was added, giving a final incubation volume of 5·1 ml. Incubation was continued for up to 5 min. At various times 1-ml. samples of the reaction mixture were removed into 1 ml. lots of ice-cold 10 per cent (w/v) TCA, chilled in ice for 5 min and centrifuged. ATP and inorganic phosphate were determined[7,11] in the supernatant after separation by paper chromatography[9]. The acid-insoluble residue was fractionated[7,10] to yield five major groups of phosphate compounds, together accounting for the total acid-insoluble phosphate: phosphoprotein, phospholipid, nucleic acid phosphate, 'residual organic phosphate' and 'acid-precipitable phosphate'. Phosphate and specific activity (counts/min/μmole phosphorus) were determined for each fraction by methods already described[7,11]. The post-mitochondrial supernatant preparation used contained 85 per cent of the RNA, 72 per cent of the phosphoprotein, 30 per cent of the residual organic phosphate, 18 per cent of the phospholipid and 10 per cent of the acid-precipitable phosphate present in unfractionated cortex[7].

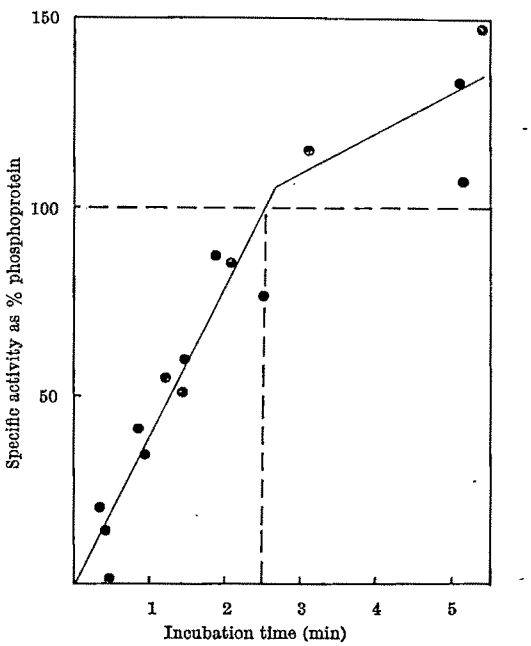


Fig. 1. Specific activity of inorganic phosphate as percentage of that of phosphoprotein at various times. Points from independent experiments

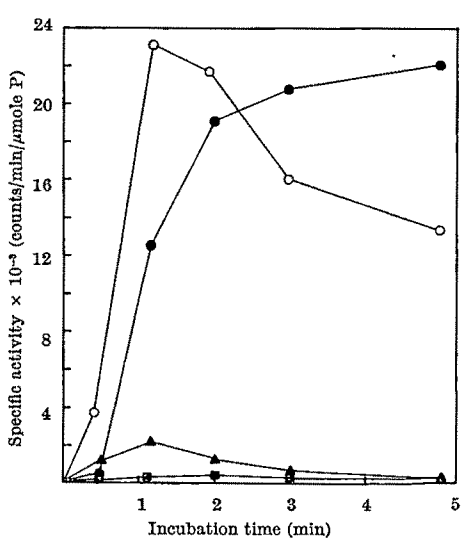


Fig. 2. Specific activity of phosphate fractions after various times. Graph typical of seven independent experiments. ●, Inorganic phosphate; ○, phosphoprotein; ▲, residual organic phosphate; ■, phospholipid, nucleic acid, acid-precipitable phosphate

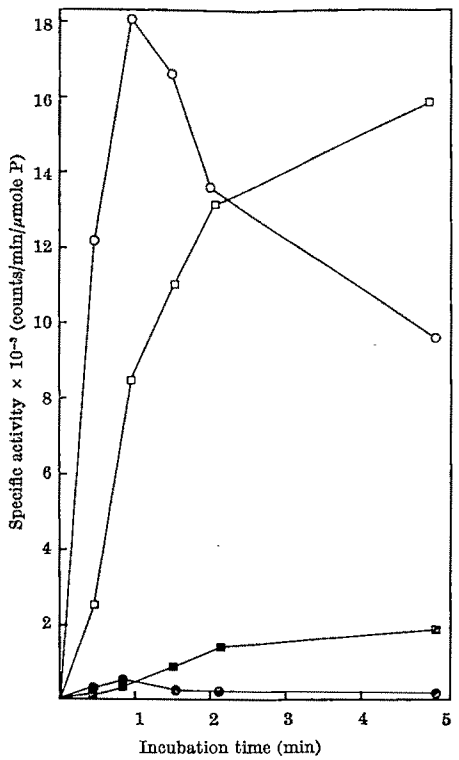


Fig. 3. Effect of Mg^{2+} and EDTA on incorporation of phosphate-32 into phosphoprotein and inorganic phosphate. ○, Phosphoprotein (standard medium); □, inorganic phosphate (standard medium); ●, phosphoprotein (medium—Mg^{2+}, + 1 mM EDTA); ■, inorganic phosphate (medium—Mg^{2+}, + 1 mM EDTA)

Under the incubation conditions described, ATP was rapidly broken down; 50 per cent of the radioactivity initially present as the γ-P of ATP was lost within 30 sec; after 5 min only 7 per cent remained. Examination of the other phosphate fractions showed that radioactivity appeared to a significant extent in only two, phosphoprotein and inorganic phosphate. After 2 min, the specific activity of residual organic phosphate, nucleic acid, acid-precipitable phosphate and phospholipid were respectively 3, 1·5, 1·5 and 0·5 per cent of phosphoprotein; in absolute terms, the counts incorporated into each of these four fractions were only 50–100 per cent above background. The time-course of incorporation into phosphoprotein and inorganic phosphate is shown in Figs. 1 and 2. Phosphoprotein rapidly became labelled, and for the first 2 min of incubation its specific activity

exceeded that of inorganic phosphate, afterwards declining. Incorporation into inorganic phosphate, on the other hand, increased steadily but more slowly, equalling the specific activity of phosphoprotein only after 2·5 min (Fig. 1), after which time it remained not greatly altered for periods of up to 15 min. 1 per cent of the radioactivity present in the γ-phosphate of ATP appeared in the phosphoprotein within 20 sec; after 5 min incubation 130 per cent of radioactivity initially present as γ-labelled ATP was found in the inorganic phosphate fraction, indicating that some β-labelled phosphate was also being removed from ATP. In 7 independent experiments, the maximum specific activity attained by the inorganic phosphate fell on or within 10 per cent of the curve representing phosphoprotein specific activity (Fig. 2); this relationship is characteristic[12] of a precursor-product relationship between phosphoprotein and inorganic phosphate, and indicates that during breakdown of ATP the major portion if not all of the liberated phosphate is transferred to phosphoprotein before being released as inorganic phosphate.

The microsomal ATPase is known to be absolutely dependent on Mg^{2+} (refs. 1–6). Omission of Mg^{2+} from the incubation medium was without effect on either phosphoprotein turnover or inorganic phosphate release; however, addition of the chelating agent EDTA at 1 mM effectively abolished incorporation into either phosphoprotein or inorganic phosphate (Fig. 3). Without EDTA, enough Mg^{2+} must remain bound to the large amounts of tissue present in the incubation medium to nullify the effects of the omission of Mg^{2+} from the medium. Similar observations have been made by Aldridge[5]. Incorporation into residual organic phosphate was also 70 per cent inhibited by EDTA; that into nucleic acid, phospholipid and acid-precipitable phosphate was not significantly changed.

These results are taken as providing evidence for the participation of a phosphoprotein fraction in the splitting of ATP by cerebral microsomes. Further details of the reaction, and its possible relation to the mechanism of cation transport, are being investigated.

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A Hypothesis linking the Succinic Dehydrogenase System to an Intermediate of Oxidative Phosphorylation

Recent work[1] has demonstrated that incubation of heart mitochondria with succinate, inorganic phosphate and NAD for short periods at 0° yields a highly labile mono-phosphorylated derivative of NADH. This derivative, capable of phosphorylating ADP, has the properties required for an oxidative phosphorylation intermediate and it is suggested that during its formation the nicotinamide ring has been opened[1]. (Abbreviations: NAD and NADH, oxidized and reduced nicotinamide adenine dinucleotide, respectively; ADP and ATP adenosine-5′-di- and tri-phosphate, respectively.)

It is the purpose of this communication to suggest a hypothesis to account for the present experimental results. The hypothesis provides: (1) a pathway for the interaction of the succinic dehydrogenase system and NAD; (2) a reaction scheme with structures for the formation of the new monophosphorylated derivative of NADH and its participation in the phosphorylation of ADP.

Work[2] on the succinic dehydrogenase system has indicated that only one hydrogen of a removable trans pair[3] of hydrogens of L-chlorosuccinate and of succinate is exchanged with hydrogen of the medium during anaerobic exchange experiments. This evidence together with other evidence has led to the concept[2] that oxidation of succinate occurs by transfer of a hydrogen and a pair of electrons to succinic dehydrogenase, and in support of this it has been noted that succinate oxidation requires less oxygen when acetoacetate is present[4]. With the foregoing in mind, the hypothesis outlined schematically in Fig. 1 may be approached.

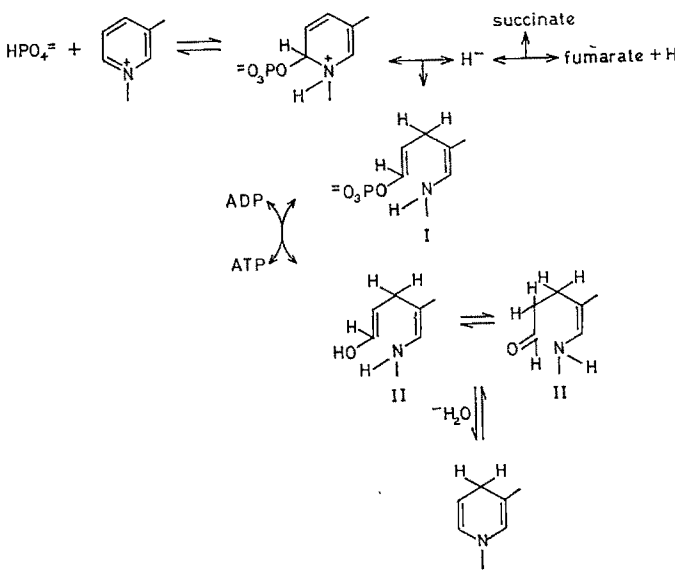


Fig. 1. Interaction of the succinic dehydrogenase system, NAD phosphate and oxidative phosphorylation

It is considered that first inorganic phosphate adds across an N to C double bond of the nicotinamide moiety of NAD, and the addition is written across the 1,6-double bond. This addition has previously been considered[5] as the starting point for oxidative phosphorylation and some supporting evidence has been adduced[6]. It is quite possible that such an addition is enzyme mediated and occurs via an NAD derivative. It is also possible that an enzyme group adds, and is afterwards displaced by, phosphate to yield compound I, the monophosphorylated derivative obtained by Griffiths and Chaplain[1]. Further, this addition may require ATP. In any event, addition is followed by transfer of a hydride ion from the succinic dehydrogenase system and this transfer results not only in reduction of the nicotinamide ring but also in ring opening to give compound I. Now, compound I, a vinyl phosphate, meeting the requirements of Griffith and Chaplain's observations, should be capable by analogy with phosphoenolpyruvate of phosphorylating ADP to ATP. Loss of the phosphate group by transfer to ADP results in transformation of compound I to compound II, which may be considered as a vinyl alcohol or an aldehyde or as both, in equilibrium. Compound II then undergoes ring closure to NADH. This is in keeping with the observations of Griffiths and Chaplain, who showed that on transfer of phosphate to ADP their monophosphorylated derivative was transformed into NADH.

It may be pertinent to enquire how the succinate half-cell with a much less negative E'_0 than the NADH half-cell[7] can be used for reduction of NAD and generation of ATP. It is possible if the driving force for the reaction comes from re-oxidation of NADH to NAD by a component of the succinic dehydrogenase system or the electron transport system which is greater in oxidation potential than the moiety reducing NAD.

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Effect of Protein Depletion on Amino-acid Activating Enzymes of Rat Liver

Experiments conducted with ^{35}S-methionine and ^{14}C-lysine provide support of the hypothesis that depletion of protein induces a modification in the distribution and utilization of amino-acids for protein synthesis.

Protein-depleted animals exhibit, in fact, relatively less incorporation of labelled amino-acids in muscles and carcass and a greater incorporation in the viscera, particularly in the liver[1–3].

Two tentative hypotheses may be formulated to explain this effect in protein depletion: (1) Protein depletion exerts a direct effect on the incorporation of amino-acids, by enhancing protein synthesis in certain particular tissues or organs, as for example in the liver. (2) Protein depletion exerts its primary effect on the distribution of amino-acids either by modifying the flow of blood through the tissues, or by modifying the cell permeability in certain organs or tissues.

To test the validity of the first hypothesis, an examination of the different steps of protein synthesis in liver of animals, kept for varying amounts of time on a protein-free diet, was undertaken.

This communication describes work on the first step in protein synthesis, namely. activation of amino-acids.

Sprague–Dawley male rats, weighing 200 g, were divided into three groups and fed the following diets: group 1, *ad lib.* 20 per cent casein diet; group 2, *ad lib.* 0 per cent casein diet; group 3, 20 per cent casein diet pair-fed with rats of group 2.

The animals were kept on the protein-free diet up to a maximum of 60 days (after this time about 50 per cent of the animals died).

The amino-acid activating enzymes were determined, for practical reasons, on a 10,000*g* liver supernatant, since preliminary testing had shown that the values obtained with a 105,000*g* (ref. 10) and a 10,000*g* supernatant were in agreement.

The amino-acid activating enzymes were determined by measuring the rate of exchange between $P^{32}P$ and ATP in the presence of four added amino-acids, namely, leucine, isoleucine, tyrosine and methionine (5 μM each), according to the method of De Moss and Novelli[11].

The results were expressed as μM exchange/h/mg protein (Table 1) and, also, as μM exchange/h/mg DNA-P (Table 2). Calculation of results in terms of DNA-P content, which is proportional to the cell number, renders possible evaluation and comparison of the enzymatic activity in normal and protein-depleted animals, against a standard of reference, unaffected by the diet. The DNA-P content was determined according to the method described by C. B. Mendes and J. C. Waterlow[12].

Table 1. Effect of Protein Depletion on the Activity of Rat Liver Amino-acid Activating Enzymes
μM exchange/mg supernatant protein/h (The values are given as percentage of pair-fed controls)

15 days	30 days	45 days	60 days
362	394	204	167
358	173	457	414
291	186	708	265
143	754	307	391
—	277	—	—
—	624	—	—
Average 289	401	419	309

Each entry is the average value of three determinations for single rat.

Table 2. Effect of Protein Depletion on the Activity of Rat Liver Amino-acid Activating Enzymes
μM exchange/mg DNA.P/h (The values are given as percentage of pair-fed controls)

15 days	30 days	45 days	60 days
289	166	200	175
188	115	374	263
200	86	407	170
107	611	341	442
—	333	—	—
—	349	—	—
Average 196	277	330	265

Each entry is the average value of three determinations for single rat.

Results in Table 1 show that the amino-acid-dependent exchange in the liver supernatant of protein-depleted rats is 3–4 times greater than in normal rats. These results can be explained, but only in part, assuming that a loss of liver proteins, other than amino-acid activating enzymes, occurred in the depleted animals.

Results in Table 2, expressed in terms of DNA-P content or, which is the same, in terms of average liver cell, substantiate this interpretation and clearly show that, following protein depletion, the level of amino-acid activating enzymes is not only fully retained but also undergoes a net increase.

Our results, obtained even with animals just before death (60 days' protein starvation), would suggest that the reported inability to build body proteins by dogs in an advanced stage of protein starvation[3] cannot be ascribed to a lessened efficiency of the first step, at least, of the machinery of protein synthesis.

In order to find out if the caloric intake might influence the amino-acid PP⇌ATP dependent exchange, the results relative to rats on 20 per cent casein diet, fed *ad lib.* or pair-fed with rats on the protein-free diet, were compared (Table 3). The results, similar in both cases, show that caloric restriction does not influence the amino-acid-dependent exchange.

Table 3. Effect of Calorie Intake on the Activity of Rat Liver Amino-acid Activating Enzymes
μM exchange/mg DNA.P/h (Values obtained with pair-fed controls are given as percentage of controls fed *ad lib.*)

15 days	30 days	45 days	60 days
92	95	97	99
108	141	130	107
88	128	81	125
115	108	99	123
—	84	—	—
—	114	—	—
Average 100	112	102	113

Each entry is the average value of three determinations for single rat.

In conclusion, the sum of evidence obtained seems to suggest that protein depletion exerts a direct effect on protein synthesis, enhancing the activity of amino-acid activating enzymes, possibly through one of the following mechanisms: (1) *de novo* synthesis of the enzymes; (2) activation of pre-existing enzymes; (3) release of these enzymes from the particulate fractions of the cell, such as the mitochondria, where the presence of amino-acid activating enzymes has been demonstrated[13] to the soluble fraction.

We are inclined to believe that the third hypothesis may be discarded in so far that preliminary results, obtained with starving rats (Table 4), have not shown

Table 4. Effect of Six Days' Starvation on the Activity of Rat Liver Amino-acid Activating Enzymes
Values are given as percentage of control mean value μM exchange/mg DNA P/h

	No. of rats	%
Controls	5	100 ± 13
Starved rats	8	92 ± 14

any increase in the level of amino-acid activating enzymes in liver supernatant, although following starvation the mitochondria becomes structurally damaged.

An effect of protein depletion, mediated through the adrenals, may as well be discarded, since Smith *et al.*[14] have shown that cortisone is without effect on the amino-acid activating enzymes.

An attractive suggestion is that the level of these enzymes in the liver is regulated, almost homeostatically, by the total pool of free amino-acids. If this pool decreases, the level of liver amino-acid activating enzymes increases and, consequently, increases the fraction of amino-acids engaged in protein synthesis.

Finally, the results obtained seem to indicate that higher incorporation of amino-acids into liver proteins of protein-depleted rats[1–9] is due, at least partially, to the increased activity of liver amino-acid activating enzymes.

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Inhibition by Trypsin Inhibitors of Dissociation of Embryonic Tissue by Trypsin

Trypsin is used extensively for dissociation of tissues and in the preparation of suspensions of living cells[1]. The cell-dissociating effect of this enzyme has been attributed to tryptic degradation of cell binding materials[1]; however, theoretically, other possibilities exist and the question whether the characteristic enzymatic properties of trypsin are actually involved in cell separation seems not to have been adequately tested, though such information would be of some general interest. Furthermore, since trypsin attacks selectively peptide linkages adjacent to lysyl and arginyl residues, clarification of the relationship between its cell-dissociating activity and its lytic properties would be useful in considerations of the mechanism of cell binding. Trypsin inhibitors offer a direct approach to this matter. It was reported that trypsin inactivated by diisopropylfluorophosphate (DFP) did not dissociate adult rat liver cells[2]. In the work recorded here, the effects of soybean and ovomucoid trypsin inhibitors on the dissociation by trypsin of embryonic tissue were examined. The interaction between these inhibitors and trypsin is considered to be specific and stoichiometric[3–5]; at concentrations equimolar to and higher than that of the enzyme, the inhibitors rapidly and completely abolish its tryptic activity. According to available results[3,5], the molecular weights of trypsin and trypsin inhibitors used in this investigation were of the following order of magnitude: trypsin—24,000; soybean trypsin inhibitor—24,000; ovomucoid trypsin inhibitor—28,000.

The tissue used in these assays was the neural retina from 10-day chick embryos. This thin sheet of non-fibrous and avascular tissue can be readily removed intact from the bisected eye. Half a retina from one eye was used in each assay. Standard cell dissociation procedure was adopted[6]. The tissue was incubated for 10 min in calcium- and magnesium-free solution (CMF[6]; Difco) at 38° C, then transferred into 1 ml. of trypsin solution, or into 1 ml. of solution of trypsin with the inhibitor, and incubated for 15 min at 38° C under 5 per cent carbon dioxide–air mixture. In all the tests trypsin (crystalline-lyophilized, Worthington Biochem. Corp.) was used as a 5·0 mg/ml. solution in CMF, that is, at about twice the minimal concentration required for effective dissociation of this tissue. Soybean trypsin inhibitor (5 times crystallized-lyophilized) and ovomucoid trypsin inhibitor (both from Worthington Biochem. Corp.) dissolved in CMF were added to the trypsin solution at concentrations listed in Table 1. Stability of intercellular adhesions in the treated tissue was tested by flushing the tissue briskly 15 times (for 30 sec) in and out of a Pasteur pipette (0·75 mm orifice), each intake measuring 0·5 ml. The resulting suspension was evaluated microscopically for the degree of cell separation, size of clusters and number of free cells.

Table 1

Tissue treated with:			Result
CMF			No dissociation
Trypsin 5·0 mg/ml. in CMF			Complete dissociation
Trypsin 5·0 mg/ml. in CMF with 15·0 mg/ml.	soybean trypsin inhibitor, or ovomucoid trypsin inhibitor		No dissociation
Trypsin 5·0 mg/ml. in CMF with 10·0 mg/ml.	,,	,,	No dissociation
Trypsin 5·0 mg/ml. in CMF with 5·0 mg/ml.	,,	,,	No dissociation
Trypsin 5·0 mg/ml. in CMF with 2·50 mg/ml.	,,	,,	Partial dissociation (fragments and cells)
Trypsin 5·0 mg/ml. in CMF with 1·25 mg/ml.	,,	,,	Partial dissociation (small fragments and cells)
Trypsin 5·0 mg/ml. in CMF with 0·63 mg/ml.	,,	,,	Complete dissociation (a few small fragments)
Trypsin 5·0 mg/ml. in CMF with 0·32 mg/ml.	,,	,,	Complete dissociation

Tissue treated with 5·0 mg/ml. trypsin was readily dispersible into single cells. The dissociating effect of trypsin was completely abolished both by the soybean and by the ovomucoid inhibitor at concentrations of 15·0, 10·0 and 5·0 mg/ml.; the lowest of these concentrations is in the range of equimolarity with the concentration of trypsin. Tissues incubated in trypsin with progressively lower concentrations of the inhibitors yielded increasingly larger numbers of free cells and, finally, suspensions of single cells were obtained (Table 1). Thus, at concentrations of inhibitor that would be expected to inactivate all the trypsin in the test medium, the tissue-dissociating effect of the enzyme was wholly abolished; at lower inhibitor concentrations, and correspondingly higher amounts of active trypsin, partial and complete dissociations were obtained.

The results demonstrate that the natural trypsin inhibitors tested in this work abolish the cell-dissociating activity of the enzyme. This accords with the reported absence of cell-separating activity in DFP-inactivated trypsin[2]. While the exact mode of inactivation of trypsin by various inhibitors is not completely clear, there is good evidence that they combine with the enzyme at, or near, its active site[6,7]. It may thus be inferred that the characteristic enzymatic properties of trypsin are causally related to its cell-dissociating effect and, therefore, that degradation of a trypsin-sensitive substrate is involved in the separation of cells by trypsin. This concurs with assumptions on the role of such substrates in

the mutual attachment of cells in tissues susceptible to dissociation with trypsin.

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Differences in the Disposition of Endogenous and Exogenous Substances by Cells

During recent years, evidence has been accumulating that cells often may discriminate between a substance that they synthesize and the same substance that is preformed and assimilated from the environment. This capacity to respond differently to a compound introduced from the extracellular fluid, as compared to that formed within the cell, may be attributable to the compartmentation of intracellular events.

In culture, neoplastic mast cells of the mouse synthesize histamine and 5-hydroxytryptamine and also concentrate these amines from the medium[1]. The endogenous and exogenous amines, neither of which is catabolized, are eliminated from the cell at markedly different rates[2]. It is known that the major portion of the endogenous amines in these cells is found in particulate material[3]. Density-gradient centrifugation in sucrose of homogenates of these cells grown in a medium containing radioactive histamine or radioactive 5-hydroxytryptamine showed that these labelled, exogenous amines are found in fractions sedimenting at different densities from the particulate fractions containing the endogenous amines[3]. The different compartments for the endogenous and exogenous amines can explain the differences in their rates of elimination from the cell.

The separation of the endogenous and exogenous pools of amines in these cells is not complete, however, for the exogenous amines have access to the endogenous pool. In cells with no detectable levels of endogenous histamine and 5-hydroxytryptamine, the exogenous amines are localized in sub-cellular fractions which normally contain the endogenous amines[3]. The extent to which the exogenous amines enter the endogenous compartment is a function of the levels of endogenous amines already present in that pool. These observations are in accord with the idea that the binding sites for endogenous amines, when not occupied by these amines, are accessible to exogenous amines[3].

The implications of these experiments probably are not restricted to neoplastic mast cells, nor are they likely to be limited to an explanation of the kinetics of an eliminative process. In intact brain[4] and in lung minces[5], endogenous and exogenous histamine are methylated at different rates. An obvious, though not inevitable, implication of these observations is that the endogenously formed histamine is held in a cellular compartment that is not readily accessible to the methylating enzyme. In brain[6] and in lung[7,8] endogenous histamine is found in particulate material, whereas the methylating enzyme is found in the soluble cytoplasm[9]. It is suggested that the exogenous histamine may not be bound within particles but instead is held in the clear cytoplasm, accessible to the methylating enzyme.

It should be noted that these experiments on the methylation of histamine were carried out on lung minces and intact brain, in both of which cellular integrity is preserved. Other experiments have shown that the organization of the cell must be retained to reveal the true kinetics of histamine-methylation. *In vivo*, male rats have a greater capacity to methylate histamine than do female rats[10]. This difference is manifest by kidney minces[10] and slices[11], but not by kidney homogenates[12]. In the intact cell, a rate-limiting reaction may exist that is not apparent in homogenized cells.

Conversely, conditions that may appear to be rate-limiting *in vitro* may not obtain within the intact cell. Intact muscle continues to phosphorylate glucose in the presence of an intracellular concentration of glucose-6-phosphate that, *in vitro*, would inhibit almost completely the hexokinase present in the muscle[13]. A possible explanation for this paradox is that only a portion of the glucose-6-phosphate in the cell is in contact with hexokinase.

Endogenous and exogenous amino-acids also differ in their metabolism. In yeast (*Candida utilis*)[14], fructose serves as a precursor of amino-acids, which are incorporated into protein. The yeast also concentrates from the medium exogenous amino-acids, which also are incorporated into protein. Although the amino-acids which the cell takes up exchange with the amino-acids in the medium, amino-acids synthesized from fructose do not exchange with amino-acids in the medium. Furthermore, the performed acid-soluble amino-acids in the cell are extractable with distilled water, which does not extract the endogenously formed acid-soluble amino-acids. Thus, in this yeast, exogenous amino-acids are held in a pool which is distinct from that containing endogenous amino-acids. The endogenously produced amino-acids are preferentially incorporated into protein, perhaps because the enzymes forming the endogenous amino-acids are in more favourable juxtaposition with the components involved in protein synthesis.

In neoplastic mast cells in culture, endogenous 5-hydroxytryptophan is more rapidly decarboxylated than is exogenous 5-hydroxytryptophan[2]. The more rapid decarboxylation of endogenous 5-hydroxytryptophan may indicate that it is present in a compartment more readily accessible to the decarboxylating enzyme.

The existence of different pools for endogenous and exogenous purines and pyrimidines also may be inferred. In *Bacillus cereus*, chloramphenicol appears to inhibit the incorporation into nucleic acid of adenylate arising from exogenous purines without inhibiting the incorporation of adenylate arising from the synthesis of purines *de novo*[15]. Similar findings have been obtained in investigations of Ehrlich ascites carcinoma cells with ethidium bromide[16]. In a uracil-dependent mutant of *E. coli*, exogenous deoxycytidine serves as a precursor of all the pyrimidines of nucleic acids, whereas the deoxycytidine produced from deoxycytidylate in the medium is found only in the thymine of deoxyribonucleic acid[17].

Even cell-free systems may discriminate between endogenous and exogenous substances. Particulate fractions prepared from bacteria incorporate amino-acids into protein, making use, as a source of energy, of adenosine triphosphate (ATP) produced by oxidative phosphorylation in these particles; this endogenous source of ATP could not be replaced by exogenous ATP[18,19]. In homogenates of pigeon-breast muscle, synthesis of glycogen depended on the ATP generated by oxidative phosphorylation, but was not supported by exogenous ATP or by an added ATP-generating system[20]. It would appear that a close structural relationship must exist between the ATP-synthesizing system and the ATP-requiring reaction.

Implicit in these observations on intact cells and subcellular systems is the importance of intracellular localization and the contiguity of sub-cellular substances and enzyme systems in the control of cellular events. But more specific conclusions emerge from the foregoing examples, although other conclusions may not hold for all substances in all systems. First, the turnover rate of a substance—whether it is catabolized, using in a synthetic

process, or eliminated unchanged by the cell—depends on whether it is of endogenous or exogenous origin. Second, some of the effects of drugs on intermediary metabolism differ if the metabolite is of endogenous or exogenous origin. Third, the disposition of substances of exogenous origin is governed by the intracellular concentration (the steady-state level) of the same endogenous substance, in part because of limited and relative accessibility to an intracellular compartment. Fourth, the disposition of an exogenous substance is altered as its concentration increases, not only because of limited activity of an enzyme system, but also because of limited capacity of a cellular compartment to accommodate the substance.

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Effects of Alloxan Diabetes and Adrenaline on Concentrations of Free Fatty Acids in Rat Heart and Diaphragm Muscles

In alloxan diabetes rates of glucose phosphorylation, glycolysis and pyruvate oxidation in rat heart and diaphragm muscles are subnormal, and evidence has been presented that these changes in carbohydrate metabolism are due to greater availability of fatty acids for oxidation in muscle in diabetes[1,2]. The evidence was that changes in carbohydrate metabolism, similar to those seen in muscle in diabetes, could be induced in normal muscle by *in vitro* addition of fatty acids[2]; and that measurements of glycerol output by rat heart and diaphragm muscles *in vitro* suggested that the breakdown of glycerides to free fatty acids and glycerol in muscle from diabetic rats was accelerated[2,3]. We have now sought to obtain more direct evidence for this idea by measuring the concentration of free fatty acids in these muscles.

Estimates of the free fatty acid concentration in muscle are few[4,5] and have been considered unreliable[6]. Difficulties in making such estimates include breakdown of lipids during extraction of free fatty acids[7]; limited sensitivity of microtitration methods of free fatty acid[8]; and interference by phospholipids in both titrimetric methods[8] and those spectrophotometric methods based on the solubility of the copper salts of free fatty acids in organic solvents[9]. With these points in mind we have estimated free fatty acid concentrations in muscle as follows.

Alloxan diabetes was induced in rats by injection of alloxan (60 mg/kg) into a tail vein under ether anæsthesia; the animals were used 48 h later. Diaphragms (from female albino Wistar rats of 150–200 g) were incubated for 60 min, and hearts (from male albino Wistar rats of 200–260 g) were perfused for 15 min (by drip through) by techniques described in detail elsewhere[10,11]. At the end of incubation or perfusion the tissues were frozen in acetone solid carbon dioxide mixture and powdered in a percussion mortar at −70°. The powder was weighed and ground in a mortar with 5 ml. of iso-propanol/*n*-heptane sulphuric acid (40 : 10 : 1, v/v). After addition of further *n*-heptane (3 ml.) and water (3 ml.-volume of muscle water which is about 0·8 times weight of powder in g) the mixture was ground once more, the upper phase (heptane phase) separated by centrifugation and 3 ml. taken and dried at 37° under a stream of nitrogen. The lipid residue was then subjected to thin layer chromatography on silica gel 'G' (Merck) as described by Malins and Mangold[12] but using the solvent system *n*-hexane/di-ethyl ether/methanol/acetic acid (90 : 20 : 2 : 3, v/v)[13]. Desaga apparatus for thin layer chromatography was obtained through CamLab (Glass), Ltd., Cambridge. Lipids were detected by their fluorescence in ultra-violet light (258 mμ) after spraying with an ethanolic solution of 2′,7′-dichlorofluorescein, 0·2 g/100 ml. The R_F values of the separated classes were phospholipids 0·00; cholesterol 0·20; diglycerides 0·25; free fatty acids 0·3–0·4; triglycerides 0·55–0·75 and cholesterol esters 0·80–0·90. The free fatty acid zone was scraped off the plate, shaken with 7 ml. of chloroform and the supernatant separated by centrifugation. Free fatty acids were assayed spectrophotometrically at 440 mμ in a Beckman *DU* spectrophotometer on 5 ml. of the supernatant by the method of Duncombe[9]. Recoveries of palmitic, oleic and linoleic acids (0·025–0·2 μmole) added to muscle powder were in the range 90–100 per cent. The advantages of the procedure described include the short time required for extraction, specificity due to separation of free fatty acids from phospholipids and sensitivity to 0·01 μmole fatty acids.

The concentration of free fatty acid in diaphragms from fed rats incubated for 1 h in medium containing glucose (1·5 mg/ml.) and insulin (0·1 unit/ml.) was 156 ± 15 (6) (mμmoles free fatty acid/g of wet muscle; mean ± *S.E.*; number of observations in parentheses). The concentration of free fatty acid was increased in diaphragms from diabetic rats to 450 ± 64 (6); but in hypophysectomized rats the concentration of free fatty acids in diaphragm was not increased by the induction of alloxan diabetes (hypox 200 ± 26 (6); hypox diabetic 150 ± 18 (6)). When insulin and glucose were omitted from the incubation medium, the concentration of free fatty acids in diaphragm from fed rats was increased (from 156 ± 15 (6) to 370 ± 60 (6)). Under these conditions the concentration of free fatty acids was increased to 620 ± 70 (6) in diaphragms from alloxan diabetic rats. The addition of adrenaline (20 μg/ml.) to medium lacking glucose or insulin increased the concentration of free fatty acids in diaphragms from rats starved 48 h from 380 ± 40 (6) to 700 ± 50 (6), but did not significantly increase the concentration in diaphragms from fed rats (370 ± 60 (6) to 420 ± 25 (6)).

The concentration of free fatty acids in hearts from fed rats perfused for 15 min with medium containing glucose (1 mg/ml.) and insulin (0·1 unit/ml.) was lower than that of diaphragm, being 45 ± 5 (24) mμmoles/g of wet muscle. The concentration of free fatty acids was increased in alloxan diabetes to 103 ± 18 (6); and by omission of glucose and insulin to 63 ± 7 (6).

The present investigations have shown that the concentration of free fatty acids in rat heart and diaphragm

muscles perfused or incubated with medium containing glucose and insulin is increased by the induction of alloxan diabetes in normal but not in hypophysectomized rats. Moreover, they show that the concentration of free fatty acids in these muscles is diminished by the presence of glucose and insulin in the medium and may be increased in diaphragm muscle by adrenaline *in vitro*. These findings provide more direct evidence for the ideas[2] that abnormalities of glucose phosphorylation, glycolysis and pyruvate oxidation in muscles of diabetic rats are secondary to greater availability of fatty acids for oxidation; that adrenaline can accelerate breakdown of glycerides to fatty acids and glycerol in muscle; and that glucose and insulin can promote esterification of free fatty acids in muscle.

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Biosynthesis of Sucrose with Sugar Cane Leaf Preparations

On the basis of results from photosynthetic experiments with $^{14}CO_2$, Buchanan *et al.*[1] first suggested that uridine diphosphate D-glucose (UDPG) is the immediate precursor of sucrose in green plants. Later, Leloir and co-workers[2] and others[3] demonstrated that extracts from wheat germ, peas, sugar beets, and other plants contain enzymes capable of catalysing the synthesis of sucrose from UDP-D-glucose by two similar reactions:

UDP-D-glucose + D-fructose ⇄ sucrose + UDP

UDP-D-glucose + D-fructose 6-phosphate ⇄ sucrose phosphate + UDP

In these reactions the equilibrium lies in the direction of sucrose or sucrose phosphate formation[3]. It was also shown that wheat germ contains a phosphatase capable of converting the sucrose phosphate to sucrose. The relative importance of the two mechanisms of sucrose synthesis in plants is not clear at present.

Pandya and Ramakrishnan[4], and Shukla and Prabhu[5] reported the presence of a sucrose phosphorylase in sugar cane plants which is similar to that found in the micro-organism *Pseudomonas saccharophila*[6]. According to these authors, the synthesis of sucrose in sugar cane occurs by the reaction:

α-D-glucose 1-phosphate + D-fructose ⇄ sucrose + inorganic phosphate

In the past, various attempts in this and several other laboratories to obtain a preparation from sugar beets, peas, carrots, and other plants that would utilize α-D-glucose 1-phosphate as a donor for the D-glucose moiety for *in vitro* biosynthesis of sucrose met with failure. Turner[7] in 1953 reported the synthesis of sucrose from these substrates with a preparation from peas when a yeast extract was added to the reaction mixture. However, he[8] afterwards found that sucrose was synthesized from UDP-D-glucose and D-fructose as previously demonstrated by Cardini *et al.*[2]. Turner[8] attributed sucrose formation in earlier experiments to the presence of UDP-D-glucose in the yeast juice added to the reaction mixture.

Shukla and Prabhu[5] reported that the main difference in the sucrose phosphorylase of sugar cane and of *Pseudomonas saccharophila* is that the equilibrium constant of the bacterial enzyme reaction is 0·058; whereas the K of the sugar cane reaction is 5·6. This equilibrium constant is clearly inconsistent with that of the sucrose phosphorylase reaction.

In view of these facts, we decided to re-examine the *in vitro* biosynthesis of sucrose with enzyme preparations from sugar cane leaves. Results of a preliminary investigation on this subject are reported in this communication.

We were unable to confirm the results of Pandya and Ramakrishnan[4] and Shukla and Prabhu[5] that sugar cane contains a sucrose phosphorylase capable of synthesizing sucrose from α-D-glucose 1-phosphate and D-fructose or D-fructose 6-phosphate. Consistent with the results of previous investigations[2,3] on other plants, the only sucrose synthesizing enzyme that could be demonstrated in sugar cane extracts was a transglycosylase that transfers the D-glucose moiety from UDP-D-glucose to D-fructose to form the disaccharide.

Approximately 50 g of immature sugar cane leaves were homogenized in a Waring Blender, the homogenate was centrifuged at 10,000*g* for 10 min, and the supernatant solution was fractionated with saturated ammonium sulphate at *p*H 7·0. The precipitate obtained at 30–60 per cent saturation was dissolved in water and the solution dialysed overnight against 0·05 M *tris* buffer, *p*H 7. This enzymatic preparation (5 ml.) was used in the sucrose synthesis experiments.

The reaction mixture contained 0·5 μmole of ATP, 0·25 μmole magnesium chloride, 2·5 μmoles phosphate buffer, *p*H 6·7, 0·5 μmole D-fructose or D-fructose 6-phosphate, $6·7 \times 10^{-4}$ μmole (0·025 μc.) UDP-D-[^{14}C] glucose, or $1·6 \times 10^{-3}$ μmole (0·06 μc.) D-[^{14}C] glucose 1-phosphate, and 20 μl. of the enzyme preparation in a total volume of 45 μl. The mixture was incubated for 60 min at 37° C and then subjected to electrophoresis on acid-washed paper (Whatman No. 1) in 0·2 M ammonium formate buffer, *p*H 3·7.

The non-mobile radioactive compounds were eluted from the paper, and the eluate was passed through a mixed 'Amberlite *MB*3' resin (H^+, OH^-) form. The eluate containing the radioactive compounds was mixed with 1 μmole of non-radioactive sucrose and chromatographed with butanol/pyridine/water (6: 4: 3 v/v). Radioactivity on the chromatograms was detected by exposure of the paper to X-ray film (Kodak-'No screen'). The unlabelled sugars were detected on the paper chromatograms with silver nitrate reagent[9]. The enzymatically synthesized radioactive compound from UDP-D-[^{14}C] glucose and D-fructose was found to co-chromatograph with authentic sucrose. The radioactive spot was eluted and hydrolysed with invertase. Subsequent chromatographic separation of the products revealed that the radioactivity resided in the glucose moiety. The recovery of activity of carbon-14 in the sucrose amounted to 68 per cent of the UDP-D-[^{14}C] glucose used as substrate.

Sucrose labelled with carbon-14 was formed only when UDP-D-[^{14}C] glucose and D-fructose were incubated with the enzyme. When D-[^{14}C] glucose 1-phosphate was substituted for UDP-D-[^{14}C] glucose in the reaction, sucrose could not be detected; nor was radioactive sucrose or sucrose 6-phosphate synthesized when D-fructose 6-phosphate was used as the D-glucosyl acceptor in the reaction with D-[^{14}C] glucose 1-phosphate.

In another experiment with sugar cane leaves, the leaf extract was separated with ammonium sulphate into three fractions of 0–30 per cent, 30–60 per cent, and 60–75 per cent saturation. When these preparations were used as a source of enzyme for the synthesis of sucrose, it was found that only the 30–60 per cent fraction was effective in synthesizing sucrose from ^{14}C-labelled UDP-D-glucose and D-fructose. As in the previous experiment, sucrose was not formed when ^{14}C-labelled D-glucose 1-phosphate was substituted for radioactive UDP-D-glucose in the reaction. The other ammonium sulphate fractions were ineffective for the synthesis of sucrose from ^{14}C-labelled UDP-D-glucose and D-fructose or D-fructose 6-phosphate; or from ^{14}C-labelled D-glucose 1-phosphate and the same D-glucose acceptors.

When sugar cane chloroplasts were used as an enzyme source, sucrose was synthesized from ^{14}C-labelled UDP-D-glucose and D-fructose or D-fructose 6-phosphate to about the same extent as with the 30–60 per cent ammonium sulphate fraction (approximately 65 per cent). However, although D-fructose 6-phosphate could serve as a glucose acceptor, no sucrose phosphate could be detected. It appears that a phosphatase is present in the chloroplasts that either hydrolyses the enzymatically synthesized sucrose phosphate or hydrolyses the D-fructose 6-phosphate to D-fructose, which then serves as an acceptor for the glucose of the sugar nucleotide.

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A Non-enzymatic Oxidative Degradation of Cellulose occurring in Preserved Cabbage Tissue

IT is recognized that plant materials preserved in dilute acetic acid exhibit a slow loss of tissue strength as a result of the structural components undergoing hydrolysis. This is caused either directly by the low *p*H of the medium, or indirectly from the presence of hydrolytic enzymes secreted by micro-organisms previously associated with the tissue[1]. An additional form of tissue breakdown, which has not apparently attracted previous attention, has been observed in the work recorded here. This is in no way connected with microbial action, and is oxidative in character.

This form of softening is, in fact, to be seen to a small extent in many chemically- and heat-preserved fruits and vegetables; but in the case of red cabbage—shredded and preserved in acetic acid—deterioration is rapid and storage for three weeks at 30° C is sufficient to bring about a radical change in the texture of the shreds. The essential role of oxygen in this breakdown is apparent from the fact that when shredded cabbage in acetic acid (*p*H 3·25) is placed in a loosely sealed container, it is the uppermost shreds which are the first to soften. Furthermore, hermetically sealing the container under vacuum prevents this from occurring.

In order to obtain some indication of the nature of the softening reaction, the effect of adding a number of substances to shredded red cabbage in acetic acid (*p*H 3·25) under aerobic conditions was observed. Certain substances were found to inhibit or retard softening. These could be classified as follows: the strong reducing agent sulphur dioxide which had a marked effect even at a concentration of 100 p.p.m.; the oxidizing agent iodine; sequestering agents such as ethylenediamine tetraacetic acid and pyrophosphates; and general precipitating agents such as alcohol, formaldehyde and phosphomolybdic acid. None of these results is inconsistent with the oxidation of pectin and cellulose by ascorbic acid investigated by Deuel[2]. Pasteurization, so that the shreds were held at a temperature of 82° C for 20 min, failed to reduce softening, thus suggesting that the reaction was non-enzymatic.

Analysis of the total pectic substances in softened and crisp shreds of the same age, taken respectively from the upper- and lower-most regions of containers over a period of time, indicated that softening was accompanied by a loss of pectic substances in the tissue (Table 1). A rise in the pectic content of the liquid contents, approximately equivalent by weight to that lost by the softened shreds, also occurred.

Table 1

Days' storage at 30° C	Percentage total pectic substances (as calcium pectate)		
	Topmost shreds	Bottommost shreds	Liquor
3	0·66	0·70	0·018
17	0·63	0·70	0·034
31	0·49	0·74	0·034
45	0·50	0·70	0·040
62	0·47	0·70	0·042

Estimation of the crude fibre content, as an indication of cellulose content, of soft and crisp shreds showed a loss of cellulose from the former. The means of the results from 7 identical containers were 0·84 per cent and 1·17 per cent respectively, the difference being statistically significant at the level $P = 0{\cdot}02$ approximately.

Using methods based on the assay of polygalacturonases[3], and also a method involving the measurement of the strength of pectic acid–aqueous cabbage extract gels (*p*H 3·5), no evidence was obtained that water-soluble constituents of cabbage were capable of degrading pectic acid appreciably in the presence of oxygen over a period of several weeks at 30° C.

Utilizing sodium carboxymethylcellulose as a substrate, as in the assay of cellulolytic enzymes[4], but using a Ferranti viscometer, it was found that red cabbage extract brought about a marked reduction in the viscosity of solutions of this cellulose derivative. Oxygen was essential for the reaction to proceed, anaerobic conditions resulting in its complete cessation. In Table 2 figures are given relating to the apparent viscosity of a 1·5 per cent solution of sodium carboxymethylcellulose in 3 per cent acetic acid (*p*H 3·6), containing 50 per cent aqueous cabbage extract, stored at 30° C over a period of time.

Table 2

Days' storage at 30° C	Apparent viscosity in centipoises	
	Aerobic conditions	Anaerobic conditions
0	37·0	37·0
6	25·0	35·0
13	19·5	35·0
27	14·5	35·0
42	10·5	34·0
69	6·0	33·0

Subsequent tests showed that the foregoing reaction was inhibited by the same substances as prevented the softening of shredded cabbage in acetic acid and that boiling the cabbage extract prior to its use, to inactivate any enzymes present, had no effect on the rate of the reaction.

It is concluded from this initial investigation that a factor exists in red cabbage which is non-enzymatic and which is capable of directly or indirectly carrying out an oxidative degradation of cellulose *in situ* under acid conditions. The change of protopectin to soluble pectin which apparently accompanies the loss of cellulose from the tissue could be explained either on the assumption of a similar oxidative factor affecting protopectin but not pectin, or alternatively that the insolubility of the protopectin present derived from its association with cellulose, a hypothesis most recently reviewed by Joslyn[5].

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PHYSIOLOGY

Reducing Agents and Absorption of Iron

Iron absorption has been shown to be increased by a variety of substances. Ascorbic acid is perhaps the best known of these[1]. The activities of inosine[2], succinic acid[3], fructose[4] and cysteine[5,6] have been discovered more recently. Several different hypotheses have been offered in explanation of the action of these substances. (1) That ferrous iron is more readily absorbed than ferric iron and that the ferrous form is maintained by an intra-luminal intestinal reducing agent (ascorbic acid); (2) that iron transport is linked with oxidative metabolism (succinic acid); (3) that iron transport depends on a carrier or chelating agent (fructose, cysteine).

It has not been generally appreciated that all these substances are reducing agents, either of themselves or in the milieu of the cell's interior. Succinic acid can reduce the prosthetic group of succinic dehydrogenase; fructose can reduce DPN when it enters glycolysis as glyceraldehyde-3-phosphate; inosine can reduce the prosthetic group of xanthine oxidase. All these substances can change the redox balance of the absorbing cell. A common action is also suggested by the quantitatively similar effect each exerts on iron absorption, a 1·5–2-fold increase.

We suggested that when the redox balance within the absorptive epithelium is tipped in the direction of reduction, iron absorption is increased. As a preliminary test of this hypothesis we administered by mouth iron plus hydroquinone, a reducing agent without known or postulated metabolic function. Iron absorption was examined in Walter Reed strain rats, weighing 250–400 g, utilizing repeated total-body counting to estimate absorption[7]. Control rats were dosed with 250 μg of iron as ferric chloride with iron-59 as tracer and with 1·6 mg of ascorbic acid, all dissolved in 1 ml. of distilled water. Experimental rats were dosed with the identical solution except for the addition of hydroquinone in varying doses. The small excess of ascorbic acid was included to preserve the ferrous state of intraduodenal iron in both control and experimental groups so that differential formation and absorption of ferric iron would not be in question.

Table 1. Iron Absorption in Control and Experimental Rats

Group	No. of rats	Hydroquinone dose (mg)	Percentage absorption (mean)
I	8	25	21·0
I (control)	8		12·7
II	8	45	21·0
II (control)	10		13·8

Absorption was increased in the hydroquinone-dosed rats (Table 1). The different doses of hydroquinone were similar in their effects on iron absorption. A *t*-test comparison between the combined hydroquinone-dosed rats and the controls showed the difference to be significant, with $P < 0{\cdot}005$. Separate experiments showed that the ascorbic acid used was sufficient to maintain the ferrous state of intraduodenal iron for at least 2 h. Hydroquinone, if it indeed functions as a reducing agent in increasing iron absorption, appears to work within the absorbing cell.

The 50 per cent increase in iron absorption obtained with hydroquinone is similar to that obtained with other compounds. This increase is relatively small compared with the increase which may occur with iron deficiency. The role which the intracellular redox potential may play in the absorption of iron in normal and abnormal states requires further definition.

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A Theoretical Model for the Computation of Decompression Tables for Divers

Discussions on the mechanism of decompression sickness have been focused mainly on the saturation and desaturation of the tissues with inert gases. To assess the capacity of the organism to free itself of an abundance of inert gas, experimental methods have usually been applied. Thus it has been possible to define a limited number of nitrogen-elimination rates which are spoken of as representing different types of tissues. Such information is, however, of limited value for the estimation of the relative importance of a certain tissue fraction (in terms of half-saturation time) for the genesis of decompression sickness. In measurements of nitrogen elimination the volumetrically large tissue fractions dominate the picture in a degree which is not necessarily related to their importance as bends tissue.

The conception of different histological types of tissues as representing different elimination rates is an oversimplification. Obviously, cells which are located at a larger distance from capillaries in a tissue will have slower nitrogen-elimination rates than those close to the walls of perfused vessels.

A more fruitful approach to the problems of inert-gas exchange in the organism seems to be to consider the body as composed of an unlimited or very large number of fractions as regards nitrogen-elimination rates. The ex-

tremes of such a spectrum of fractions would be one representing some imaginary cells with an infinitely rapid elimination rate and another representing cells with an extremely slow elimination rate. Neither of these extremes would present problems as bends tissue after a period of increased pressure followed by decompression, whereas it is possible to conceive of a fraction somewhere between them which would have critical over-saturation with inert gas.

A mathematical model has been devised which makes it possible to apply the concept of a great number of rates of gas exchange in the body to evaluate different programmes for pressure exposure. With this model it is possible to take into consideration different times of exposure and varying pressures and solubility of different breathing gases. For practical reasons, however, it must be presupposed that the gas-exchange rate of a certain tissue fraction is constant.

The possibility of symptom-producing changes in a certain tissue fraction is assumed to be related to the following main factors: (1) inert gas tension in relation to surrounding pressure (oversaturation ratio); (2) the magnitude of surrounding pressure; (3) the time that oversaturation exists.

In covariation with these factors there are probably others, for example, amount of dissolved gas, susceptibility of the tissue to deformation, and diffusion rate of different gases. Consequently a theoretical investigation of the connexion between each of the above-mentioned factors separately and the risk of decompression sickness is of little practical value.

However, to make possible a rational experimental investigation of what over-saturation ratios are acceptable under different conditions and in different tissues, it is necessary to be able to calculate the optimal decompression programme which fits in with the criterion (the over-saturation ratio, etc.) to be tested. The optimal decompression programme would then be the one in which the chosen criterion exists all the time in at least one tissue fraction, without being exceeded.

Mathematical considerations. The well-known exponential equation for the equilibration of differences in gas tensions between a tissue and breathing gases at constant external pressure has been modified (see footnote) to be valid for varying external pressure.

As the mathematical model is adopted for use with several breathing gases, the different tissue fractions are characterized not only by the elimination constant (k) (for explanation of symbols, see footnote) of one gas but also by its relation to the constants of the other gases. By application of the above-mentioned equation the tension of each gas is calculated, after which the total gas tension of each tissue fraction can be obtained by summation. To allow the factor of external pressure change ($U+H$) to be considered as linear, the course of the pressure programme must be split into sufficiently short time intervals. For each interval the following factors must be defined: the time of the beginning of the interval (t_0), external pressure at the beginning of the interval (a), external pressure change (b) per time unit within the time-interval, relative partial pressure of each breathing gas in the time interval (U_1, U_2, etc.). It should be noted that the value of the gas tension at the end of an interval constitutes the initial gas tension (P_0) of the next interval. When the aforementioned parameters which are valid for a dive are applied in the calculations, the over-saturation ratios for any tissue fraction at all intervals can be tabulated.

When an optimal decompression programme is to be determined, the maximal ascent (b) per time unit for each time-interval and tissue fraction which fits in with the chosen criterion is calculated. The computation technique calls for iterative calculation of the ascent which can be allowed within the time-interval, as the change in pressure modifies the conditions for gas exchange in the tissue. The tissue fraction in which the criterion is reached with the smallest ascent limits the decompression that is tolerable during the interval. The programme for the diver's ascent from the dive is obtained when the calculations have been performed and tabulated for all the time intervals of the dive.

The need to make the calculations, some of which have to be iterated, for a great number of tissue fractions and time-intervals makes the use of an electronic computer necessary if a series of any practical importance is to be worked through.

A number of combinations of different criteria are now under computation according to the rules given here with the view of their being subjected to experimental testing.

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$$P = UH - \frac{Ub}{k} + \left(P_0 - Ua + \frac{Ub}{k}\right) \exp - k\,(t - t_0)$$

P, pressure of gas dissolved in tissue fraction at time t; U, fraction of pressure exerted by component of gas mixture in the lungs; H, external pressure at time t. $H = a + b\,(t - t_0)$; a, external pressure at time t_0; b, external pressure change per time unit; P_0, pressure of gas in tissue fraction at time t_0; t time variable; t_0, initial value for t; k, elimination constant.

Changes in the Motor Neurones after administering Muscle Relaxant and Convulsant Drugs

Mature female rats were given 2 mg of the muscle relaxant 'Flaxedil' (gallamine triethiodide) repeated at intervals of 24 h for three days with full clinical recovery between doses. The animals were killed with chloroform 72 h after the last injection of 'Flaxedil' and the tissues fixed by perfusion of 10 per cent formalin and 5 per cent acetic acid via the aorta. The cervical and lumbar enlargements of the spinal cord were sectioned at 7μ and stained with 0·5 per cent aqueous cresyl violet to show the Nissl substance in the anterior horn nerve cells. Only the large anterior horn cells were included in the investigation.

Morphological changes, similar to those produced by section of the axon or prolonged electrical stimulation of a nerve[1], can be demonstrated in most of the anterior horn cells of the spinal enlargements within three days of the last injection of 'Flaxedil'; cytoplasmic and nuclear swelling and early chromatolysis could be easily identified (Fig. 1*a*) but have not yet been traced beyond the three-day stage.

Treatment of a second series of rats with convulsant doses of strychnine and cardiazole produces similar chromatolytic changes in these somatic efferent neurones (Fig. 1*b*).

These observations may be of interest and value to the neurophysiologist in the assessment of the function of the nerve cell and in explaining the mystery of its abundant chromidial (Nissl) substance and its role in nerve impulse transmission.

After crushing the hypoglossal nerve, Brattgard, Edstrom and Hyden[2] investigated the chemical changes occurring in the individual nerve cells of the hypoglossal nucleus during the so-called retrograde degeneration or, more properly, retrograde reaction. Between the second and eighth day following the operation, the main features are an increase in cell volume, maintenance of the total RNA content per cell, and an increase in proteins and lipids. The visible change in the Nissl substance they regard as a transition from an active to a more active form of RNA and not as a reduction in its amount.

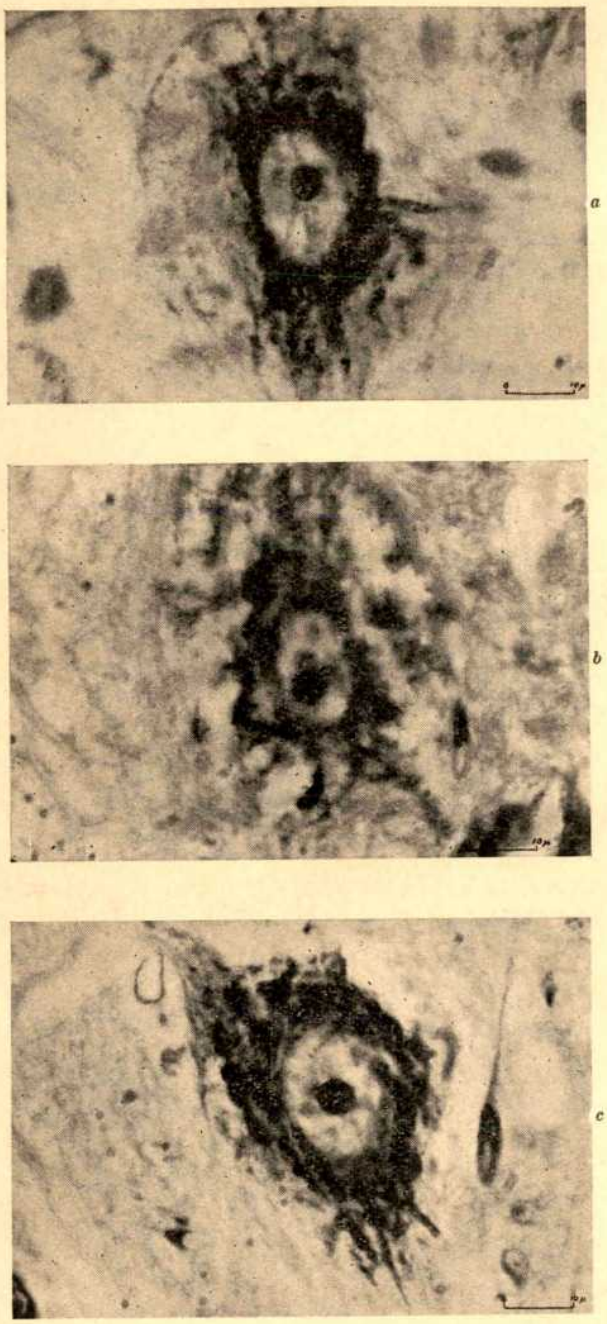


Fig. 1. Anterior horn nerve cells of the rat spinal cord stained with cresyl violet. *a*, 72 h after the last of three injections of 'Flaxedil'; *b*, 72 h after the last of three injections of strychnine; *c*, normal anterior horn nerve cell

The origin of synaptic vesicles, which are believed to carry the chemical transmitter at the synapse, has been investigated by Van Breemen, Anderson and Reger[3]. These authors suggest that the vesicles, originating from the Golgi complex (smooth-membraned vesicles) in the cell body, migrate through the axoplasm to reach the synapse. It may be, then, that in normal circumstances the ribonucleoprotein (chromidial substance) produces a secretion and thereafter the Golgi complex stores and transmits the secreted material in the synaptic vesicles along the axoplasm to the nerve ending.

Brattgard *et al.*[2] associate the production of the proteins and lipids with the regeneration of the nerve and later with the maturation of the nerve. Since the same retrograde or chromatolytic reaction can be obtained by administering a drug like strychnine where there is no call for nerve regeneration, the increased activity of the RNA and other morphological features suggest increased secretion. This is also compatible with the known function of strychnine, namely, its central stimulatory action on the nervous system resulting in increased activity of the anterior horn cells. Perhaps, then, excessive activity of the nerve cell causes such accumulation of normal secretion in the cell body that it disperses or fragments the Nissl substance, giving the chromatolytic effect.

It is difficult, however, to correlate what is apparently an increased functional state of the nerve cell with the flaccidity of the musculature after administering the muscle relaxant 'Flaxedil' unless we regard the effect of the drug as a blockade of the natural chemical transmitter at the myoneural junction accompanied by persistent hypersecretion on the part of the nerve cell to overcome the blockade.

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Flow of Uterine Blood, and the Œstrous Cycle

Although much has been written describing vascular changes in the endometrium with the œstrous cycle, little information is available regarding the changes in flow of blood to the whole uterus, which is largely myometrium, during this cyclic physiological event. Recently, it has been demonstrated that the fraction of an intravenously administered physiological dose of tritiated-epinephrine which is taken up from the circulation and bound in the uterus varies more than 4-fold between œstrus and dioestrus[1]. Since tritiated-epinephrine is rapidly removed from the circulation, such a change could be a consequence of an alteration in the fraction of the administered catecholamine which is delivered to this organ. Sapirstein's method[2] was used to evaluate the fraction of the cardiac output which perfuses the uterus at various times in the œstrous cycle. (The legitimacy of applying this method for determining uterine blood flow was confirmed by demonstrating that, during the first minute after injection of potassium-42, there was no dependence of uterine uptake upon time, despite continuing recirculation of the indicator[2].) It will be shown that during all phases of the œstrous cycle the uterus receives 0·2–0·3 per cent of the cardiac output.

Sprague–Dawley female rats weighing 180–200 g received 180,000 c.p.m. of potassium-42 in 0·2 ml. of normal saline within 2 h after a vaginal smear was taken for determination of the œstrous phase. The animals were beheaded 15 sec later; the uterus was then removed and weighed, and the potassium-42 present in this organ was assayed in a scintillation well counter (Nuclear, Chicago). Standard counts were made with 1 per cent of the injected potassium-42 every 15 min to correct for radiation decay. Vaginal smears were analysed according to Papanicolaou's method[3].

Although there was an increase in the weight of the uterus as rats approached œstrus, there was no corresponding increase in the proportion of the cardiac output which perfused the uterus (Table 1). Thus, if the cardiac output does not change during the œstrous cycle, the flow of blood to each unit weight of uterus actually decreases during the œstrus. More tritiated-epinephrine is bound in the uterus during œstrus[1]. Since alterations in the distribution of circulating blood cannot explain this phenomenon, it

Table 1. Effect of Œstrous Cycle on Uterine Blood Flow

	% injected ^{42}K*	Mean uterine weight	% injected ^{42}K/g
Diœstrus (12)	0·31 ± 0·05 (*S.E.*)	172	1·8 ± 0·23
Proœstrus (6)	0·30 ± 0·07	202	1·5 ± 0·27
Œstrus (6)	0·25 ± 0·05	253	1·0 ± 0·15†
Metaœstrus (6)	0·22 ± 0·06	158	1·4 ± 0·24

* Per cent injected potassium-42 is taken to represent per cent of cardiac output delivered to the uterus.
† $P < 0·02$ compared to diœstrus.

must be concluded that the mechanism for binding tritiated-epinephrine is altered during the œstrous cycle.

Investigations with oophorectomized rats have indicated that the atrophic uterus receives 0·045 per cent of the cardiac output[4]. Administration of œstrogen resulted in a 3-fold increase in this fraction. In these experiments flow of blood through the ovarian artery, which is responsible for a portion of the uterine blood flow, had been cut off. The value obtained for the fraction of the cardiac output delivered to the uterus 4 h after œstrogen administration, 0·2 per cent, is compatible with that reported here. The changes noted with œstrogen administration were in a situation of almost total œstrogen deprivation and uterine atrophy. It is evident that the changes produced in delivery of blood to the uterus by cyclic fluctuations in œstrogen-levels are not nearly as marked as those observed after œstrogen administration to oophorectomized rats.

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Fœtal Myoglobin in the Urine of an Adult

A fœtal form of myoglobin has been shown in newborn animals[1] and human infants[2], and it has a different electrophoretic mobility from myoglobin in adults. This communication describes the presence of fœtal myoglobin in the urine of an adult with idiopathic myoglobinuria.

The patient was a 28-year-old female with a long history of muscle pain associated with the passage of dark urine. The diagnosis of myoglobinuria was established during an acute attack, although it was noted that her urinary myoglobin precipitated in 80 per cent saturated ammonium sulphate. Since fœtal myoglobin is not as soluble in ammonium sulphate as the adult protein[1], the presence of a fœtal form of myoglobin was suspected.

Fœtal myoglobin was prepared from psoas muscles of stillborn infants, and adult myoglobin was obtained from the psoas muscle of an adult who died of a non-myopathic disease. The muscle tissues were homogenized in the cold with distilled water and the cellular detritus centrifuged out. The two respective muscle liquors and the patient's urine were purified simultaneously by column chromatography, utilizing carboxymethylcellulose according to the method of Åkeson and Theorell[3]. Following elution from the column with phosphate buffer (*p*H 6·9), the myoglobins were reduced to the met-forms with potassium ferricyanide and then dialysed against distilled water. Electrophoresis was performed on cellulose acetate at 120 mV with barbital buffer (*p*H 8·6, ionic strength 0·1 M) for 110 min. Concurrently, samples of the patient's serum and hæmoglobin were subjected to electrophoresis. The resulting separations were stained with benzidine for iron, as well as with nigrosin for protein.

The results (Fig. 1) demonstrate the presence of a urinary iron-containing protein in the same electrophoretic region as purified fœtal myoglobin. In addition, the patient's urine had an iron-staining protein corresponding

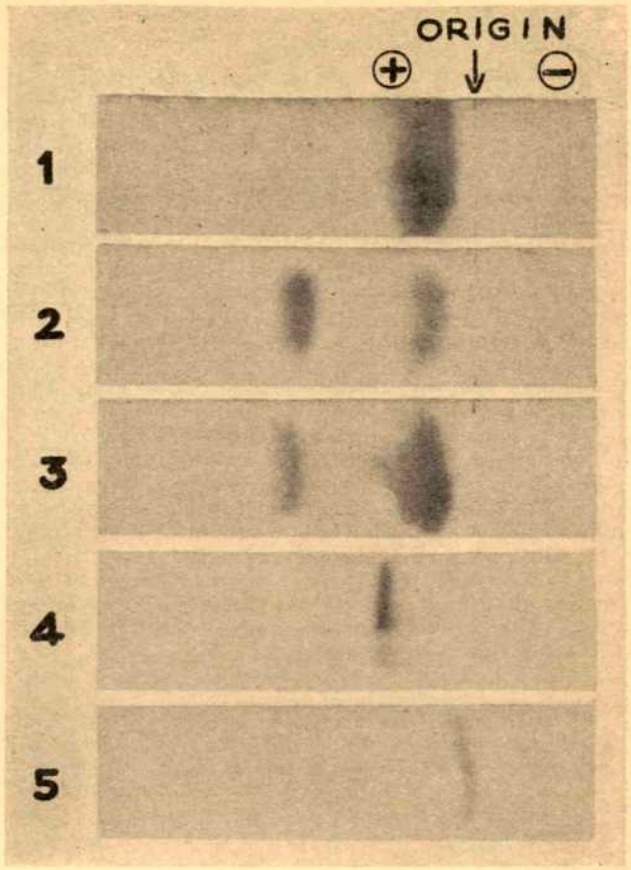


Fig. 1. Electrophoresis on cellulose acetate with barbital buffer (*p*H 8·6; 0·1 M ionic strength) and stained with benzidine. 1, Adult myoglobin; 2, fœtal myoglobin; 3, patient's urinary myoglobin; 4, patient's hæmoglobin; 5, patient's serum

in mobility to the adult myoglobin. Comparison of the stained areas by densitometry, to ascertain relative content of the two urinary proteins, showed 43 per cent fœtal and 57 per cent adult form. The serum and hæmoglobin patterns did not show iron-containing proteins in the same areas as either fœtal or adult myoglobin. The strips stained with nigrosin for protein gave the same patterns, except in the instance of the serum sample, which showed a normal pattern.

These findings indicate that during an attack of myoglobinuria this patient excreted a urinary protein which was about one-half fœtal and one-half adult myoglobin. Although muscle biopsy was not performed, it is presumed that this abnormal myoglobin originated in the patient's muscles, and that it represented persistence of the fœtal protein into adulthood. Further, it is speculated that this molecular defect may be responsible for the myoglobinuria.

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Some Statistical Aspects of Blood Sugar Regulation

In the examination of physiological regulations we are often concerned with averages observed on successive occasions with short time-intervals. Other statistical parameters could be also used, since they throw some light on regulatory mechanisms. This can be shown by the following results.

Long-term reliability of fasting blood sugar-level is very low ($r = 0{\cdot}32$), but if the time elapsed between the first and the second taking of blood is short (about 1 h) correlation appears to be relatively high: $r = 0{\cdot}79$. This figure has been obtained on fasting men at rest. If before the second blood-letting they were made to walk on a tread-mill (15 min at 7 km/h), averages did not change, but the correlation between the successive blood sugar-levels determined with the same time-interval fell to 0·57. Therefore something happened which was not reflected by averages, but interfered with correlations.

If $r = 0$, blood sugar variations on successive occasions would be completely independent and no regulatory influence for a given time-interval could be supposed. If $r > 0$, it has no obvious meaning: it may be due to regulatory activity or to passive maintenance of the same blood sugar-level during a short period. But if correlations between successive occasions change in an orderly way, their variations may be meaningful.

Tables 1 and 2 show what happens with half-hourly intervals after absorption of glucose (30 g).

Table 1. Blood Sugar-levels. Adult Women, $n = 52$ (g/100 ml.)

	Arith. mean	σ	C.V.
Fasting	1·02	0·14	13·72
After glucose:			
30 min	1·47	0·26	17·68
60 min	1·39	0·28	20·14
90 min	1·13	0·22	19·46
120 min	0·97	0·13	13·40

Table 2. Correlations between Blood Sugar-levels Adult Women, $n = 52$

		1	2	3	4	5
1	Fasting	—	0·25	0·05	0·25	0·51
2	30 min		—	0·55	0·38	0·37
3	60 min			—	0·67	0·43
4	90 min				—	0·77
5	120 min					—

Half an hour after a glucose meal, blood sugar-level attains its highest average. Absolute and relative variabilities rise simultaneously and the correlation with the fasting-level appears to be very low, much lower indeed than short-term fasting reliability. After 1 h the average begins to decrease whereas both variabilities are at their peak value. Thereafter, averages and variabilities decrease simultaneously. There is an increase of 'disorder' followed by a progressive decrease.

Correlations grouped in Table 2 have two prominent features. In the upper row, correlations between fasting-level and after-glucose-levels disappear at the 60th min, then rise again, and the last figure is twice as great as the first one and is significant. On the other hand, figures in the lowest diagonal show that correlations between successive blood sugar-levels, beginning with a very low value, rise steadily and at the end we find a coefficient as high as fasting short-term reliability.

The steady increase of correlations in the lowest diagonal probably reflects the growing efficiency of regulatory process. In the upper row the disappearance of the correlation between the fasting and after-glucose level at the 60th min, followed by a remarkable rise, means that after a break there is a tendency to restore the initial fasting-level. Nevertheless, even after 2 h this result is not completely achieved: in column 5, the first figure is still lower than the last one which, as I have already pointed out, is as high as fasting reliability.

Moreover, in Table 1 the last average is lower than the first; the difference is small, but significant. A similar difference has been observed more than once, and some authors, in order to define the fall of the last observed value below the fasting-level, used words such as 'hyper-correction'. This word seems to mean that physiological regulations should in all circumstances restore a fixed 'normal' value. If for some reason they cannot 'correct', at least they must 'hypercorrect' the departure from the 'normal' point.

In accordance with some other results my figures have a less-metaphysical meaning. The comparison of averages, variabilities and correlations show that 2 h after a glucose meal a new slightly different orderliness is attained. This may be explained in many ways, which are not alternative.

The fall of blood sugar-level below the fasting value can be due to the fact that 2 h after a glucose meal the subjects are still hungry. The same fall has been observed twice in fasting men at rest as well as after a tread-mill walk (first average: 0·95 g/100 ml.; second average: 0·90). Perhaps this small difference can be explained also by psychological interferences, the first blood-letting being apparently feared more than the second or the third. If this is so, 'normal' values, usually represented by the first fasting-level, may be a little exaggerated by emotive hyperglycæmia.

We cannot standardize either laboratory animals or human subjects. Alimentary habits, activities preceding the test and possible psychological perturbations are seldom taken into account. Of course, it would be difficult to ascertain them in every case. Therefore, we are left with an unavoidable margin of uncertainty. Nevertheless, my figures agree with conclusions already suggested by the examination of intra-individual variability[1]. In fact, the living organism is never in equilibrium, but struggling for it. In continuously changing situations physiological regulations tend to maintain a limited (statistical) orderliness, not necessarily to restore the previous one.

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PHARMACOLOGY

Thymectomy in Dogs with Renal Homografts treated with Drugs

Miller[1] has reported an additive effect of X-irradiation and thymectomy in depressing immunological mechanisms in adult animals. Accordingly an investigation was undertaken to determine whether thymectomy improved the functional survival of homologous renal transplants in dogs treated with drugs.

The experimental preparation and assessment of results were identical to that reported previously elsewhere[2]. Using pentobarbitone anæsthesia with air-insufflation of the lungs, the thymus was removed from mongrel dogs of either sex, via a right thoracotomy through the second inter-space. At the same time, a kidney from an unrelated dog was transplanted to the right iliac fossa and the animal's own two kidneys were removed. The drugs were administered as follows: actinomycin *C* (10 γ/kg) intravenously on the day of operation and at weekly intervals. 6-mercaptopurine suspension 5 mg/kg intramuscularly on the day of operation, followed by 'Imuran' daily by mouth, 10 mg/kg on the first and second day after operation and 5 mg/kg on subsequent days. The dose was reduced if the total white cell count fell below 5,000 cells/mm.^{3}. This drug combination has been found previously to be effective in prolonging canine renal homograft survival[2].

Table 1

Experimental groups	Total No. of animals	Animals dying with marrow depression	Animals surviving beyond 14 days and those dying before 14 days with microscopic rejection	Animals surviving beyond 20 days with good renal function	Animals dying after 14 days without microscopic rejection	Animals surviving beyond 3 months	Mean survival time
Thymectomy/actinomycin *C*/'Imuran'	8	4	7	4	1	0	17
Actinomycin *C*/'Imuran' (series II)	11	5	8	3	4	0	17

The results of the experiments are shown in Table 1, where a comparison is made with similar experiments performed without thymectomy.

The control experiments were performed concurrently in the same laboratory and have been reported elsewhere[3]. It can be seen that thymectomy has not improved the length of survival of functioning renal transplants. Examination of the thymuses of the animals treated with the thiopurine and actinomycin drug combination showed a very marked diminution in size of the organs and a severe depletion of lymphoid follicles.

In the context of these experiments it would appear that if the thymus has an important role in the rejection of canine renal transplants in adult animals, then the drugs used in this study can inhibit this effect so that surgical thymectomy has no additional advantage.

I thank Miss Leigh Brown for assistance, and Dr. G. H. Hitchings and his colleagues of Burroughs Wellcome, Inc., for their co-operation in this work and also for providing the purine analogues. I also thank Dr. D. Whitfield, of F.B.A. Pharmaceuticals, Ltd., for providing the actinomycin *C*. This work was financed by grant *G*–62–29 from the Life Insurance Medical Research Fund.

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Degraded Carrageenan and Experimental Acute Gastric Ulceration in the Guinea Pig

It has been shown[1] that histamine-induced peptic ulceration in the intact unanæsthetized guinea pig can be prevented or diminished by the oral administration of the sulphated polysaccharide, degraded carrageenan; severe duodenal ulceration could be completely prevented by a dose which at the same time approximately halved the severity of gastric ulceration. It was suggested that degraded carrageenan might augment the natural protection afforded by mucin by complexing with it, so providing a more robust mucosal coating. It has since also been found[2] that in the guinea pig the volume and acidity of histamine gastric secretion is reduced by prior oral administration of degraded carrageenan.

In the experimental results reported here another factor in the protective action of degraded carrageenan on the stomach is described. Acute gastric ulceration caused by histamine has been decreased by the intraduodenal injection of a solution of degraded carrageenan in random bred, male albino guinea pigs of one strain (weight range 300–500 g), where the junction of stomach and duodenum has been sealed by application of a clip or by ligation.

Gastric ulceration was produced in the anæsthetized ('Nembutal' sodium 30 mg/kg intraperitoneally) guinea pig by the subcutaneous administration of histamine acid phosphate (5·0 mg/kg in saline). The peritoneal cavity was opened and an artery clip or ligature placed on the duodenum above the bile duct, to prevent communication between the contents of the duodenum and stomach. 200 mg degraded carrageenan in 1 ml. water were injected into the duodenum in the direction of the pylorus 0·5 h before administration of histamine, and again immediately after the histamine was given; control animals received 1 ml. water instead of degraded carrageenan solution. In a further group of control animals treated similarly but receiving neither histamine nor degraded carrageenan the stomach was apparently healthy at the end of the experiment. The peritoneal cavity was closed by artery forceps and the animal left covered in the supine position on a warm table until 1 h after the injection of histamine, when it was killed by ether. The stomach was removed with the clip in position and the gastric secretion collected. It was filled with water and examined by transmitted light for ulceration. Only clearly established ulcers were considered and these appeared as clean, virtually transparent areas sharply bounded by a whitish opaque margin. This corresponds to the established ulcer carefully described by Watt[3].

Table 1. Anti-gastric Ulcer Effect of Degraded Carrageenan

Treatment	No. of animals	Average severity of gastric ulceration
Control (water)	14	3 +
Degraded carrageenan	14	1·5 +

A 4+ scale was used to grade severity of ulceration: + indicates one or two small areas of ulceration; 2+, one or two larger areas; 3+, several areas of marked ulceration; 4+, extensive ulceration with confluence or impending perforation. Results were averaged for each group and are shown in Table 1.

Thus, 50 per cent reduction in gastric ulceration in 1 h is effected by the sulphated polysaccharide without gaining access to the gastric contents; and this protection compares with that reported[1] in a 24-h experiment where a rather larger (but oily and intramuscular) dose of histamine produced less-severe gastric ulceration, and where a much larger dose of degraded carrageenan was used.

It is suggested that the protection from gastric ulceration given by degraded carrageenan is brought about by events which are initiated in the intestine and probably in the duodenum.

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HÆMATOLOGY

Evidence that 'Normal Incomplete Cold Antibody' is not a Gamma-globulin

Dacie[1] discovered that if normal human red cells were left at 0° C with their own fresh serum and afterwards washed in warm saline, they could be agglutinated by anti-human globulin serum, and he postulated that serum normally contained an incomplete antibody reacting at 0° C with human red cells. Later, Dacie, Crookston and Christenson[2] produced evidence that the positive anti-globulin reactions were not due to the direct detection

of the antibody, but to a reaction with complement which was evidently bound to the cells at 0° C and was not removed by the subsequent washing.

Crawford, Cutbush and Mollison[3] showed that normal incomplete cold antibody had anti-H specificity. Thus the strongest reactions were obtained by incubating group AB serum with group O red cells, and the reactions could be inhibited by the addition to the serum of H-containing saliva.

It has been shown that normal incomplete cold antibody is present in the serum of new-born infants[4,5]. Since it is believed that the only antibodies present in cord serum are those which have been derived from the mother by placental transfer, and since the only γ-globulin transferred is 7*S* γ-globulin (for review, see ref. 6), these observations might suggest that normal incomplete cold antibody is a 7*S* γ-globulin, but this would be surprising since natural antibodies are, at least usually, 19*S* γ-globulin.

The problem was further investigated in the following way: samples of normal adult serum were fractionated by DEAE-cellulose chromatography[7] using a modification described elsewhere[8] and the fractions tested for the presence of normal incomplete cold antibody; in testing fractions, in order to overcome the difficulty of having to supply complement without normal incomplete cold antibody, horse serum was added as a source of complement to the fractions, and the cells were finally tested with immunoconglutinin serum to see if complement had been bound[9]. The normal incomplete cold antibody was recovered in the fractions eluted with 0·10–0·12 M phosphate buffers and was not present in the 0·02 M fraction, in which the bulk of the 7*S* γ-globulin is eluted.

Because normal incomplete cold antibody is thus apparently not a 7*S* γ-globulin, and because it is present in cord serum, the possibility must be considered that normal incomplete cold antibody is not a member of the γ-globulin family at all. Two further pieces of evidence support this view. First, we have found that red cells sensitized with normal incomplete cold antibody are not agglutinated either by potent anti-7*S* γ-globulin or by anti-19*S* γ- (γ_{1M}-) globulin sera. Secondly, we have found that normal incomplete cold antibody is present in approximately normal amounts in the serum of patients with hypogammaglobulinæmia; thus it was present in samples from 10 out of 11 patients, even though some of these samples had been stored at −20° for some years. Table 1 shows three examples; in all three, concentrations of 7*S* γ-, γ_{1M}- and γ_{1A}-globulin were very low, and none of the samples contained iso-agglutinins or the common cold auto-agglutinin anti-I; but all contained normal amounts of normal incomplete cold antibody.

Normal incomplete cold antibody differs in various ways from classical antibodies; thus we have found that it is relatively heat-labile, being destroyed in 30 min at 63° C, that it is absorbed by zymosan (as previously noted by Dr. F. Stratton, personal communication), and that it cross-reacts with certain bacteria. In these ways, normal incomplete cold antibody resembles properdin, and incidentally properdin is present in cord serum[10] and in the serum of patients with hypogammaglobulinæmia[11]. However, we have found that normal incomplete cold antibody and properdin differ in many respects; for example, whereas normal incomplete cold antibody is best absorbed by red cells at 0° C and is absorbed by zymosan just as well at 0° C as at 37° C, properdin is absorbed by zymosan only at 17° C or higher; again, whereas a wide range of bacteria absorb properdin[12], only a few bacteria (for example, *E. coli* C_3) absorb normal incomplete cold antibody.

Table 1

Initials	γ-Globulins (% normal)*			A B O blood group	Anti-A	Anti-B	Anti-I	Titre of normal incomplete cold antibody (reciprocals)
	7*S* γ	γ_{1A}	γ_{1M}					
S.S.	0·8	0·5	17	O	nil	nil	nil	64
W.T.	2	0·2	1	B	nil	—	nil	64
C.V.†	11	3	6	A	—	nil	nil	16

* Concentration expressed as percentage of standard normal sera.
† This sample had been stored for almost three years; the other two had been stored for only a few weeks.

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Quantitation of Erythropoiesis

It was not until recently that a method for the quantitative estimation of erythropoiesis became available[1]. The method introduced requires the application of high doses of radioactive iron, a very stable and sensitive measuring equipment and protracted measurements. The authors showed that the usual calculation of erythrocyte formation from plasma iron-turnover (PIT) values is frustrated by a substantial return of radioiron from the bone marrow into the plasma, starting at about 8 h after injection of radioiron. This finding and the results of Lamerton *et al.*[2] induced me to block the re-utilization of radioiron by injecting inactive iron. Under such conditions PIT would correctly measure iron uptake by the organs of the body, and the radioiron incorporation into erythrocytes would be a correct indicator of the amount of iron built in into erythrocytes. The quotient between the amount of iron contained in the total circulating hæmoglobin and the amount built in into erythrocytes would then be a measure of the effective life-time of the erythrocytes.

Five hæmatologically healthy subjects were examined and their PIT measured[3]. Inactive iron as iron-dextrin ('Ferrigen', kindly supplied by Astra, Södertälje) was slowly injected intravenously, in 100-mg doses, at the indicated intervals. Radioiron incorporation into the erythrocytes was followed for at least 2 weeks to obtain the maximum value. The results obtained are given in Table 1. As even in normal individuals some random destruction of erythrocytes occurs, which according to Mollison[4] is calculated to be about 10 per cent, and as about 1 per cent of the injected radioiron remains in the plasma 8 h after injection, the values calculated were arbitrarily increased with 10 per cent.

It can be seen from Table 1 that the method described leads to values for the erythrocyte life-time which lie within the normal range reported for human beings[4]. The blocking with inactive iron thus proved to be successful in the individuals studied. A complete inhibition of re-utilization cannot be obtained by applying reasonable iron doses; it can, however, be shown that re-utilization

Table 1. EXPERIMENTAL VALUES FROM FIVE HEALTHY INDIVIDUALS

No.	Hgb (g %)	Hct* (%)	Serum-Fe (μg %)	^{59}Fe T½ (min)	PIT (mg/day)	^{59}Fe incorp. (%)	RBC–IT (mg/day)	Hgb (tot. g)	Iron in Hgb (tot mg)	RBC life† (days)	RBC life‡ (days corr.)	App. ^{51}Cr T½ (days)	100 mg Ferrigen
1	14·6	42	169	146	28·1	66·2	18·6	616	2·10	113	124	/	8, 24, 36 h 2, 3 days
2	15·1	37	108	86	35·3	72·3	25·6	674	2·29	90	99	/	8, 24, 36 h 2, 3, 5, 11 days
3	14·2	39	56	60	26·1	81·3	21·2	650	2·21	104	115	/	8, 24, 36 h 2, 3, 5, 13 days
4	13·1	39	95	93	26·8	78·9	21·2	578	1·96	93	102	33	24 h, 1, 4, 11 days
5	12·3	35	73	71	22·8	71·1	16·2	428	1·46	90	99	27	24 h, 1, 5 days

* Hct corrected for venous/body hæmatocrit ratio (8 per cent) and plasma trapping (2 per cent).
† $\frac{\text{mg iron in total Hgb.}}{\text{RBC–IT mg/day}}$
‡ Corrected with 10 per cent as described.

becomes insignificant. Four doses of 100 mg of inactive iron each given at 8 h, 1, 4 and 7 days after injection of radioiron suffice to give the needed blocking effect.

The method described permits the quantitative determination of effective erythropoiesis. From the results obtained the effective erythrocyte life-time can be calculated. In the case of a non-steady-state, appropriate corrections have to be made.

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PATHOLOGY

Transplantation of Polyoma Tumours in Rats

IT has been shown that polyoma tumours induced in mice and in hamsters might contain a new cellular antigen[1–4]. The same phenomena were suggested in rats by comparative examination of the transplantability of polyoma and carcinogen-induced and spontaneous tumours in rats[5]. In the work reported here an attempt was made to demonstrate the presence of this antigen in transplantable polyoma tumours in rats and to verify its persistence in tissue cultures derived from primary polyoma tumours. The same antigen was also looked for in rat embryo fibroblasts which underwent a neoplastic transformation *in vitro* by the polyoma virus. No cytopathic effect was observed in this tissue culture, and no virus could be detected after the second *in vitro* passage.

Rats of the inbred strain *R* and Lewis were used. In a first experiment an attempt was made to induce resistance in adult rats to polyoma tumour isografts by pre-treatment (three weekly subcutaneous inoculations) with a homologous transplantable polyoma kidney sarcoma (LKS_1). For challenge, isografts consisting of a transplantable kidney sarcoma (RKS_7), a continuous tissue culture derived from a primary polyoma kidney sarcoma (*38K*) and an *R* rat embryo fibroblast cell line rendered neoplastic *in vitro* by the polyoma virus (*REPT*) were used. No polyoma virus could be recovered from the transplantable tumours or from the continuous tissue cultures. All the isografts were inoculated one week after the last inoculation with homologous tumours and at the minimal cell dose. As control, three other groups of adult rats were treated prior to isografting respectively with a homologous transplantable benzpyrene sarcoma ($LBPS_1$), normal trypsinized and washed Lewis kidney cells and with control medium (Eagle's basal medium with 10 per cent calf serum). The control groups as well as the group treated with a homologous polyoma tumour were also challenged with isologous tumour cells derived from a carcinogen-induced mammary tumour in an *R* rat (*DE7R* cells) and cultured *in vitro*. In a second experiment three groups of adult *R* rats were, prior to isografting, inoculated (three times at weekly intervals) either with a homologous polyoma tumour (LKS_1), with polyoma virus (10^7 $TCID_{50}$) partly purified by one cycle of absorption-elution on cavia erythrocytes, or with control medium. The fourth group consisted of *R* rats inoculated subcutaneously at birth with the virus and isografted with polyoma tumour cells when 2–3 months old. In this group, about half the animals had died of polyoma tumours before the isografts were inoculated. These grafts were a tissue culture derived from a primary subcutaneous fibrosarcoma originally induced by the polyoma virus in an *R* rat (*33F* cells) and the *in vitro* transformed *R* rat embryo fibroblast line (*REPT*).

Table 1. DEVELOPMENT OF RAT POLYOMA TUMOUR ISOGRAFTS IN *R* RATS PRE-TREATED WITH HOMOLOGOUS TUMOURS OR WITH POLYOMA VIRUS

	Immunization material	Isologous tumours			
		RKS_7 5×10^5 cells*	*38 K* 5×10^5 cells	*REPT* 5×10^5 cells	*DE7R* 1×10^4 cells
Exp. 1	LKS_1 (Lewis polyoma kidney sarc.)	(0) 1/6†	(0) 1/4	(0) 3/6	(2) 4/4
	$LBPS_1$ (Lewis benzpyrene sarc.)	(3) 5/6	(2) 3/4	(2) 6/6	(2) 4/4
	L kidney cells	(2) 4/4	(3) 4/4	N.T.	(3) 4/4
	Control medium	(4) 6/6	(3) 4/4	(2) 6/6	(2) 4/4
		33 F 1×10^6 cells	*33 F* 5×10^7 cells	*REPT* 5×10^5 cells	
Exp. 2	LKS_1	(0) 2/16	(9) 13/16	(1) 2/4	
	Polyoma adults	(0) 1/16	(6) 9/16	(0) 1/4	
	Polyoma new-borns	(4) 12/24	(14) 16/16	N.T.	
	Control medium	(7) 16/16	(15) 16/16	(2) 4/4	

* Minimal cell dose of isograft to obtain a tumour *in situ* of the inoculation.
† No. of rats bearing tumours 20 days after isografting on total number inoculated. Figures in parentheses indicate number of tumours palpable 12 days after the inoculation.

The results are summarized in Table 1. They indicate (Exp. 1) that pre-treatment of adult rats with a homologous polyoma tumour gives a partial resistance to isografts derived either from a transplantable polyoma tumour, from a continuous tissue culture of polyoma tumour cells or from an *in vitro* transformed rat embryo fibroblast line. They also show that this resistance is only directed against polyoma tumour isografts, is not related to an organ or a connective-tissue specific antigen and is not dependent on the presence of virus in the tumour cells. The results obtained in Exp. 2 show that partial resistance to polyoma isografts is also found in adult rats pre-immunized with polyoma virus and in rats inoculated at birth with the virus. They also indicate that this resistance is highly dependent on the cell dose inoculated as isograft. In the group rats inoculated at birth with the virus and which were still alive at the time of isografting, seven had developed primary polyoma tumours at the end of the experiment. Five of them showed resistance to isografting with 10^6 *33F* cells and one did not. One primary polyoma tumour was detected in the group inoculated with 5×10^7 cells.

The presence of resistance to polyoma tumour isografts in adult rats pre-treated with the virus might be due to the induction by the virus of the same antigen in the cells it infects. These antigenic transformation leads to the rejection of these cells in the adult and immunological competent animal whereas in new-born rats these cells may go further proliferating and eventually give rise to a tumour[3]. There remains, however, to explain why rats injected at birth and tested when adult may show a slight but significant resistance to polyoma tumour isografts. When they do not develop polyoma tumours, one may speculate that the cells infected by the virus, and thereby antigenically transformed, pre-immunized the animal but did not acquire a neoplastic character or were rejected afterwards. When they do develop polyoma tumours and yet are found resistant to the isografts, it becomes difficult to understand why these animals do not reject their own tumours. Although highly speculative, one could, however, consider the hypothesis that rats injected with polyoma virus at birth do not reject neoplastic transformed cells, being immunologically non-reactive, but do not either become tolerant for these cells. At the time their immunological system is built up, in most instances the polyoma tumour cells could be too numerous to be rejected but could pre-immunize the animal sufficiently to enable it to reject a very small inoculum of transplanted isologous polyoma tumour cells.

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A Glycosidase Abnormality in Synovial Membrane in Joint Disease

Polysaccharide–protein complexes are known or suspected to undergo depolymerization or incomplete polymerization in rheumatoid arthritis[1], osteoarthritis[2] and intervertebral disk prolapse[3].

We have, therefore, investigated in synovial membrane the activity of enzymes participating in the degradation of oligosaccharides derived from hyaluronic acid[4], and have found an apparent relationship between β-*N*-acetylglucosaminidase activity, and what can be termed clinically the 'reactivity' of various disease processes.

Specimens of synovial membrane were removed at operation from the knee joints of patients suffering from four clinically distinguishable conditions, namely, internal derangements in the acute and in the quiescent state, osteoarthritis, and rheumatoid arthritis.

No true 'normals' can be quoted, as autopsy material was considered not comparable, but it is thought that the group of interval menisectomies—that is, those in whom a torn semi-lunar cartilage was being removed as an elective procedure without any recent trauma—must approximate closely to normal. In order to limit the number of variables involved, all patients were in the age group 25–45 years: senile osteoarthritis is therefore not included, and the group of 'degenerations' comprises those joints in which there was some predisposing cause, such as an old fracture or internal derangement, accompanied by visible fibrillation or actual loss of articular cartilage.

The specimens for assay, identified by serial number only, were placed in dry sterile containers and immediately refrigerated at 4° C. They were then homogenized and assayed for β-*N*-acetylglucosaminidase activity using ρ-nitrophenyl-β-*N*-acetylglucosamine as the substrate[5].

Table 1. β-*N*-Acetylglucosaminidase activity in 35 consecutive specimen. of synovial membrane in four different abnormalities of the knee-joints The results are expressed as micrograms of ρ-nitrophenyl liberated per hour per gram of tissue (wet weight) at 37° C; and are arranged in descending order of activity

No.	Interval menisectomy	Menisectomy with naked-eye hyperæmia of the synovium	Degeneration of meniscus or of articular cartilage	Rheumatoid arthritis
1				36,300
2				30,500
3				28,370
4				26,450
5				25,520
6				18,910
7				17,020
8				16,040
9				14,000
10			13,890	
11				13,720
12				12,470
13			12,420	
14			11,600	
15		9,970		
16				9,200
17		8,820		
18				8,090
19			7,690	
20				7,020
21		6,770		
22			6,370	
23		5,120		
24			5,010	
25			4,870	
26		3,990		
27				4,300
28		3,986		
29				3,350
30	2,900			
31	2,540			
32	2,160			
33	1,880			
34	1,330			
35				1,060

Thirty-five consecutive specimens gave the results shown in Table 1. No detailed analysis can be undertaken on the basis of this number of estimations, but the general pattern is clear. β-*N*-Acetylglucosaminidase has never been shown to be other than a catabolizing enzyme; and the figures, therefore, confirm that breakdown of tissue is increased in all three abnormalities above, and suggest that the turnover of polysaccharide is much accelerated, both in the so-called degenerative conditions and in rheumatoid arthritis. The wide scatter of results in this last group is puzzling, and may perhaps be associated with cyclical activity of the disease. High enzyme activity tended to be associated with clinical acuteness, as judged on the heat, swelling, and tenderness of the affected joints. There was no apparent correlation with Rose Waaler titre.

The possible relationship of this abnormal enzyme activity to the ætiology of the various processes is interesting, particularly in rheumatoid arthritis, where the enzyme activity is so markedly increased, and where already, on general grounds, there is evidence that enzyme-determined factors are important[6].

The synthesis of 2-acetamido-2-deoxy-gluconolactone, a highly-specific, non-toxic inhibitor of β-*N*-acetylglucosaminidase[5], provides a possible theoretical method of restoring the enzymological balance and perhaps affecting the disease process. However, the clinical results of administration of this enzyme inhibitor to patients with rheumatoid arthritis and allied diseases have so far been unreliable.

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Anoxia and Cerebral Water Content in the Adult Rat

ALTHOUGH there is adequate evidence that anoxia can damage the brain, the precise method by which it does so is not known. It has been suggested that deprivation of oxygen can lead to death of the cell by direct action on the neurone itself[1]. However, an alternative mechanism has been postulated by Lindenberg, namely, that cerebral œdema follows anoxia and this in turn leads to interference with the blood supply of the nerve cells in certain vulnerable areas[2].

Anoxia in man and experimental animals is followed by an increase in water content of the brain[3–5]; but the importance of this in relation to the pathogenesis of brain damage is not understood. This communication describes experiments in which the effects of post-anoxic cerebral œdema and cerebral œdema induced by administration of water were compared, in order to examine some of the possible factors involved in this problem.

The experimental animals were white Wistar rats of either sex, weighing 220–300 g. Unilateral anoxic forebrain lesions were induced in 50 animals by the method of Levine[6], in which the right common carotid artery in the neck was ligated under ether anæsthesia, and the animal exposed to an atmosphere of nitrogen with a sufficient admixture of air to maintain regular respiration but keep the animal comatose. This was maintained for 45 min. More than 70 per cent of the surviving animals were made hemiplegic by this procedure.

At 24 h the brains of the survivors showed swelling of the right forebrain with displacement of the midline structure to the left. There was neuronal degeneration in the cerebral cortex and hippocampus of the right hemisphere, with nerve cell loss especially in the hippocampus. Proliferation of microglia and astroglia was also seen. 60 per cent of the animals did not survive the first 24 h. Some of these died in the nitrogen and death was preceded by fits in about half of them. The rest died 10–20 h after the anoxic episode, and all had convulsions within 2 h before death. No neuronal loss was observed in these rats, but there was shrinking and pycnosis of the cells of the right hippocampus.

A further similar set of experiments was performed, but nine animals were killed immediately after the onset of fits and the brain removed. The water content of the left and right halves of the forebrains was determined by weighing immediately after drying to constant weight at 110° C. The normal water content was similarly determined in the brains of untreated rats. Fits during the anoxic episode were not accompanied by cerebral œdema. However, all rats developing this disturbance 10–20 h after anoxia had a significant increase in cerebral water content ($t = 6{\cdot}5$, $P < 0{\cdot}001$) (Table 1).

Table 1. WATER CONTENT OF FOREBRAINS OF ADULT RATS SUFFERING FROM CONVULSIONS FOLLOWING WATER ADMINISTRATION (150–175 ML/KG INTRAPERITONEALLY) AND ANOXIC–ISCHAEMIC BRAIN INJURY

(Mean values given ± 1 standard deviation, expressed as g water/g wet weight of tissue)

	Control	Convulsions after water administration	Convulsions after anoxic–ischæmia
Water content of forebrain	78·4 ± 0·4	81·0 ± 0·9	Left hemisphere 78·3 ± 1·0 Right hemisphere 81·9 ± 1·3
No. of animals	7	7	9

In the final set of experiments cerebral œdema was produced by the administration of water alone. Convulsions were induced in adult rats by the intraperitoneal injection of 150–175 ml./kg of distilled water, 10 min after intramuscular injection of 1 U. of vasopressin B.P. The average water content of the brain at the commencement of fits was found to be 81·0 ± 0·9 per cent (seven animals). At this stage further injections of water were found to be fatal. 24 h after this, six animals were killed and the brain examined histologically. No loss or degeneration of nerve cells, or glial reaction, was seen.

These findings indicate that, in the rat, cerebral œdema follows both anoxic–ischæmic encephalopathy and overhydration. The results suggest that convulsions and subsequent death in the early post-anoxic period could be due to an increase in the cerebral water content alone, because in both the anoxic and overhydrated groups the degree of œdema was similar when convulsions began. Previous work in this laboratory supports this interpretation[5], as it was shown that there was significantly fewer deaths from severe anoxic–ischæmic encephalopathy following administration of 30 per cent urea, which decreased the degree of cerebral œdema in all affected animals.

Cerebral œdema was not found in animals dying during the initial anoxic episode, in whom other metabolic or physiological factors must cause death. As degeneration and loss of nerve cells did not follow cerebral œdema produced by water administration, it may be concluded that brain swelling alone cannot lead to the destruction of neurons. This does not necessarily disprove Lindenberg's hypothesis, because anoxic brain injury could be the consequence of several factors acting together, and in the presence of circulatory and metabolic disturbances, œdema could potentiate their effect.

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HISTOCHEMISTRY

Changes of Coenzyme (TPN) Diaphorase and TPN-linked Dehydrogenase during Axonal Reaction of the Nerve Cell

EXAMINATION of the axonal reaction of the nerve cell has revealed a high level of metabolic processes. Lipid, protein and RNA content[1,2], increase and changes in the activity of various enzymes have been described[1,3,5,6,8]. Most of the histochemical investigations of the enzymes in this field were carried out on anterior horn cells or on dorsal root ganglia after cutting the sciatic nerve. A disadvantage of this is the normal variability of enzymatic activity, especially in the spinal ganglia. Difficulty is also encountered in cryostat material in differentiating a normal cell from a chromatolytic cell.

In order to avoid these difficulties, investigations were carried out on the nucleus facialis after unilateral transection of the nerve.

30 adult guinea pigs were used. The n. facialis was cut at its exit from the skull at the foramen stylomastoideum. Animals were killed after 1, 3, 5, 6, 7, 8, 9, 10 days and after 2, 3, 4, 5, 6, 7, 8, 10, 14 weeks.

20-μ thick cryostat (model Dittes–Duspiva) sections were incubated with the appropriate substrate and the tetrazolium salt nitro-BT or tetra-nitro-BT according to the technique of Nachlas *et al.*

The following enzymes were investigated: TPN-diaphorase (TPN-D), the dehydrogenase of 6-phosphogluconate (6-PG) and glucose-6-phosphate (G-6-P). Activity of TPN-D, which is very weak in normal cells of the nucleus facialis, increased significantly from 5–50 days. During this interval, G-6-P-dehydrogenase shows a marked increase of enzymatic activity while that of 6-PG-dehydrogenase increased slightly. Changes in the activity of TPN-D and G-6-P-dehydrogenase were relatively stronger and more persistent than DPN-diaphorase and DPN-linked dehydrogenases.

The results recorded here suggest a relation between the increase of enzymatic activity and the well-known changes in lipid and ribonucleotide content[2,7]. The increased activity of G-6-P-dehydrogenase and 5-PG-dehydrogenase was interpreted as an indication of an increased activity of the pentose phosphate pathway. Thus it may be possible to yield more pentose for an increased RNA-synthesis. On the other hand, the 'Horecker'-cycle is also a source of TPN-H, which is of importance for fatty acid synthesis by delivering 'reductive equivalents'. This may be the basis for increase of lipids in the chromatolytic nerve cell.

The histochemically demonstrated increase of enzymatic activity well supplements microchemical findings. There is no support for an enzymatic depletion of regenerating neurones as postulated by Friede[4]. Only some degenerative neurones show a decrease of oxidative enzymes.

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HISTOLOGY

Minute Structure of the Inner Surface of an Embryonic Central Nervous System

F. Sauer[1], referring to mitosis in the neural tube, wrote: "The attachment of the neural tube cells to each other at the surface bordering the lumen appears to play an important part in the mechanics of the development of the neural tube". Having been preceded in general conception by other authors[2–4], he stated that elements of neural epithelium were attached to each other at the surface bordering the lumen by a terminal bar net, and that there was "no internal limiting membrane other than the cytoplasmic membrane of cells". In spite of this statement, the old conception of His[5] describing the internal limiting membrane as a felt-work of fibrils into which passed the cytoplasmic fibrils of the spongioblasts had been generally accepted. Later on, Sauer's suggestion was revived by further authors[6–11]; but none of them elaborated the minute structure of internal limiting membrane or considered its possible significance in ontogenesis.

We tackled this problem during an investigation of the phenomenon of 'overgrowth' of the neural tube and had occasion to examine the structure of internal limiting membrane by means of electron microscope, thin membranous preparations and replica method. Our work with histological preparations, verified by mathematical calculations, has shown the internal limiting membrane to consist of two types of polygonal fields arranged into two layers, representing terminal enlargements of cytoplasmic end-feet of all germinal zone cells (Fig. 1). The fields of the superficial layer are designated as 'principal', that of the deeper layer as 'subsidiary' fields. The 'principal' fields are attached to each other by distinct cementing borders of the first order, the 'subsidiary' ones by finer cementing borders of the second order. Amid every field is situated a low cytoplasmic hillock with a subjacent centriole. The whole system, in geometrical abstraction, is shown in Fig. 2. The mean field area does not remain constant but progressively diminishes in the course of development as a result of our measurements carried out on the mesencephalic region of chicken embryos, and illustrated in Fig. 2. The rate of the decrease is maximal during the period of neurulation. From the third day of incubation, the absolute mean size of fields practically does not change at all. From these observations several suggestions may be put forward:

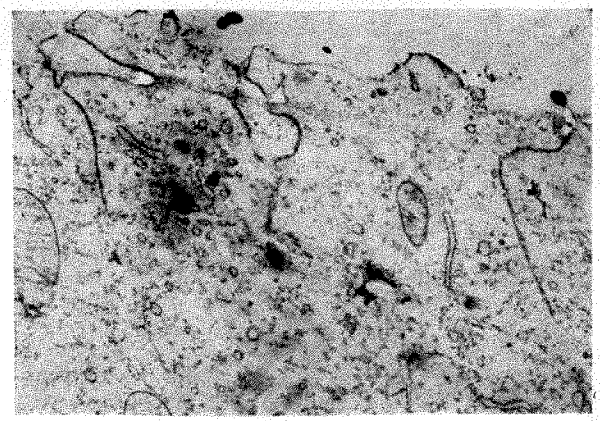


Fig. 1

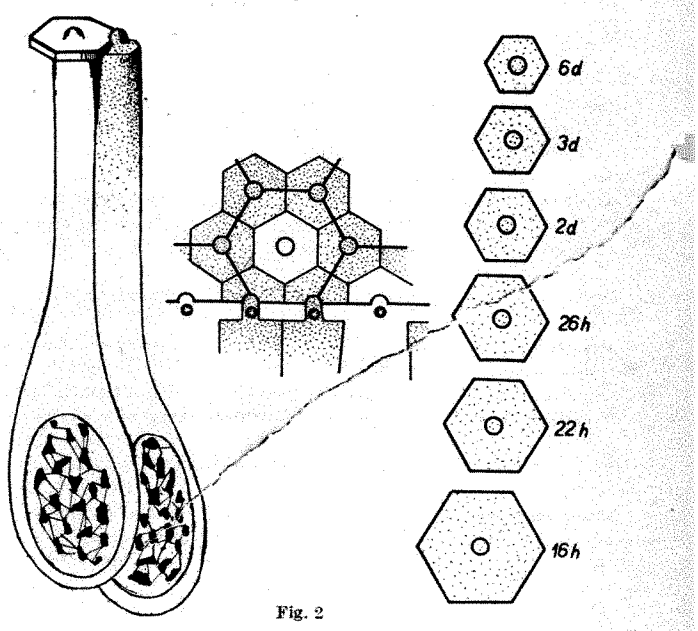


Fig. 2

(1) The internal limiting membrane system determines polarity of the neural tube proliferation structure, anchoring the cytoplasmic 'feet' of all intermitotic cells.

(2) The mean field area rapidly diminishes during neurulation, indicating the ensuing disproportion between areal extension of the neural plate and volume of cells anchored in its internal limiting membrane system. It is suggested that this process is essential for closure of the neural tube.

(3) The number of cells bound in the internal limiting membrane system is limited by the surface extension of ventricular cavities and the mean field area. With the constant field area, lateral migration of released neuroblasts, constituting the mantle zone, and surface growth of cerebrospinal fluid spaces are interdependent phenomena.

(4) At the end of proliferation phase, the 'principal' elements differentiate into ependymal cells, while the 'subsidiary' ones change either into sub-ependymal layer or migrate towards the periphery.

Experimental evidence has been obtained that regeneration of the internal limiting membrane system was limited to initial stages of neural development only. On the following stages it may be easily disintegrated by a wide range of noxious factors, resulting in distortion of proliferation structure with serious effects on later morpho-

genesis of the central nervous system[12–14]. Destruction of the internal limiting membrane system often causes and regularly accompanies the development of malformations of the central nervous system.

The possible relation between relative field area and proliferation intensity, and origin of the internal limiting membrane system in the process of induction are the matter of further investigation.

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Another Variable to be observed in Autoradiographs

Autoradiography is a technique which reveals the sites of radioactivity in an object by means of a photographic emulsion. The object is brought into close contact with the emulsion and after a suitable period of time the emulsion is processed by photographic development. In such a preparation the sites of radioactivity are indicated by the presence of silver grains in the emulsion. At present, regions of an autoradiograph are regarded as different if the densities of their silver grains, in terms of some attribute of the object, are different. It may be possible to use another property of the grains to show that regions are different. This property is the way the grains are distributed in clusters.

The silver grains in autoradiographs of biological tissues prepared for light microscopy are not all present as single grains; some are present in clusters. The clusters can be arranged in sizes according to the number of grains they contain. It is possible to decide how many grains are in a cluster by examination of its outline. The number of clusters of each size can be counted over one area if a suitable micrometer is in the ocular of the microscope. When all the grains have been counted in one area a table of the frequency with which they are present in each size of cluster can be prepared.

Consider the hypothetical situation in which an isotope is present in a layer on a microscope slide and the isotope is distributed at perfectly regular intervals in the layer. It is possible for all the tracks of emission from the isotope to be only just separate. If this were so the frequency with which grains in the autoradiograph occurred in the various size of cluster would be determined by: (1) the thickness of the layer containing the isotope; (2) the ability of the layer to filter the emission; (3) the energy spectrum of the emission; (4) the thickness of the photographic emulsion; (5) the way in which the halide crystals are packed in the emulsion; (6) the number of halide crystals per unit area of emulsion; (7) the size of the halide crystals; (8) the number of developed grains per unit area.

Now consider the hypothetical situation in which the isotope is concentrated in small particles which are distributed at regular intervals in the layer. If the number of grains per unit area is the same as before, then the tracks of the emission must overlap. The frequency of single grains is decreased and that of clusters increased in comparison to their frequencies when the distribution of the isotope is perfectly even. The extent of the effect would be determined by the amount of the isotope in each particle and the size of the particles.

In practice the variations inherent in the technique are controlled best when observations on clusters are made under the following conditions: (1) regions of the same tissue section are compared which have the same number of grains per unit area; (2) the emulsion is affected by a single emission; (3) when more than one section is used, they should be on one microscope slide, covered by a single continuous layer of emulsion, and processed together.

If these precautions are taken, any difference in the frequency with which grains occur in the various sizes of cluster will reflect a difference in the distribution of the isotope in the section. With short-range emissions, like that of tritium, the greatest change can be expected in the frequencies with which grains are present as single grains and triple grain clusters.

Observations on this variable have been made in an experiment on the incorporation of tyrosine into the growing hair of rats. The tyrosine was generally labelled with tritium. Autoradiographs prepared from 2 rats killed 30 min after injection of the labelled tyrosine show three regions of the hair cortex over which the number of grains per unit area is the same. The three regions are the matrix of the hair bulb, the cortex of the upper bulb at the level of the top of the hair papilla, and the cortex just below the level of its complete keratinization. As a different process is performed in the cells at each of these sites it is reasonable to expect that the incorporation of tyrosine might be carried out in different manners. A table of the frequencies with which the grains are present in the sizes of cluster up to triple grains was prepared for each of the three levels. This was done for three hairs in each animal. The results from all the hairs are similar to those shown in Table 1. The percentage of single grains is lower for the shaft than for the matrix and upper bulb. The percentage of grains in clusters of three is higher for the shaft than for the matrix and upper bulb. The percentage of grains in clusters of two is similar for all three levels. No difference is apparent between the frequencies of the matrix and the upper bulb.

Table 1

	Matrix	Upper bulb	Shaft
Percentage of single grains	40	45	24
Percentage of grains in cluster of 2	22	35	25
Percentage of grains in cluster of 3	17	8	37

The percentage of the total number of grains present in the size of cluster indicated in three regions of one growing hair.

The constant difference between the shaft and the other regions in the six hairs is shown to be significant at the 5 per cent level ($P = 0{\cdot}016$) by the sign test. It is concluded that the labelled tyrosine incorporated into the hair cortex is distributed more evenly in the matrix and upper bulb than in the shaft just below the level of full keratinization. The difference could be produced by the presence of the isotope in different cell components, or the presence of the isotope in the same components under different spatial conditions. A full report and discussion of this experiment on protein synthesis will be published elsewhere.

The experiment illustrates the use of the method to show that regions of a tissue have incorporated a tracer in a different way at the same time interval after its administration. On suitable material it may be possible to demonstrate changes in the same region at different time intervals by this method.

I thank Prof. Leblond for his advice on the experiment with radioactive tyrosine which was performed at McGill University during the tenure of a Wellcome research travel grant. The work was supported by a grant from the Medical Research Council of Canada.

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BIOLOGY

A Strain of *Peronospora tabacina* Pathogenic to Tobacco Lines with Resistance derived from *Nicotiana debneyi* and *N. goodspeedii*

The occurrence of strains of *Peronospora tabacina* Adam. (blue mould of tobacco) as suggested by Hill[1] has now been confirmed.

Conidia of *P. tabacina*, from susceptible varieties growing at Canberra, A.C.T., and at Myrtleford, Victoria, were used as inoculum for separate lots of plants of the susceptible variety Virginia Gold and the resistant variety SO_1 (ref. 2). After 4 or more days, depending on temperature, symptoms of the disease were obvious on leaves of the susceptible variety and extensive sporulation occurred on the following morning. Small chlorotic flecks that failed to sporulate appeared on young leaves of the resistant variety. Three or more days later, one to three sporulating lesions appeared on some of the resistant plants that had been inoculated with conidia from either source.

Conidia produced on SO_1 plants following inoculation with the pathogen from Canberra were used to inoculate alternate leaves of three SO_1 plants from which the flowers, and all except 4–5 leaves, were removed. The remaining leaves, inoculated with conidia from a susceptible variety, served as controls. Results at 8 days after inoculation are shown in Table 1. An SO_1 plant destroyed by blue mould is shown in Fig. 1.

Table 1

Plant *A*		Plant *B*		Plant *C*	
Leaf size (cm)	No. of sporing lesions	Leaf size (cm)	No. of sporing lesions	Leaf size (cm)	No. of sporing lesions
30 × 18	24	26 × 16	18	27 × 16*	0
31 × 18*	0	29 × 18*	0	34 × 20	42
35 × 18	12	32 × 19	19	41 × 20*	1
34 × 17*	0	36 × 19*	0	41 × 19	28
		38 × 18	56	43 × 21*	0

* Leaves inoculated with conidia from Virginia Gold plants. Other leaves inoculated with conidia from SO_1 plants.

In the susceptible variety, the disease appeared earlier, and its progress was more rapid, when the plants were inoculated with conidia from susceptible varieties than when inoculated with conidia from SO_1 plants. Rates of development were similar in SO_1 seedlings inoculated with conidia produced on SO_1 plants and in Virginia Gold seedlings inoculated with conidia produced on Virginia Gold plants.

Seedlings of the resistant lines *Bel*-61-12 (ref. 3) from the United States, Fixed *A*2 hybrid[4] and two lines[2] with resistance derived from *N. goodspeedii* Wheeler were all susceptible when inoculated with conidia produced on SO_1 plants.

Resistance in the SO_1 variety is derived from *Nicotiana debneyi* Domin. In another test, a line with resistance derived from *N. goodspeedii* was inoculated with conidia from a susceptible variety, and sporulating lesions indistinguishable from those reported for SO_1 were produced.

The results show that the *P. tabacina* used in these experiments is a mixture of a major and at least one minor component. When propagated on the normal susceptible commercial varieties, the major component appears to have a selective advantage over the minor component. However, propagation on genotypes resistant to the major component excludes the operation of this selective advantage and the minor component builds up rapidly.

As *P. tabacina* in Europe is considered to be of Australian origin, results obtained in Australia may be applicable in Europe. In Australia, resistant lines of tobacco have not been released for commercial production, consequently the situation in the fields will remain unchanged. However, it would appear that extensive use of resistant varieties now becoming available could be followed by a build-up of the strain to which they are susceptible.

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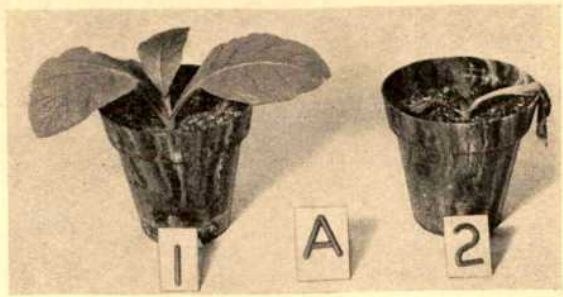


Fig. 1. Resistant variety SO_1 photographed 11 days after inoculation with conidia from: (1) Virginia Gold plants; (2) SO_1 plants

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[2–4] Seed supplied by D. Wark, C.S.I.R.O., Canberra, Australia. *Bel*-61-12 was originally from the U.S. Department of Agriculture and Fixed *A*2 hybrid from Mr. H. Lea.

Nitrogen Fixation by the Leaf-nodule Endophyte of *Psychotria bacteriophila*

Investigations of the symbioses in root-nodules of legumes and non-legumes have established that nitrogen fixation occurs in the nodular tissue[1]. Although some reports have appeared describing the leaf-nodule symbiosis in *Psychotria*[2–4] no isolate of the endophyte is at present available, and it has not been unequivocally established that nitrogen fixation is involved. We now report that the isolated endophyte fixes atmospheric nitrogen in pure culture and, apparently, in the plant as well. It is known that plants either naturally or experimentally freed of the endophyte are dwarfs[2,3]. Nitrogen fixation does not fully account for these observed effects.

The endophyte has been repeatedly isolated in pure culture from germinating seedlings on either nitrogen-free mineral agar or trypticase soya agar. Although the isolates obtained from both media were identical the N-free medium is preferable for isolation purposes, since contaminating non-fixing micro-organisms are thereby eliminated. The isolate, which we have identified as a species of *Klebsiella* by serum agglutination tests, was a Gram-negative, motile, non-acid fast, non-spore forming rod with terminal refractile granules.

Growth in nitrogen-free glucose broth was delayed for 4 or 5 days until the evolution of gas was observed. When an 80 per cent nitrogen–20 per cent helium mixture was used as the gas phase there was little delay in growth and both the growth rate and the total crop were greater than that obtained with cells grown in air.

Nitrogen fixation was demonstrated directly using a nitrogen-15 enriched gas phase. Cells were grown on nitrogen-free mineral agar for 4 days, aseptically suspended in sterile 0·1 M, *p*H 7 phosphate buffer and inoculated into 4–5 l. of the nitrogen-free culture solution in an 11-l. carboy. High-purity nitrogen was sparged through the culture for 48 h. The cells were collected aseptically by centrifugation and 10 ml. of a thick suspension of cells (≈3 mg nitrogen) in a mineral salts solution were treated with various gas mixtures as indicated in Table 1. Leaf homogenates were made by cutting small pieces of surface sterilized leaves with a razor blade followed by grinding in an all-glass TenBroeck homogenizer in 0·1 M. *p*H 7 phosphate buffer. After exposure to the labelled gas, the cells were digested by conventional Kjeldahl method using a mercury catalyst. The digests were distilled into hydrochloric acid and converted to nitrogen

Table 1. NITROGEN FIXATION BY AN ENDOPHYTE OF *Psychotria*

Exp. No.*	Tissue	Gas phase (atm.)	Hour shake 28° C	Atom % Excess ^{15}N †
1	Homogenized nodulated leaves			0·029
	Homogenized nodulated leaves, boiled	0·7 $^{15}N_2$ 0·1 O_2 0·2 He	16	0·000
	Isolated endotype, not adapted			0·039
2	Isolated endophyte, adapted	0·8 $^{15}N_2$ 0·2 He	24	0·049
3	Isolated endophyte, adapted + Na pyruvate	0·5 $^{15}N_2$ 0·5 vac.	24	0·849

* In experiment 1 the $^{15}N_2$ was 95 atom per cent excess ^{15}N; in experiments 2 and 3, 30 atom per cent excess ^{15}N.
† Average results of duplicates. Corrected for air contamination by mass 32 peak.

gas by alkaline hypobromite oxidation according to the method of Sims and Cocking[5]. Isotope ratios were determined with a Consolidated Electrodynamics model 21–130 mass spectrometer.

The results in Table 1 show that cells grown in tryptic case soya broth had a slight enrichment of nitrogen-15 which was increased by growing the cells without combined nitrogen. The addition of 10^{-3} M sodium pyruvate greatly increased the isotope incorporation in the cells. There was a small but significant fixation of nitrogen in homogenates of nodulated leaves, but this was not readily reproduced. In this experiment, oxygen was in the gas phase since it has not yet been established that fixation in the isolated endophyte occurs under anaerobic conditions. Since nodulated plants have been maintained in vermiculite supplied with nitrogen-free nutrient solution for more than 6 months without any signs of nitrogen deficiency, it is clear that nitrogen fixation undoubtedly occurs in these plants.

Although nitrogen fixation is a factor in this association, it does not explain the striking effect of the endophyte on the growth of the plants because non-nodulated dwarf plants do not recover even when supplied with various forms of combined nitrogen or when potted in soil. Since the dwarfing effects are modified but not completely reversed by the application of gibberellic acid, it is likely that the main effect of the association involved the production of plant growth substance(s) as yet unidentified.

So far as we are aware, this is the first demonstration of a micro-organism in association with a higher plant in which the endophyte fixes atmospheric nitrogen in pure culture and presumably also in the plant. A more detailed account of these studies will appear elsewhere.

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Note added in proof. Cell-free extracts of the *Klebsiella* convert pyruvate to hydrogen, carbon dioxide and acetyl phosphate. Electron paramagnetic resonance spectra of these extracts show strong signals for manganese (gauss 2·01) and for non-hæm iron (gauss 1·94) and are similar to the spectra observed for extracts of *Clostridium pasteurianum* that fix nitrogen gas (Nicholas, D. J. D., Wilson, P. W., Heinen, H., Palmer, G., and Beinert, H., *Nature*, **196**, 433; 1962).

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Tissue Cultures of Juvenile and Adult Specimens of Ivy

Robbins[1] and Wareing[2] have suggested that chemical or cellular differences are the basis of the striking dimorphism of the juvenile and adult growth phases of some plants. The following experiments support this view and show that differences can persist through repeated sub-cultures of tissues *in vitro*.

The English ivy, *Hedera helix* Linn., exhibits marked dimorphism in the different growth-phases. The juvenile forms are vines with flattened stems, with dorsiventral leaf arrangement, with palmately lobed leaves and aerial roots. Red pigments are also common in stems and petioles. On the other hand, the mature or adult form produces a shrubby, upright growth with entire leaves and the stems rarely produce roots.

The following experiments present comparisons of tissue cultures grown both from adult specimens and from juvenile reversion shoots on the same plants. These were occasionally observed to occur spontaneously at the bases of adult plants by Stoutemyer and Britt[3].

Stems approximately 0·5 cm in diameter were washed and the epidermis stripped off. Pieces of tissue approximately 2 mm² and weighing 1–2 mg were removed with a sterile scalpel and the cambial side laid on agar slants. Cultures were sometimes started from cross-sections of thin stems. The cultures were grown in diffused light or in darkness at temperatures of from 20° to 32° C. A temperature of 27° C permitted excellent growth of all types of tissue. At 24° C or lower, sub-cultures were difficult to maintain except when taken from young seedlings. Sub-cultures likewise deteriorated at 32° C.

Extensive trials of numerous culture media led to successful sub-cultures of *H. helix*. The two best media have been White's[4] medium or a modification based on a suggestion of Shantz and Steward[5] in which potassium nitrate was reduced to 47·0 mg per litre and 60 mg of ammonium chloride was added. The latter was used in the following experiments. With both formulations, the following supplements were added to each litre: (*A*) fresh coconut milk was filtered through a No. 1 Whatman paper, autoclaved at 15 lb. for 30 min and stored in the refrigerator. It was re-filtered and 100 ml. was added before the prepared medium was autoclaved; (*B*) enzymatic casein hydrolysate powder, 200 mg, and inositol, 100 mg. Requirement of the latter has not been established; (*C*) naphthalene acetic acid, 1·0 mg.

6 g Difco 'Bacto Agar' were added to each litre of the nutrient, which was prepared with glass-distilled water. The medium was adjusted to *p*H 5·8 with 0·1 N sodium hydroxide before autoclaving. 4- or 6-dram flint glass vials with plastic caps were most convenient for the sub-cultures.

The appearance of typical adult and juvenile tissue cultures of *Hedera helix* after 24 weeks from the initial inoculation is shown in Fig. 1. Sub-cultures were stable and consistent in performance if transfers were made at monthly intervals, and were used for quantitative comparisons of growth-rates. The behaviour of sub-cultures of juvenile and mature stems from the same

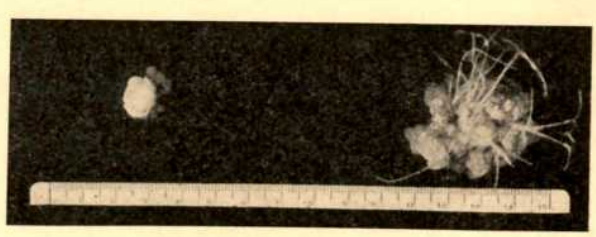


Fig. 1. Growth of tissue culture from adult and juvenile stems from same plant of *Hedera helix* showing appearance after 24 weeks. Left, mature tissue, weight 0·588 g; right, juvenile tissue, weight 6·780 g with abundant root formation

Table 1. Growth of Tissue Cultures from English Ivy Stems expressed as Ratios of Final to Initial Weights. All Means are based on 100 Cultures, Except where Stated

Order of transfer	Source of tissue	Temperature 24° C	Temperature 27° C
First sub-culture	Young seedling	(30) 11·75	(10) 16·19
	Juvenile reversion	4·01 ± 0·31	8·20 ± 0·72
	Adult	1·44 ± 0·17	4·06 ± 0·50
Second sub-culture	Young seedling	—	10·96 ± 0·60
	Juvenile reversion	—	7·69 ± 0·46
	Adult	—	5·50 ± 0·36

plant is shown in Table 1, together with a similar culture taken from a young open pollinated seedling derived from the same plant. The weighings of the tissues were made on a small torsion balance using sterile techniques. Standard errors were calculated from 10 values each representing the mean of 10 cultures.

The higher temperature was preferable, particularly with cultures of mature tissues. The exceptionally high growth-rate of the cultures from the very young seedling should also be noted.

These results explain those reported by Privat[6], who, using a similar culture medium, was not able to establish sub-cultures from ordinary juvenile or mature stems of ivy, although the original cultures grew well. However, when very young seedlings were used, tissues from these stems could be sub-cultured successfully. Apparently the range of temperatures (22°–25° C) was too low to permit success with any but the latter type of tissue.

Sub-cultures from juvenile reversion and adult stems of *Hedera helix* transferred through nine generations at monthly intervals continued to show differential rates of growth. Typical responses are shown in Table 2.

Table 2. Growth of Tissue Sub-cultures from English Ivy Stems expressed as Ratios of Final to Initial Weights (Means of 50 Cultures at 27° C)

Order of transfer	Juvenile reversion	Adult
Seventh sub-culture	5·6 ± 0·4	4·4 ± 0·3
Eighth sub-culture	4·8 ± 0·4	3·9 ± 0·5
Ninth sub-culture	6·8 ± 0·5	5·7 ± 0·4

Experience with more than 13,000 sub-cultures during the past year has shown that those from juvenile stems maintained more than 20 per cent higher growth-rates than those from adult type tissue. Another marked difference was the considerably greater amount of root formation in the cultures taken from juvenile stems.

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Systemic or Semi-systemic Infection of Wheat with *Puccinia graminis tritici*

Eriksson[1] proposed the mycoplasm theory according to which rusts could persist in wheat seeds and thus produce systemic infection of wheat. Hungerford[3] and Mehta[4], however, could not substantiate the theory of mycoplasm.

Apparent systemic or semi-systemic infection in wheat seedlings with *Puccinia graminis tritici* was produced in test-tubes using the technique of inoculation described by me[2]. The variety of wheat used in these investigations was a club type from Afghanistan, locally known as 'Lucchak', and the stem rust race used for this work was a biotype of race 21 isolated from the country. This biotype differed from the key race 21 in that it produced a 1+ type of infection on Vernal, whereas the key race produces an O type of infection on that differential variety.

In an experiment with fifty seedlings, uredospore suspension of the stem rust fungus was injected with a hypodermic needle No. 18 near the growing point of 60–72-h. old wheat seedlings on May 22, 1962. All the seedlings showed infection with stem rust on the first leaf on May 29–31, 1962. However, in two of the seedlings in which rust pustules appeared on May 29, stem rust appeared on the second leaf on June 6, 1962, on the third leaf on June 11, and on the first tiller on June 14. Similar infections were also obtained in 3–4 per cent of the inoculated seedlings in test-tube experiments carried out in June 1962. This, therefore, shows that in those seedlings the infection with stem rust was of a systemic or semi-systemic type.

In another experiment seedlings were raised from surface disinfected seeds, and the inoculated plants when they showed infection on the first and second leaves were pulled out of the agar in the tube. The leaves and the rudimentary leaves enclosing the growing point were removed right from the base of the growing point so far as possible and the growing point tissue crushed on a slide. The undifferentiated tissue material on staining with cotton blue revealed the presence of hyphæ and uredospores of the rust fungus. It would, therefore, be seen that if stem rust of wheat could be established in the growing point of wheat seedlings, subsequent unfolding leaves would show infection with rust fungus.

Systemic infection of a mature wheat plant with stem rust was observed by me under field conditions in wheat variety Purdue 5759*B*4–5–3 listed as entry No. 93 of the International winter wheat rust nursery of 1962, seeds of which were received from Dr. W. Q. Loegering, of the U.S. Department of Agriculture. One wheat plant of this variety with 7 tillers showed 40 per cent rust infection on all the leaves and heads on May 15, 1962, when other varieties and the local varieties did not show a trace of stem rust infection. Stem rust must have appeared on this variety at least 10 days earlier. Stem rust infection was first noted in traces on the local variety and some other varieties on May 29, 1962.

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Effect of Raw Wheat Germ on Nutrition of the Chicken

During our investigations on the nutritional aspects of human sprue designed to find an experimental animal which might help in our basic understanding of this disease, we noted reports by Creek[1] that wheat germ had a deleterious effect on growth of chickens and that this was associated with an apparent increase in fat content of fæcal droppings. After further examination of his experiments[2] and after seeing the degree of 'contamination' of the raw wheat germ with flour as it comes from the mills, we felt that his results could possibly be accounted for by the adverse effect of gluten in the adherent flour.

Early experiments confirmed Creek's findings of poor growth and the fæcal fat[3] was as high as 20 per cent of fat intake in the wheat germ groups. When gluten was given as a substitute for the corn in the diet, however, we found that the birds fed gluten grew slightly better than controls ($P < 0{\cdot}0125$) and there was no difference in fæcal fat content.

Table 1

	Day 1		Day 28		Day 7–14
	No. of birds	Mean wt. (g and S.E.)	No. of birds*	Mean wt. (g and S.E.)	Fæcal fat as % fat intake
Basic diet + raw wheat germ (1)	36	38·3 ± 0·47	33	461·0 ± 9·8	24·6
Basic diet + raw wheat germ (2)	36	38·3 ± 0·52	34	487·6 ± 12·6	20·3
Basic diet + corn (controls)	36	38·3 ± 0·48	33	567·0 ± 12·5	7·9

* 8 birds died.

In further experiments (Table 1) carried out with the co-operation of the Poultry Division of Michigan State University, we re-confirmed these findings. Three replicates of 12 Arbor Acre males were used for each diet. The controls were fed a basal corn diet, the test groups had a similar diet, but raw wheat germ (40 per cent) was substituted for corn. The raw wheat germ was from two different sources. In similar experiments we were able to show that by autoclaving the raw wheat germ before adding it to the diet the effects were no longer evident (Table 2). All diets contained 15 per cent fat.

Table 2

	Day 1		Day 21		Day 7–14
	No. of birds	Mean wt. (g and S.E.)	No. of birds*	Mean wt. (g and S.E.)	Fæcal fat as % fat intake
Basic diet + autoclaved wheat germ	36	34·9 ± 0·26	28	220·8 ± 6·8	9·72
Basic diet + raw wheat germ	36	35·5 ± 0·26	31	165·8 ± 5·4	21·10
Basic diet + corn (controls)	36	35·4 ± 0·23	33	199·8 ± 3·7	9·73

* 16 birds died.

Creek believes that these results might be caused by a thermolabile toxin in the raw wheat germ which is destroyed on heating[4].

From close observations during all our experiments where raw wheat germ was part of the diet it became obvious that during and after feeding the food formed a paste on the beaks in combination with salivary mucus and water and that these birds ate correspondingly less and hence grew less well. We also repeatedly noted the way the birds cleaned their beaks on the wires of the flooring, thus adding sufficient food fat to the droppings to account for the difference in fæcal fat figures between control and wheat germ groups.

In one experiment where the wheat germ formed 55 per cent of the diet, the stickiness was so apparent and the depression of growth so great ($t = 12{\cdot}8$, $P < 0{\cdot}0005$) that the birds died of starvation. Autoclaving the raw wheat germ alters its consistency markedly so that it becomes drier and does not form a sticky paste; the birds eating it without difficulty.

In these experiments there were no macroscopic, microscopic or electron microscopic changes in the bowel mucosa such as those seen in human cœliac-sprue. We have thus been able to confirm Creek's original work, but think our observations offer a simple and reasonable alternative explanation for the effects of raw wheat germ and we believe can save a fruitless search for a non-existent toxin.

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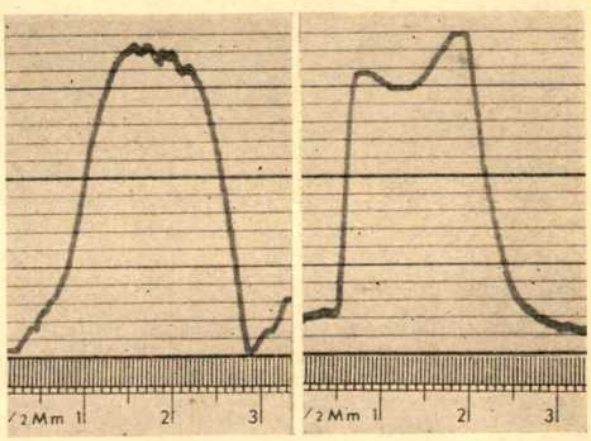
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Use of Ear Chamber with Uterine Muscle Transplant to differentiate Myometrial and Vascular Activity

The Sandison[1,2]–Clark[3] rabbit ear chamber technique has been used extensively for the study of mammalian tissues *in vivo*. The type of ear chamber used in the present investigation[4] was constructed mainly of 'Perspex' as in the Sandison–Clark chamber but has been adapted for removal of coverslip for implantation of grafts after the method of Williams and Roberts[5]. It has been shown that within such grafts the vasculature of the donor organ persists[6].

It occurred to us (J. D. M. and W. J. C.) that prolonged investigations of uterine action could be made by this method, for in the past it has been difficult by conventional methods to study vascular activity in relation to myometrial activity in the uterus. The possibility that in some instances the uterine contractility might be secondary to vasoconstriction has been suggested because of the delayed occurrence of contractions produced by ergotamine as compared with oxytocin or ergometrine[7].

An ear chamber was therefore inserted in a non-pregnant female rabbit's ear under sterile conditions. The chamber was observed frequently following its insertion, and it was noted that new vessels gradually filled the observation space. After complete healing, minute grafts of uterine muscle were placed in the chamber. Following re-establishment of circulation in the grafts the animal was mated, and progressive hypertrophy of the graft was observed



A *B*

Fig. 1. Photoelectric record showing vasoconstriction (*A*) and myometrial contraction (*B*) of uterine muscle graft

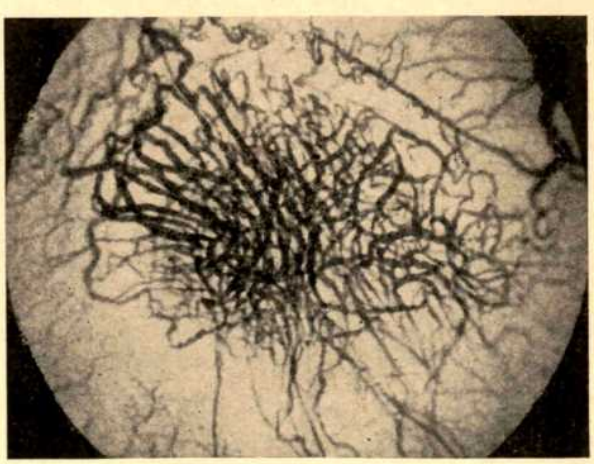


Fig. 2. Uterine muscle graft (normal appearance, × *c.* 18)

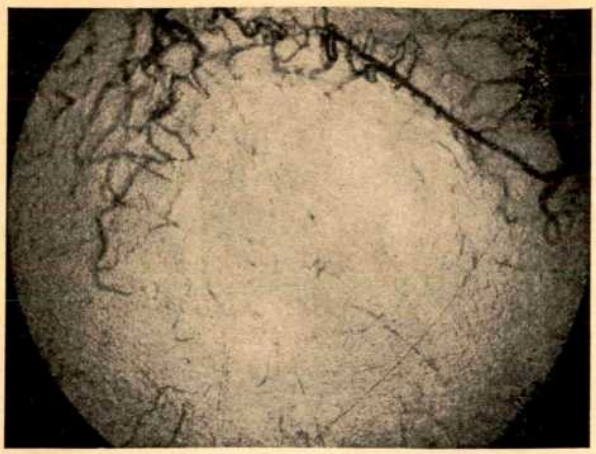


Fig. 3. Uterine muscle graft (vasoconstriction, × *c.* 18)

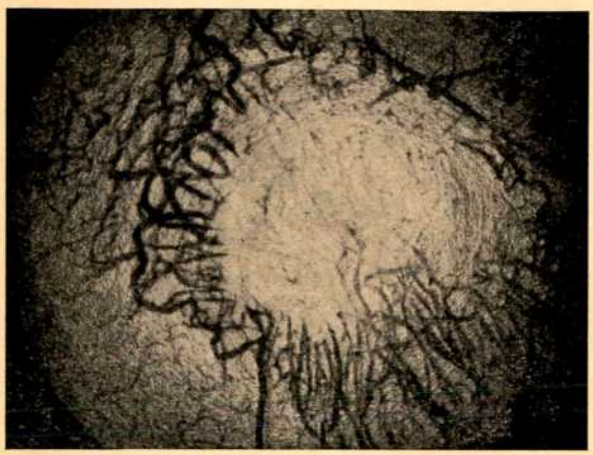


Fig. 4. Uterine muscle graft (myometrial contraction, × *c.* 18)

during pregnancy and involution was seen to occur *post-partum*.

On the administration of drugs or stimuli these grafts showed two types of response—vasomotor and myometrial contractions. Either led to blanching of the field. However, myometrial contractions produced a movement of the muscle into a spherical shape and a reduction in size of the entire graft. Photographic records were made of the preparation and observations were made on the unanæsthetized animal.

A simplified method of observation suggested by Dr. C. N. Smyth was to record the changes in transmission of light through the chamber as the vessels dilated or constricted. Using infra-red reflecting and green filters and a cadmium sulphide photo-resistive cell placed above the microscope optical cross-over outside the eyepiece, two types of tracing were obtained which could be correlated with simultaneous optical observation of the responses. A binocular microscope with the recorder attached to one eyepiece made simultaneous observation possible.

In 'pure' vasoconstriction the rate of light transmission change was slower than in 'pure' myometrial response (see Figs. 1*A* and 1*B*, which correspond to the changes between Figs. 2 and 3 and 2 and 4, respectively). As a preliminary investigation the effect on the graft of loud noises, oxytocin, ergometrine, ergotamine, serotonin, adrenaline and noradrenaline have been examined before, during, and after pregnancy.

The tentative conclusions of this first investigation were that this was a useful method of observing the mode of action of oxytocic drugs. The uterine graft became progressively more sensitive to oxytocin as pregnancy advanced and its vasoconstrictor responses could be abolished by chlorpromazine, enabling study of the muscle action alone.

The work is being continued by one of us (C. A. M.) with the help of a grant-in-aid from the Fotheringham Research Fund of the Australian Regional Council of the Royal College of Obstetricians and Gynæcologists and by another (W. J. C.), who is a Research Fellow of the National Heart Foundation of Australia. We thank Mr. R. Fleay for making the electronic recording apparatus.

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Temperature and the Rate of Ageing in Poikilotherms

Two theories, which I shall call the 'rate of living' and the 'threshold' theories, have been suggested to explain the fact that poikilothermous animals live longer at low than at high temperatures. In this communication these theories are explained, and an experiment described which decisively contradicts the rate of living theory.

The rate of living theory (Fig. 1*A*), put forward by Pearl[1], suggests that the greater longevity at low temperatures arises because the rates of ageing processes, like those of chemical reactions, are greater at higher temperatures. This theory has recently been reformulated by Shaw and Bercaw[2], who also report some experimental results on *Drosophila* which they believe to support it. By analogy with chemical reactions, they assume:

$$dy/dt = -ky^n \qquad (1)$$

where y is a 'longevity substance', such that when y falls to a final value y^f the animal dies; k is a constant which depends on the temperature. They are at some pains to show that if an individual is kept for a time x at a low temperature and is then transferred to a higher one, its age at death, l_3, is given by

$$l_3 = l_2 + x(1 - l_2/l_1) \qquad (2)$$

where l_1, l_2 are the life-spans at the low and high temperatures respectively. From this they conclude that if in fact l_3 is found to be a linear function of x, this will support the idea that ageing is due to a simple temperature-dependent process of the kind described in equation (1). They describe an experiment in which male *D. melanogaster* were kept for varying periods at 25° C and then transferred to 29° C. In this experiment they found l_3 to be a linear function of x.

The first point to be made is that equation (2) follows immediately from much more general conditions than those assumed by Shaw and Bercaw. All that is required is to suppose:

(1) Individuals kept at two different temperatures pass through an identical series of states, $a_0\ a_1 \ldots a_r \ldots a_n$, and die when they reach the terminal state a_n; and (2) the time taken to pass from any one state to the next, $a_r \rightarrow a_{r+1}$, at the higher temperature bears a constant ratio to the equivalent time at the lower temperature; if so, clearly the ratio is l_2/l_1.

With these assumptions, suppose that an individual spends a time x at the lower temperature and (l_3-x) at the higher, dying at age l_3. It does not matter how these periods are broken up; for example, the individual might live at the higher temperature first, or spend alternate days at the high and low temperature. Then:

$$l_1 = x + (l_3 - x)l_1/l_2$$

which is the same as equation (2). Thus observational agreement with this equation would not support any particular equation describing the ageing process, but only the very general conditions set out here. By the 'rate of living' theory, therefore, I mean the theory that these conditions are at least approximately satisfied. Such a theory can be tested by an observational check of equation (2).

Unfortunately, Shaw and Bercaw have not established that their observations agree with this equation. They did not measure l_1, the life-span at 25° C, so it is impossible to tell whether the graph of l_3 against x does or does not have the expected slope, although from other work on *D. melanogaster* their slope does seem to be about right.

Finally, they appear to be unaware that a number of experiments[3–5] had previously been published in which the rate of living theory was tested by keeping *D. subobscura* for varying periods at different temperatures. These experiments showed:

(1) Flies kept first at a high temperature and then transferred to a lower one die at the same age as do flies kept continuously at the lower temperature (this statement is only strictly true of males and ovariless females, since the life-span of normal mated females is increased by a preliminary period at a high temperature).

(2) If flies are kept first at a low temperature and are then transferred at various ages to a higher one, then initially their expectation of life at the high temperature is reduced by one day for every day spent at the lower temperature, until the expectation of life at the high temperature has fallen to approximately half its initial value in young flies. Subsequently the expectation of life at the high temperature does not alter substantially with increasing age at the low temperature.

These results are clearly incompatible with the rate of living theory. To explain them, Clarke and Maynard Smith[4,5] put forward what I shall call the threshold theory (Fig. 1*B*). The essential feature of this theory is that if at a lower temperature an individual starts to die when it reaches a state a_n, then at a higher temperature a similar individual will start to die at an earlier state a_r. In more detail, it is assumed:

(1) The life-span is divided into two phases, 'ageing' and 'dying'.

(2) The rate of the ageing process is approximately independent of temperature. It continues until the 'vitality' of the individual has fallen to a threshold-level below which the individual can no longer maintain itself at the temperature at which it is living. This threshold is higher at higher temperatures, so that individuals which can maintain themselves at a low temperature may be unable to do so at some higher temperature.

(3) In individuals whose vitality has fallen below the threshold appropriate to the temperature at which they are living, a second process, 'dying', commences and terminates in death. This dying process is temperature-dependent in its rate, and, provided it is not allowed to terminate in death, can be reversed at lower temperatures.

The evidence supporting these assumptions has previously been published. But the results reported by Shaw and Bercaw, although they may not support the rate of living theory, are incompatible with our finding that the rate of ageing is approximately independent of temperature. Although it is conceivable that there are different mechanisms of ageing in the two species of *Drosophila*, this does not seem likely. It therefore seemed

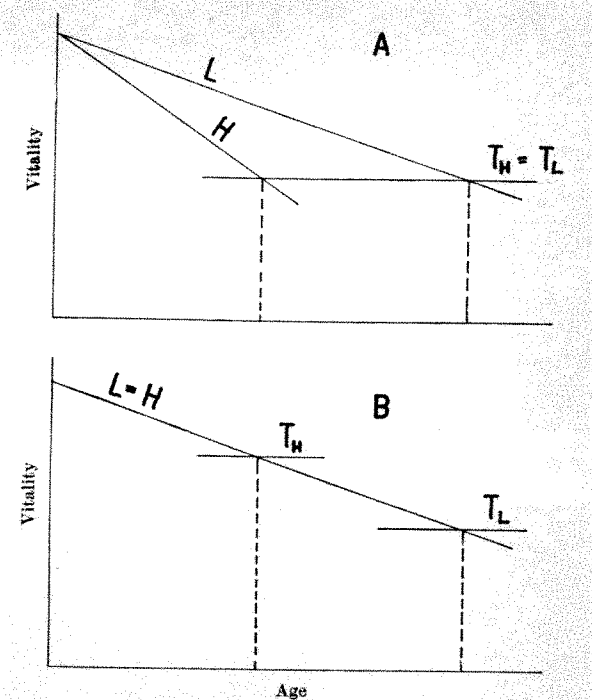


Fig. 1. *A*, the 'rate of living' and *B*, the 'threshold' theories of ageing. In both theories ageing is supposed to lead to a decline in 'vitality' with chronological age, until a threshold is reached, when the individual dies, or starts to die. *L,H* represent the decline in vitality at low and high temperatures respectively; T_H, T_L represent the threshold-levels at low and high temperatures respectively

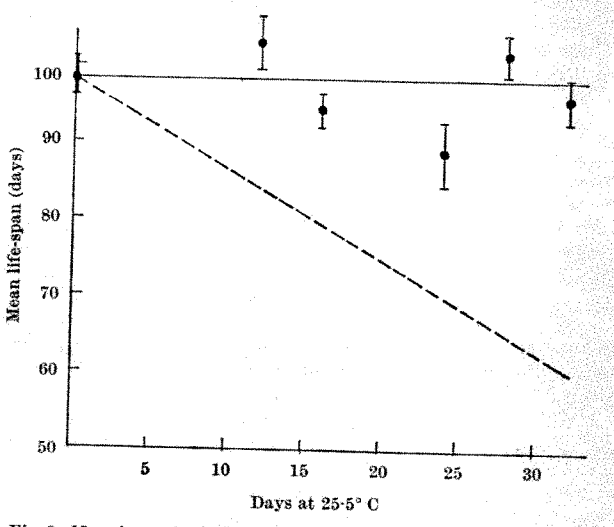


Fig. 2. Mean longevity in days of male *D. subobscura* kept first at 25·5° C and then at 20° C, as a function of time spent at 25·5° C. ●, Observed values; full line, expectation according to threshold theory; broken line, expectation according to rate of living theory

desirable to perform a further experiment to decide between the two theories; this seemed all the more necessary because, in the absence of evidence to the contrary, it is only natural to assume that the rates of ageing processes would be temperature-dependent. At the same time an opportunity has been taken to meet a criticism made by Strehler[6] of our earlier experiments. Strehler argues that in those experiments in which flies were kept first at a high temperature and then transferred to a lower one, the initial temperature, 30·5° C, was so high that the flies were dying of temperature stress and not of ageing: this criticism does not apply to the experiments in which flies were kept first at a low temperature

and then transferred to a high one, since in some of these experiments 26° C was used as the higher temperature.

An experiment has therefore been performed in which flies were kept first at 25·5° C and then transferred to 20° C. The species can reproduce at both these temperatures, although the former is close to the maximum. F_1 hybrid males between the *B* and *K* inbred lines of *D. subobscura* were raised at 20° C, and afterwards kept, 2 males per vial, in 3 in. × 1 in. diameter shell vials containing food medium. The flies were transferred to fresh food vials every two days, since we have found that this procedure gives more repeatable results than our earlier practice of 4-daily transfers. Flies were kept for four days after emergence at 20° C and then, with the exception of a control group, were transferred to 25·5° C. A group of 110 males which were kept continuously at 25·5° C until they died survived for a mean of 43·1 days at that temperature. Other groups, each consisting of 20 flies, were kept at 25·5° C for 12, 16, 24, 28 and 32 days, and were then transferred to 20° C and kept at that temperature until they died. The mean ages at death, with standard errors, of each of these groups are plotted in Fig. 2 against days spent at 25·5° C. Also shown in Fig. 2 are the expectations according to:

(1) The threshold theory, assuming the rate of ageing to be independent of temperature; on these assumptions, all groups returned to 20° C should die at the same chronological age as the controls, regardless of how long they had previously spent at 25·5° C.

(2) The rate of living theory, that is, equation (2), assuming a mean longevity of 43·1 days at 25·5° C.

The results of the experiment contradict the rate of living theory, and are fully consistent with the threshold theory suggested in earlier papers.

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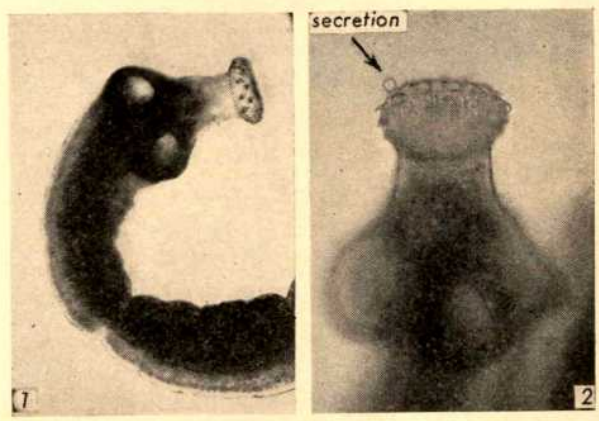
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Secretory Activity by the Scolex of *Echinococcus granulosus in vitro*

During recent work on the *in vitro* cultivation of the hydatid organism, *Echinococcus granulosus*, observations were made on living 35-day-old strobila freshly removed from the intestine of dogs. Strobila were examined as quickly as possible after autopsy on a warm stage in hydatid fluid or Parker's 199 medium. The scolex showed a very marked activity, one phase of which was characterized by the rostellum region being expanded to its limit—a process resulting in the hooks being raised to form an anterior disk (Fig. 1). This phase may be only momentarily observed for it rapidly changes to one in which the hooks are lowered. This represented a picture of the behaviour pattern of the *Echinococcus* scolex not evident from text-book illustrations, which appear to be based on fixed specimens.

Continual high-power *in vitro* observations of the living scolex has revealed a previously undescribed phenomenon—the secretion of small viscid droplets into the medium from the hook region (Fig. 2). This phenomenon was peculiarly difficult to observe due to the activity of the scolex, but once seen was found repeatedly in many specimens from different dog hosts; the precise site of secretion was not observed. The phenomenon has not been found in strobila earlier than 32-days' development in the dog, and the appearance of the secretion may be related to the metabolism of the later stages of maturation only. Detailed cytological examination of the rostellar region of the scolex[1] has revealed the presence of a group of cells, lying just beneath the anterior tip of the rostellum apparently not figured in early morphological accounts. These cells contained droplets from which the secretions have clearly originated. The existence of gland cells in the scolices of some cestodes[2] has been described; but active secretion from the cestode scolex—nor indeed from any other region of a cestode—does not appear to have been observed or reported. It may prove to be a common phenomenon and may have implications necessitating a revision of our concept of cestode physiology.



Figs. 1 and 2. (1) Scolex of living *Echinococcus granulosus* freshly removed from the dog intestine showing characteristic expansion of rostellar region (electronic flash microphotograph); (2) anterior tip of rostellum of living *Echinococcus granulosus* showing release of a secretion droplet (electronic flash microphotograph)

The nature and possible function of this secretion from *E. granulosus* can be only a matter of speculation at this stage and these questions are being further investigated. In view of the close contact of the scolex with the intestinal mucosa[1], the secretion may be important from the immunological point of view, and for this reason, especially, warrants further investigation.

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VETERINARY SCIENCE

Nicotinamide Adenine Nucleotides in Ovine Liver

A knowledge of the levels of nicotinamide adenine nucleotide coenzymes in ruminant liver may be of value in understanding the ætiology of bovine ketosis and ovine pregnancy toxæmia. In particular, the possibility that a deficiency of the reduced form of the dinucleotide phosphate ($NADPH_2$) may be associated with the hyperketonæmia which is characteristic of these metabolic disorders has been suggested by several workers[1–4]. Because $NADPH_2$ is an essential cofactor for fatty acid biosynthesis[5] a deficiency of it would result in impaired lipogenesis and consequently an increased ketogenesis[6,7].

There are few reports in the literature, if any, of the measurement of nucleotide coenzyme-levels in biopsy

samples of ovine liver or of the rates of hepatic $NADPH_2$ formation. The purpose of this communication is, therefore, two-fold: (1) to offer data which indicate the similarity in coenzyme levels of pregnant ewe and rat livers, the rat being taken as a typical non-ruminant; (2) to show that the hyperketonæmia of undernourished ewes is not apparently accompanied by a significantly reduced capacity to form $NADPH_2$ compared with well-nourished control animals.

A slight modification of the enzyme-spectrophotometric method of Holzer *et al.*[8] was used for the assay of either oxidized or reduced forms of the coenzymes in liver biopsy specimens taken from normal pregnant ewes. Results so obtained showed wide variations, and recently it has been found more convenient to use the sensitive and reproducible enzyme-colorimetric method of Slater and Sawyer[9] for the assay of both oxidized and reduced forms in a single biopsy specimen weighing approximately 400 mg. Average nucleotide coenzyme-levels for pregnant ewes estimated by the Holzer method are given in Table 1. The same relative distribution of oxidized and reduced forms was observed as in rat liver, but levels of NAD and NADP were higher. The concentration of $NADPH_2$ was never found to be very low so that apparently the ewe is not especially prone to a deficiency of this cofactor later in pregnancy. Assays made on livers of two similar post-parturient ewes using the enzyme-colorimetric method are given for comparison. Although total amounts of nucleotide so estimated were comparable with values obtained for pregnant ewes by the Holzer method, $NADPH_2$-levels were somewhat elevated. Results obtained for the rat using the same method compared well with Dr. T. F. Slater's own values for his laboratory strain of rat.

Table 1. NUCLEOTIDE COENZYME-LEVELS IN OVINE AND RAT LIVERS

Species	Physiological status	Mean nucleotide content (μg/g fresh liver) (Range of values) (No. of animals)				Method of assay*
		NAD	$NADH_2$	NADP	$NADPH_2$	
Ewe	3–4 wks. pre-partum	482 (258–628) (6)	51 (35–106) (4)	60 (33–113) (3)	183 (162–347) (4)	*H*
Ewe	0–2 wks. pre-partum	361 (295–452) (3)	51 (23–65) (3)	44 (23–65) (3)	181 (156–205) (3)	*H*
Ewe	Lactating (i)	252	114	70	294	*S*
	(ii)	334	92	49	298	*S*
Rat	Starved overnight	318 (303–347) (5)	72 (33–103) (4)	16 (7–22) (5)	168 (88–262) (4)	*H*
Rat	Starved overnight	408 (334–462) (4)	100 (95–104) (2)	31 (20–42) (2)	241 (220–262) (2)	*S*
Rat	—†	400	85	45	200	*S*

* Methods of assay: *H* = Holzer *et al.* (1958); *S* = Slater and Sawyer (1962).
† Slater, T. F. (private communication). Mean values.

Table 2. $NADPH_2$-FORMING ENZYME REACTIONS IN THE LIVER OF THE PREGNANT EWE

Enzyme activities were assayed spectrophotometrically in the supernatant fraction obtained from a 1 : 8 homogenate in 0·15 M potassium chloride by centrifuging at 4,000*g* for 1 h at 0° C. 1 unit of enzyme activity = quantity of enzyme producing 10 mμmoles $NADPH_2$/min at 20° C.

Enzyme assayed†	Well-nourished (control) ewes		Under-nourished (hyperketonæmic) ewes*		Statistical significance of difference between groups
	Days pre-partum	Activity (units/g fresh wt.) (No. of biopsies)	Days pre-partum	Activity (units/g fresh wt.) (No. of biopsies)	
ICD	13·6 ± 2·26	389 ± 22·1 (9)	13·2 ± 0·81	404·6 ± 36·2 (9)	None
G6PD	12·4 ± 2·33	17·1 ± 1·60 (10)	13·2 ± 0·81	18·2 ± 1·80 (10)	None
6PGD	12·4 ± 2·33	18·5 ± 5·12 (10)	13·2 ± 0·81	15·7 ± 8·28 (9)	None
		[Mean values ± *S.E.M.*]			

* Mean plasma concentrations at 13 days pre-partum:
Glucose — Controls 62 m/100 ml.; Hyperketonæmic 48 m/100 ml.
NEFA (non-esterified fatty acids) — Controls 990 μequiv./l.; Hyperketonæmic 1,700 μequiv./l.
Ketone bodies at levels of more than 10 mg 'acetone'/100 ml. were detected in all undernourished ewes.

† Isocitrate dehydrogenase (ICD) by the method of Ciotti and Kaplan 1951) (ref. 10). Glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) by the method of Glock and McLean 1958) (ref. 11).

No values have so far been obtained for liver nucleotide concentrations in pregnancy toxæmia but it is hoped to do so using the Slater and Sawyer method when cases become available to us. Observations, to be published in detail elsewhere, on levels of $NADPH_2$-generating enzymes in liver homogenates obtained from hyperketonæmic pregnant ewes (not exhibiting clinical symptoms) are, meanwhile, of interest. These results indicate that there is probably no serious lack of $NADPH_2$ in hyperketonæmia as judged by the *in vitro* rate of formation of this cofactor in the *iso*citrate dehydrogenase, the glucose-6-phosphate dehydrogenase and the 6-phosphogluconate dehydrogenase reactions measured under optimal conditions in livers of these animals (Table 2). Until the direct estimation of nucleotide concentrations has been made in liver samples obtained from clinical cases of pregnancy toxæmia it is impossible to say for certain whether or not $NADPH_2$-deficiency is an important feature of this metabolic derangement, but the experimental results presented here show that $NADPH_2$-levels are not normally very low late in pregnancy. Moreover, in hyperketonæmia induced in the normal pregnant ewe by dietary restriction, the capacity of the liver to form $NADPH_2$ is not significantly diminished.

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Effects of Œstrogen on Fertility in Ewes

IN a recent experiment[1] daily injection of 128 and 32 μg of stilbœstrol, prior to, and during, mating reduced fertility in Merino ewes almost to zero. Some circumstantial evidence also suggested that even 8 μg might result in reduced conception percentages and proportions of multiple births. Since intakes of plant œstrogens greater than the equivalent of 8 μg stilbœstrol (assayed by uterine weight in ovariectomized ewes) could be common in sheep-grazing pastures containing subterranean clover or red clover[2,3], it seemed to us important to assay the effects of such doses. Accordingly three groups of about 90 ewes were injected for six weeks, thrice weekly, with 1 ml. peanut oil containing the equivalent of 0, 8 μg and 16 μg stilbœstrol dipropionate (May and Baker) daily. Injections commenced on February 27, 1963. Rams were introduced on March 20 for three weeks. Vasectomized rams were kept with the ewes for a further three weeks. Matings were recorded with the aid of SIRE-SINE harness and crayon.

The ovaries and uteri were examined by laparotomy of those ewes which were found non-pregnant by vasectomized rams, and after slaughter in those judged pregnant. The results are summarized in Table 1.

Table 1. Reproductive Status of Ewes treated

	Level of œstrogen per day		
	0	8 μg	16 μg
Ewes alive	92	91	87*
Not mated	6	7	10
No. services	88	94	85
% conceptions	75	59	42
No. non-pregnant	26	36	51
% Non-pregnant, non-return	4	25	41

* 2 ewes died during the experiment.

Substantial differences between treatments were found in conception percentage and in the percentage of ewes which, although not pregnant, did not return to service within 3 or more weeks after mating.

Some further effects of stilbœstrol were evident from the examination of embryos and ovaries of the pregnant ewes, as shown in Table 2.

Table 2. Multiple Pregnancies and Corpora Lutea in Pregnant Ewes

	Level of œstrogen per day		
	0	8 μg	16 μg
No. pregnant	66	55	37
No. twin pairs	12	5	0
Single corpora lutea	47	50*	35*
Two corpora lutea	19†	5	1
Ovulation-rate	1·29	1·09	1·03
% pregnant, excluding those with two corpora lutea	64	59	41

* One of these scored as zero corpora lutea.
† One of these scored as single corpus luteum.

From these results it is evident that stilbœstrol, administered during mating even at relatively low doses, decreased conception percentages, and affected the œstrous cycle so that many ewes, although not pregnant, did not exhibit œstrus. Moreover, the ovulation rate was considerably reduced, resulting in decreased multiple pregnancies.

The decreased conception percentage was not a result of reduced probabilities of at least one conception associated with decreased multiple ovulation. Of 25 double ovulations observed 16 produced twins. If p is the probability of fertilization of an ovum, and if this is independent of other fertilizations, p is estimated as 0·8. The probability of neither of two ova being fertilized is then 0·04. Therefore the number of non-fertilized double ovulations may be neglected without serious bias. Removing all cases of multiple ovulation from our data, the percentages of pregnancies, shown in the last line of Table 2, are seen to differ to an important extent.

The reduced conceptions, and proportions of multiple pregnancies, are consonant with results reported from ewes mated on red-clover[4,5] and lucerne[6]. It seems likely that many cases of unsatisfactory fertility in sheep, especially those grazed on pastures containing appreciable quantities of red or subterranean clovers, could be caused by relatively small intakes of œstrogen during mating. Doses such as these are unlikely to be associated with permanent effects[7], obvious derangements of mating behaviour, or pathological changes in genitalia. Yet their econonic consequences could be far more serious than those reported for prolonged ingestion of relatively large doses other than during the mating period.

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CYTOLOGY

Trisomy of Autosome 16

So far as we are aware, true trisomy of autosome 16 (Denver classification) has not previously been reported, accordingly we wish to record the following case.

The patient is a female æt. 59 with certain multiple congenital abnormalities. Her buccal smears on two separate occasions gave 34 and 46 per cent single Barr bodies. The peripheral blood cultured by the technique of Moorhead *et al.*[1] gave the chromosome pattern presented in Table 1 and Fig. 1.

Table 1

Chromosome No.	43	44	45	46	47	>47	Total
Cell count	1	1	1	4	91	2	100

The following statistical analysis revealed that the 47's were trisomic for 16: variance of arm ratios (long arm/short arm) of the 3 autosomes 16 in the patient compared with each other was not significant at the 5 per cent level. Variance of arm ratios of these 3 autosomes compared with a control series was not significant at the 5 per cent level (F ratio 1·42, degrees of freedom 35, 49). Confidence limits based on this variance also indicated that the extra chromosome was unlikely to be an isochromosome of X. This was further confirmed by comparing the total length of this chromosome to the short arm of the X-chromosome.

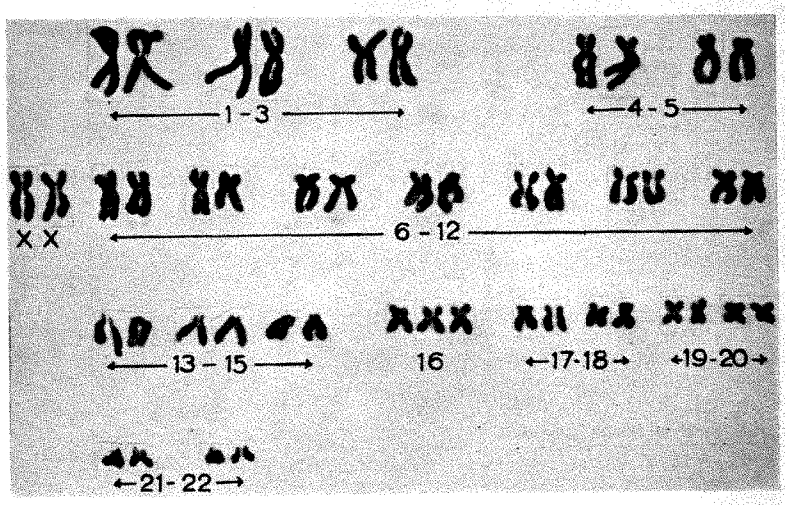


Fig. 1. Karyotype of a typical spread (case 62/750; cell 66)

(F ratio 3·19, degrees of freedom 11, 11, significant at the 5 per cent level.)

Student's t test on means of arm ratios demonstrated that the extra chromosome does not belong to the 17–18 group. ($t = 14·34$ degrees of freedom 25, highly significant at the 0·1 per cent level.) These findings appear to establish that this is in fact trisomy of 16, is unrelated to either the 13–15 or 17–18 groups, and is not an isochromosome of X.

We thank Dr. Heaton-Ward for permission to publish this case and Mr. G. M. Clarke for his help with the statistics. We also thank the Ethel Showering Fund for defraying some of the expenses.

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ENTOMOLOGY

Procedure for Rearing Houseflies

THE various methods and media used for rearing houseflies[1] each give results considered satisfactory by the individual workers or laboratory, but no rearing procedure is yet accepted as standard. The Chemical Specialties Manufacturers' Association rearing medium and procedure[2] are often accepted in North America but do not appear to be used elsewhere. The rearing procedure evolved in this laboratory[3] has since been modified in the various ways described in *a–d* below, and is now probably simpler and easier than any previously described.

The modifications of the previously described procedure[3] are: (*a*) A 2-in. diameter hole is added to the bottom lid of each cylindrical cage. This accommodates a polythene feeding cup of the same diameter and about 1·5 in. high. These cups are conveniently made by cutting away the upper portion of a 6-oz. polythene bottle. (*b*) The milk-sugar-water food fed previously often sours and requires frequent renewal. As a simpler procedure, each cage, containing up to 1,500 adult flies, is now supplied with approximately 200 ml. of 2·5 per cent sugar solution in the feeding bottles and approximately 25 g dry food in the feeding cups. Cages are charged in this way when pupæ are added for emergence; thereafter the food is changed weekly or when the supply nears exhaustion, except that flies intended for insecticide testing have both sugar solution and solid food renewed two days prior to the insecticide tests. Flies intended for insecticide testing are fed dry white cane sugar in the feeding cups. Those which provide eggs for further rearing are fed a dry mix containing equal parts by weight of cane sugar and dried whole cream milk powder to which are added 10 per cent dried yeast and 0·1 per cent cholesterol. These proportions are arbitrary, and no attempt has yet been made to establish to what extent proportions could be varied or components eliminated. Cholesterol is added as a precaution against inferior egg hatching[4]. A synthetic diet for adult houseflies is available[4]; its adoption could be a further step towards a standardized rearing procedure. (*c*) The breeding medium now used comprises equal parts dry weight of absorbent tissue paper and dried whole cream milk powder to which is added 5 per cent of dried yeast, and 2·1 ml. water/g dried mix. Each rearing jar receives rearing medium from 75 g of dry mix and this is seeded with 0·4 ml. of eggs to produce 1,300–1,500 pupæ in 7 days at 25° C. (*d*) In the earlier procedure[3] the absorbent tissue was soaked for 30 min to facilitate pulping before the dry mix was worked into the wet tissues. It has also been suggested that the tissues be shredded into pieces about an inch long[5]. In the present procedure the dry tissues are disintegrated in a cutting mill run at 3,000 r.p.m., all material passing a 12 mesh to linear in. screen before leaving the mill. The final product is a loose flock, without obvious particles of sheet paper. On mixing the paper flock with the dry components of the medium and adding the water the materials aggregate in large discrete crumbs. This wet material is then easily transferred to the rearing jars, is uniformly distributed by slight shaking and is then lightly tamped prior to addition of eggs.

The last of these modifications has transformed medium preparation from a tiring and time-consuming task to a simple instant mix easily effected with a glass rod. The physical consistency of the wet mix allows excellent penetration by the growing larvæ. The usual fly culture odours are noticeably absent, and there have been no cases of mould or bacterial invasion leading to the revolting 'cheesy' or rotten odour sometimes developed with the mashed paper medium. Pupation occurs in the medium. Pupæ are easily separated by flotation, and, because there is no entanglement, with incompletely macerated paper almost all pupæ are recovered.

Table 1. EFFECTS OF SUBSTITUTING DRIED SKIM MILK FOR DRIED WHOLE CREAM MILK AND OF ADDED CHOLESTEROL ON THE SUITABILITY OF THE STANDARD DIET

Rearing medium	No. of trials	Average values of rearing unit			
		No. of pupæ	Total weight of pupæ (g)	Pupal weight (mg)	Total eggs laid (ml.)
Normal	8	1,374	21·8	16·2	2·6
Skim milk		1,531	16·8	11·1	1·2
S.E. of mean		49·4	0·84	0·52	0·37
Significance		Sign.	H. Sign.	V. H. Sign.	Sign.
Normal	14	—	20·8	15·4	2·9
+1·0% cholesterol		—	20·7	15·4	2·8
S.E. of mean			0·70	0·37	0·16
Significance			N.S	N.S.	N.S.

The overall improvement resulting from this simple mechanical preparation of the paper has remarkably simplified rearing procedures, and it is suggested that the medium described here might well serve as a simple and approximately standard rearing medium until fully defined diets and conditions are available. Further, the dry medium could probably be mixed in bulk and stored or the wet mix prepared and deep-frozen until required, an expedient which is frequently used in this laboratory.

Two other variations have failed to effect improvements in the breeding medium. First, substitution of the cheaper dried skim milk for dried whole cream milk increased the number of pupæ per culture but so markedly decreased the average weight of individual pupæ that the biomass per culture unit was decreased. Further, the skim milk-reared flies took longer to come to egg production and the total yield of eggs per rearing unit was less than half that of those reared on the whole milk diet. Information for eight rearings is summarized in Table 1. Secondly, when cholesterol amounting to 1·0 per cent of the dry weight of yeast and milk powder was added to the medium[6] and compared with the normal rearing medium (Table 1) there were no real differences in numbers of pupæ reared, average weight of pupæ, initial egg production or the total egg yield.

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Response to Movement by *Formica polyctena* Forst.

ANTS of the *Formica rufa* group often overlook suitable prey if it does not move[1] and often attack insects not normally preyed upon if they perceive movement[2]. The following experiment was designed to obtain more accurate information on the role of prey movements in the searching behaviour of worker ants.

A small spindle was placed through the centre of the floor of a 1 m² forage area of a colony of 3,000 workers of *Formica polyctena* Forst. A portion of the spindle below the floor was fitted with vanes, allowing the spindle to be rotated by air blown through a rubber tube from a small aspirator bulb. The portion of the spindle above the floor was fitted with either a small white paper flag painted black on one side, or a small drum with black and white vertical stripes. The flag, because it was off-centre, caused a vibration when rotated. When in use these two objects were covered with one of the following: (1) an opaque cover that prevented the

ants from seeing the object or detecting any direct air currents; (2) a double 30-mesh copper screen which did not completely stop air current, but minimized the possibility of the ants seeing movement; or (3) a glass vial which allowed the ants to see the movement but prevented the detection of air currents. The tests were made by rotating the drum or flag once every five seconds for ten-minute periods. The number of ants attracted to within a 5-cm radius of the spindle during this period was compared with the number of ants passing through the same area during the 10 min both before and after the test. The number of ants in the initial control varied from 14 to 34, depending on the number foraging. In addition, the effect of smell was tested by placing freshly dissected cutworms, a normally acceptable food, under a small screen. The results of three repetitions of each test are given in Table 1.

Table 1. Ratio of the Numbers of Ants responding to Rotation of a Flag or Drum. A 10-min Period before the Test = 1

	10-min test period	10-min period after test
(*A*) Rotating flag		
(1) Opaque cover	2·2	1·3
(2) Screen cover	5·6	1·5
(3) Glass cover	7·3	1·7
(*B*) Rotating drum		
(1) Opaque cover	0·1	0·3
(2) Screen cover	0·8	0·4
(3) Glass cover	9·4	3·9

The foregoing results show that vibration and visual movement are the main factors in attracting these ants to their prey. All tests using the flag excited most of the ants in the area, but when the object was completely covered (*A*1) they had difficulty in locating the source of the disturbance. Nevertheless, the response was significant at the 0·02 per cent level. The fact that a highly significant response was obtained from the screen and flag, but not from the screen and drum, indicates the screen was a good visual barrier and the response from the flag (*B*2) was caused by vibration. A highly significant response was obtained from both tests in which the workers could see the object (*A*3 and *B*3). No response was obtained from the tests with dissected cutworms.

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GENETICS

Differential Mutagenic Activity of *l*(+) and *d*(—) Diepoxybutane

The mutagenic activity of diepoxybutane (1 : 2,3 : 4-butadiene dioxide) has been investigated in several biological systems[1–3]. In *Neurospora*, its ability to induce back mutations at the adenineless locus was found to be about twenty times higher than with X-rays[1]. On the other hand, its chromosome-breaking activity is high for both higher plants and animals[3–5]. In this respect, this difunctional compound is about 150 times more efficient than the monofunctional corresponding ethylene oxide. Two isomers of this compound were synthesized by Feit[6]. The experiments recorded here were designed to test if the two compounds have similar activity in both producing mutations and chromosome aberrations.

A mould, *Penicillium multicolor* G-M and P, was selected for checking mutation rate. Treated conidia were plated on complete Czapek–Dox medium, and all kinds of hypomorphic clones were recorded and isolated for further control of stability. No significant difference in toxicity was observed (Fig. 1). In contrast, it was found that

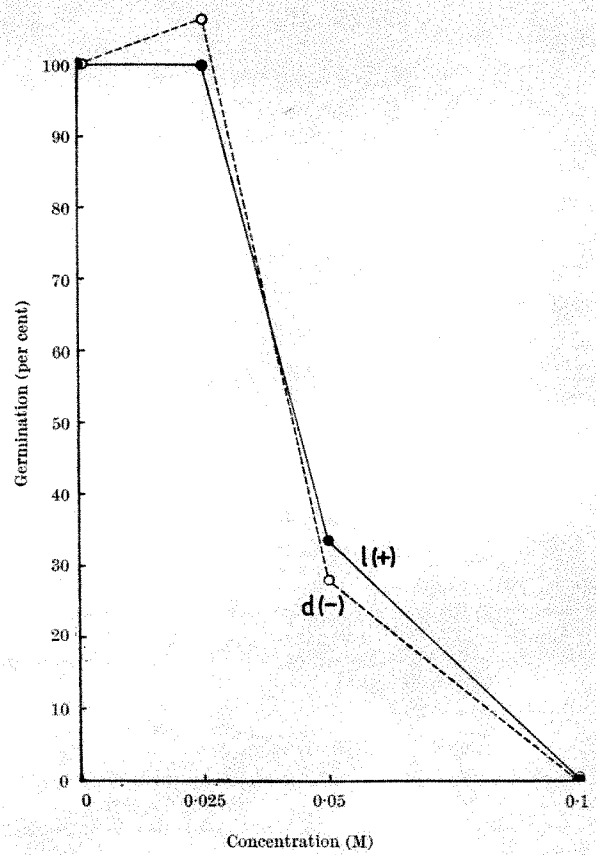


Fig. 1. Toxicity of isomers *l*(+) and *d*(−) of diepoxybutane in *Penicillium multicolor* (percentage of control)

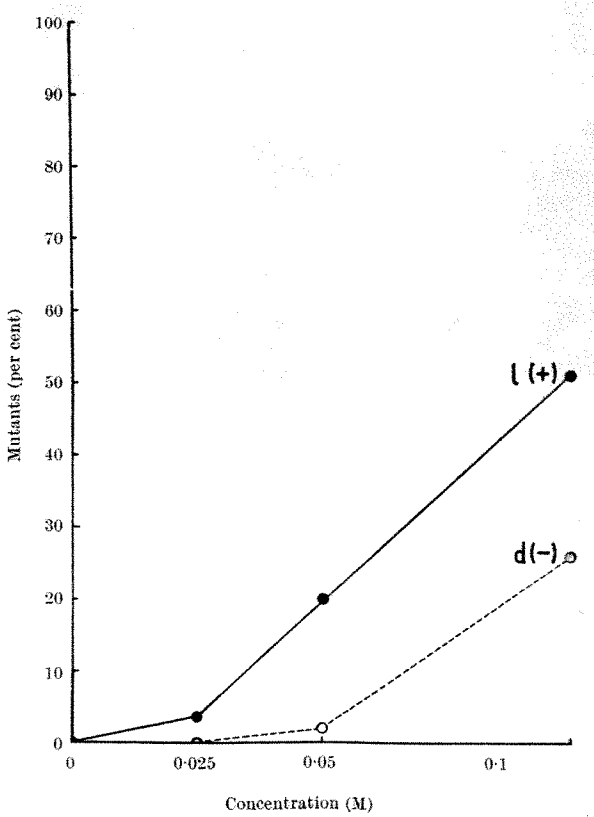


Fig. 2. Mutation-rates with isomers *l*(+) and *d*(−) of diepoxybutane in *Penicillium multicolor*

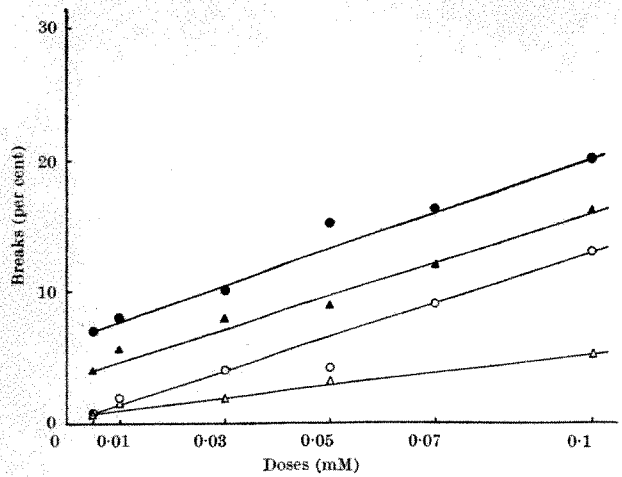


Fig. 3. Percentages of chromosome breaks induced with isomers $l(+)$ and $d(-)$ of diepoxybutane in *Vicia faba*

$l(+)$ {●, B $B + SR$ ▲, B $d(-)$ {○, $B + SR$ △, B

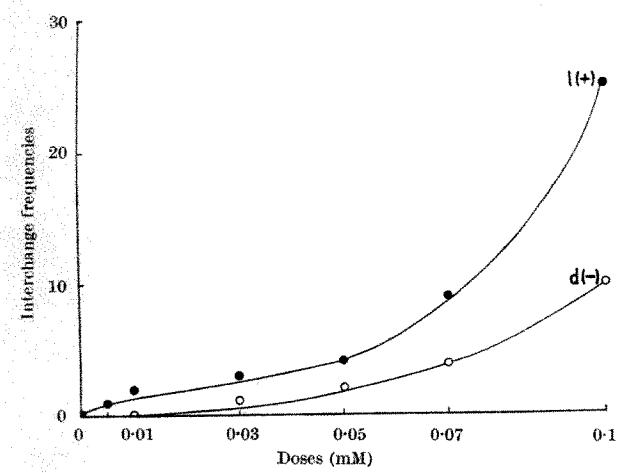


Fig. 4. Frequencies of chromosome interchanges induced with isomers $l(+)$ and $d(-)$ of diepoxybutane in *Vicia faba*

mutation rate is constantly higher for the $l(+)$ isomer. A 0·1 M for 45 min of $l(+)$ dose induced 2 times more detectable mutant clones (Fig. 2). In this organism, this dose is as efficient as 5×10^4-r. X-rays for $l(+)$ and $2{\cdot}5 \times 10^4$-r. X-rays for $d(-)$. In order to extend these results to chromosome breakage, dry broad bean seeds were treated at increasing doses. The $l(+)$ isomer was shown to produce more chromosome aberrations than $d(-)$. In Fig. 3 it can be seen that the main part of this effect is due to the increase of breaks rather than sister unions. Whether this higher break frequency originated from a decreased restitution process cannot yet be stated.

Fig. 4 clearly shows that the interchange frequency is also higher with the $l(+)$ compound. For both $l(+)$ and $d(-)$, the dose-effect curve is two hit for interchanges whereas it is one hit for breaks (Fig. 3).

These curves can be compared with those obtained with X-rays. The localization of chromosome aberrations was not observed to be different for $l(+)$ and $d(-)$ compounds. In contrast with X-rays a majority of aberrations is localized within the cephalobrachial chromosomes and all the isomers show an affinity for heterochromatic segments of the genome.

At this stage of the research, it can only be supposed that the differential activity of the isomers has a chromosomic origin. In this case, the toxicity of the compounds would not be mainly chromosome dependent. Other tests should be designed at specific loci to check whether this assertion can be extrapolated to point mutations.

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Male Sterility for Increased Outbreeding in Populations of Barley

In recent years several interesting mechanisms have been investigated which enable many plant species to maintain a mixed mating system with its variable proportions of outbreeding and inbreeding adaptively adjusted to the optimal requirements of recombination potential, hybridity and reproductive economy[1–3]. In the predominantly self-fertilized species such mechanisms seem to be of particular evolutionary significance. Using a theoretical model, Jain[4] showed that male sterility factors occurring in their populations might be favoured for an effectively increased rate of outcrossing on the male-sterile individuals. The adaptive role of cytoplasmic male sterility in the case of gynodioecious species was discussed earlier by Lewis[5]. The composite cross-bulk populations of barley in which a recessive male sterility gene (*ms*) has been introduced to facilitate continued recombination[6] provide suitable material for examining the consequences of Jain's model. In this communication an analysis of two such populations is briefly discussed in relation to the regulation of breeding system.

The observed proportion of male-steriles (*ms ms*) in various successive generations of the *C.C.XIV* and *C.C.XV* populations of barley are given in Table 1. From the results on percentage seed-setting on the male-sterile plants their probability of outcrossing (kt) was found to be 0·42 and 0·36 in the two populations respectively where the amount of outcrossing (t) in the absence of *ms ms* individuals was estimated to be 0·01–0·02 (ref. 7). The selection coefficients were estimated under two alternative sets of values, namely, (1) *Ms Ms* = *Ms ms*, x_a; *ms ms*, 1; or (2) *Ms Ms*, x_b; *Ms ms* = *ms ms*, 1 ($0 < x < 1$). The *ms ms* class being poorer in seed set, the latter set thus requires heterozygote advantage over both the homozygotes. Estimates of x_a and x_b that suggest an intense selection differential among the three genotypes at this locus are given in Table 1. In both populations the frequency of *ms ms* (Q) decreased with advancing generations except for a notable increase in the F_6 generation. However, their rate of diminution is clearly much lower than would be expected without selection. In the present material the initial selective advantage of male sterility gene seems to be eventually outweighed by the lower fertility of male-steriles. In view of the low values of selection coefficient, perhaps heterozygote advantage at several other loci may also have to be invoked for the maintenance of *ms* gene in such populations.

In order to determine the extent of increased outbreeding due to a male sterility factor, a coefficient (t') measuring the rate of outcrossing in populations such as *C.C.XIV* and *C.C.XV* may be defined as:

$$t' = t(1-Q) + ktQ$$

A point to note is that adjustments needed in the two terms $t(1-Q)$ and ktQ for the absence of pollen on *ms ms* plants counterbalance each other. Now, as shown by

Table 1. Estimates of the Relative Frequency of Male-Steriles (Q %) and the Selection Coefficient (x)*

Generation	CC XIV Q	CC XIV x_a	CC XIV x_b	CC XV Q	CC XV x_a	CC XV x_b
F_2	26·38			26·07		
		0·634	0·278		0·848	0·680
F_3	28·36			24·06		
		0·450	0·750		0·549	0·680
F_4	18·69			16·40		
		0·194	0·070		0·243	0·253
F_5	28·33			21·25		
		0·514	0·920		0·410	0·800
F_{10}	5·16			6·19		
		0·385	0·368			
F_{13}	6·17					
		0·770	0·891			
F_{14}	2·08					
		1·000	1·000			
F_{15}	Less than 1·00					

* Selection coefficients were estimated for each time interval between various generations so as to give the best fit to the observed frequencies.

Table 2. Some Theoretical Values of Q, t' and H at Equilibrium

Q	$kt = 0·42$ t'	$kt = 0·42$ H	$kt = 1·00$ t'	$kt = 1·00$ H
5·0	0·040	0·038	0·069	0·065
10·0	0·060	0·056	0·118	0·106
20·0	0·100	0·091	0·216	0·178

Wright[8], the panmictic index $(1-F)=\frac{2t'}{1+t'}$, and at equilibrium the expected amount of heterozygosity (H) at a locus with gene frequencies (p, q) is simply given by $H=2pq(1-F)$. Table 2 gives numerical examples of this relationship among Q, t' and H for two different values of kt (0·42 and 1·00) and $p=q=0·5$, $t=0·02$. Thus, for Q as large as 10·0–20·0 the amount of increase in outbreeding as well as in the levels of heterozygosity is large enough to be of adaptive significance, as postulated by Jain[4], and this contribution is proportional to k, the probability of outcrossing on male-steriles. The population genetics of such devices controlling the breeding system of a species is indeed likely to direct attention of many more investigators handling natural as well as experimental populations of inbreeding species. The adaptive maintenance of an outbreeding factor in such species has important bearing also on their controlled breeding methods.

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SOIL SCIENCE

Relationships between Mycostasis and Free Monosaccharides in Soils

Investigations of the mycostatic factor in British soils[1] have shown that mycostasis is reduced and finally masked by the addition of increasing concentrations of glucose to the soil. It has further been shown[2] that seasonal variation in mycostatic activity in British soils could be closely correlated to the level of reducing substances present: a high level of mycostatic activity corresponding to a low level of reducing substances while an increase in the level of reducing substances resulted in a low level of mycostasis.

Reducing substances were estimated as glucose equivalents on water extracts of pine forest humus layer (A horizon) from North Wales obtained by bubbling oxygen-free nitrogen gas through a soil/water mixture. After centrifugation, substances other than sugars were precipitated from the supernatant with neutral lead acetate, after which sugars were estimated by Wager's[3] modification of the Shaffer–Hartman method and measured photometrically on a Spekker absorptiometer at 580μ, using Nelson's[4] arsenomolybdate chromogenic reagent.

Qualitative analysis of the sugars present in the soil extracts was carried out chromatographically. 1-kg samples of soil from the A horizon in pine forest in North Wales were sieved through a B.S. 10 mesh and extracted:

(a) By stirring with 0·5 N sodium hydroxide for 48 h; the supernatant siphoned off and acidified with hydrochloric acid to precipitate the humic acids. The solution was passed through animal charcoal, previously treated with 0·1 hydrochloric acid, and the eluate concentrated under reduced pressure at 50° C. The residue was extracted twice with ethanol and finally reduced to dryness under vacuum. The residue was made up in 50 ml. of double distilled water passed through 'Zeo-Karb 225' and 'Deacidite FF' and further concentrated to 2 ml.

(b) By bubbling oxygen-free nitrogen gas through a soil/water mixture for 5 h, followed by centrifugation at 3,000 r.p.m. for 20 min. The supernatant was Seitz-filtered, and the tannins present were precipitated with saturated neutral lead acetate and the solution filtered through Whatman No. 50 filter paper on to sodium oxalate. The precipitated lead oxalate was filtered off and the filtrate concentrated under reduced pressure to 50 ml., after which it was passed through 'Zeo-Karb' and 'Deacidite FF' and finally concentrated to 2 ml.

Chromatograms of the two solutions obtained from (a) and (b) were run on Whatman No. 1 paper against standardized solutions of known monosaccharides in butanol/pyridine/water (6 : 4 : 3) for 48 h at 25° C. The chromatograms were dried, dipped into a solution of silver nitrate in acetone, further dried and sprayed with alcoholic sodium hydroxide. The developed chromatograms were dipped into 2 N ammonia and finally rinsed in sodium thiosulphate/sodium metabisulphite solution.

Chromatograms of the solutions (a) and (b) above showed the presence of ribose, sorbose and galactose together with an unidentified spot of high molecular weight. Similar chromatograms from the extract after hydrolysis with N sulphuric acid showed a decrease in concentration at this spot accompanied by an increase in size and intensity of the spots for ribose and galactose, indicating hydrolysis of an unidentified polysaccharide. Autoclaved soil gave much larger spots for the same three sugars with the addition of a spot with the same R_F value as xylose.

The absence of glucose in the soil solution confirms the findings of Hinson[5], who showed that glucose added to forest soils disappeared very rapidly over the course of a few days, probably due to its utilization by the soil microflora.

Recent work by Nagar[6] has shown that free monosaccharides also occur in American and Brazilian soils, and work is now in progress to establish whether or not free sugars occur in the highly mycostatic soils of Malaya[7].

Our thanks are due to the Forestry Commission for a grant to one of us (D. A. G.) which enabled this work to be carried out.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

DEMONSTRATOR (with a good honours degree in physics, chemistry, applied mathematics or some branch of engineering) IN FUEL TECHNOLOGY AND CHEMICAL ENGINEERING—The Registrar, The University, Sheffield (July 31).

RESEARCH ASSISTANTS in the following departments: COMPUTING, CYBERNETICS AND MANAGEMENT (in Statistics or Programmed Learning); APPLIED PHYSICS (one in Solid State Physics and one in Metal Physics); CHEMISTRY; CIVIL ENGINEERING AND BUILDING; MECHANICAL ENGINEERING; and ELECTRICAL ENGINEERING—The Registrar, Brighton College of Technology, Richmond Terrace, Brighton 7 (July 31).

LECTURER or ASSISTANT LECTURER (with a degree in agricultural chemistry, biochemistry or physiology, and an interest in human nutrition) IN THE DEPARTMENT OF NUTRITION—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 31).

GRADUATE RESEARCH ASSISTANT (qualified for registration for Ph.D degree, and with a special interest in organic chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (August 1).

ASSISTANT EXPERIMENTAL OFFICER (with a degree in science, horticulture or agriculture, or equivalent), for physiological studies on vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (August 3).

EXPERIMENTAL OFFICER (preferably with experience in at least one branch of spectroscopy) IN SPECTROSCOPY IN THE DEPARTMENT OF CHEMISTRY—The Registrar, The University, Manchester 13 (August 3).

HEAD (highly qualified experienced person) OF THE DEPARTMENT OF BUILDING AND CIVIL ENGINEERING—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref. 7/63/6 (August 3).

RESEARCH ASSISTANT (with a degree in agriculture) IN CROP HUSBANDRY; and a RESEARCH ASSISTANT (with a degree in zoology) IN AGRICULTURAL ZOOLOGY, for work on the potato crop—The Secretary, The School of Agriculture, West Mains Road, Edinburgh (August 3).

SPECIAL RESEARCH ASSISTANT (with a Ph.D. and interested in the physics of liquids) to Dr. V. I. Little IN THE DEPARTMENT OF PHYSICS, for work on the recoilless emission of gamma-rays from macromolecules in supercooled solution—The Secretary of Council, Bedford College (University of London), Regent's Park, London, N.W.1 (August 5).

TUTORIAL ASSISTANT IN PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (August 6).

GRADUATE RESEARCH ASSISTANT (preferably male, with at least an ordinary degree in a combination of biological subjects, including zoology) TO THE PROFESSOR OF ZOOLOGY AND COMPARATIVE PHYSIOLOGY, for work on the electron-microscopic structure and on the electrophysiology of sense organs—The Assistant Registrar (Science), The University, Birmingham 15 (August 7).

SENIOR LECTURER IN MICROBIOLOGY at the University of the West Indies —The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (August 7).

RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSICS, for work on infra-red lasers under Prof. G. F. J. Garlick—The Registrar, The University, Hull (August 9).

SENIOR RESEARCH FELLOW IN CRIMINOLOGY, to work within the Department of Social Studies—The Registrar and Secretary, The Durham Colleges in the University of Durham, 38 North Bailey, Durham (August 9).

EXPERIMENTAL OFFICER IN THE DEPARTMENT OF METALLURGY, to be responsible for the maintenance of X-ray equipment for undergraduate teaching and for research—The Registrar, The University, Manchester 13 (August 10).

INVESTIGATIONAL OFFICER (with a degree or diploma in agriculture, and preferably a knowledge of economics and accountancy) IN THE DEPARTMENT OF AGRICULTURAL ECONOMICS, mainly for the collection and initial processing of the data from farms—The Registrar and Secretary, King's College (University of Durham), 6 Kensington Terrace, Newcastle upon Tyne 2 (August 10).

LECTURER or ASSISTANT LECTURER (with a good degree in chemical or mechanical engineering) IN THE DEPARTMENT OF POLYMER AND FIBRE SCIENCE—The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester 1 (August 10).

RESEARCH ASSISTANTS (2) (with postgraduate experience) IN PLANT PHYSIOLOGY IN THE DEPARTMENT OF AGRICULTURAL SCIENCE, School of Agriculture, to undertake investigations on the physiology of the potato plant—The Registrar, The University, Nottingham (August 10).

SENIOR LECTURERS, LECTURERS, or ASSISTANT LECTURERS IN (*a*) ANIMAL NUTRITION; (*b*) ANIMAL BREEDING AND GENETICS; (*c*) ANIMAL HEALTH (registrable veterinary qualifications essential); (*d*) SOIL MICROBIOLOGY; and (*e*) TREE AND ECONOMIC CROP AGRONOMY at the University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 10).

CHIEF TECHNICIAN (with university or equivalent experience) IN THE ZOOLOGY DEPARTMENT, University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 13).

ASSOCIATE PROFESSORS (2) IN VIROLOGY AND BACTERIOLOGY in the Medical School, University of Lagos, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 15).

BIOCHEMIST or CHEMIST (with a good honours degree or equivalent and research experience) FOR THE LIPIDS BIOCHEMISTRY DEPARTMENT—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen (August 15).

LECTURER and an ASSISTANT LECTURER IN THE MECHANICS OF FLUIDS in the Faculty of Science—The Registrar, The University, Manchester 13 (August 15).

LECTURER or ASSISTANT LECTURER (preferably with special interests in hydrology, or economic geography or regional geography of North America) IN GEOGRAPHY at the University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 15).

LECTURER or JUNIOR LECTURER or JUNIOR RESEARCH FELLOW (with a higher degree, some teaching and research experience, and preferably a medical qualification) IN PHARMACOLOGY IN THE DEPARTMENT OF PHYSIOLOGICAL SCIENCES, University of Lagos—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 15).

PULMONARY PHYSIOLOGIST (graduate with honours in physiology with interest in respiratory physiology, and preferably experience of pulmonary function techniques) FOR A PULMONARY FUNCTION UNIT now being established—The Secretary, Glasgow Royal Infirmary, 84 Castle Street, Glasgow, C.4 (August 15).

ASSISTANT LECTURER and a COLLEGE LECTURER (graduates in medicine or pharmacy, and preferably with teaching or research experience in pharmacology or cognate subjects) IN THE DEPARTMENT OF PHARMACOLOGY —The Secretary and Bursar, University College, Dublin, Republic of Ireland (August 17).

HEAD (with high academic qualifications and interested in the developme t of higher national certificate and other courses suited to the needs of the growing chemical industry in the area) OF THE DEPARTMENT OF APPLIED CHEMISTRY—The Principal, College of Technology, Belfast 1, Northern Ireland (August 17).

SENIOR LECTURER or LECTURER IN THERAPEUTICS IN THE DEPARTMENT OF MATERIA MEDICA AND THERAPEUTICS—The Secretary, The University, Aberdeen (August 17).

STAFF TUTOR IN PSYCHOLOGY IN THE DEPARTMENT OF EXTRA-MURAL STUDIES—The Registrar and Secretary, King's College (University of Durham), 6 Kensington Terrace, Newcastle upon Tyne 2 (August 17).

SENIOR RESEARCH FELLOW (with a Ph.D. or equivalent postgraduate research experience) IN PARASITOLOGY IN THE DEPARTMENT OF ZOOLOGY, The Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 18).

ASSISTANT LECTURER (with medical qualifications registrable in the United Kingdom) IN ANATOMY—The Registrar, The University, Manchester 13 (August 24).

LIBRARIAN, grade IV (with at least A.L.A. or London Diploma, and an interest in biology) mainly for cataloguing in a small specialized library—The Establishment Officer, Anti-Locust Research Centre, 1 Princes Gate, London, S.W.7 (August 26).

ASSOCIATE PROFESSOR/SENIOR LECTURER IN MEDICINE at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 30).

POSTGRADUATE SCHOLARSHIP IN PROGRAMMED LEARNING—Dr. E. D. Fraser, Department of Psychology, The University, Old Aberdeen, Scotland (August 30).

READER or SENIOR LECTURER IN MICROBIOLOGY; and a LECTURER IN MICROBIOLOGY at Monash University, Victoria, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (Australia and London, August 30).

ASSISTANT LECTURER (veterinary surgeon or with a degree in physiology) IN THE DEPARTMENT OF PHYSIOLOGY—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (August 31).

DEPARTMENTAL GRADUATE ASSISTANT (with a degree or equivalent qualifications in chemistry and physics or in either subject separately), to operate a nuclear magnetic resonance spectrometer as a service for all departments in the School of Chemistry—The Registrar, King's College (University of Durham), Newcastle upon Tyne 1 (August 31).

DEPUTY REACTOR ENGINEER (with a university degree in engineering or an equivalent qualification, several years industrial experience, and preferably experience in plant operation) in connexion with the nuclear reactor to be operated jointly by the University of Liverpool and the University of Manchester—The Bursar, The University, Manchester 13 (August 31).

HEAD (with a dental qualification registrable with the South African Medical and Dental Council) OF THE DEPARTMENT OF DENTISTRY AND DIRECTOR OF THE ORAL AND DENTAL HOSPITAL, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1—(South Africa and London, August 31).

LECTURER/ASSISTANT LECTURER (preferably with experience in teaching and research) IN THE DEPARTMENTS OF ANATOMY AND PHYSIOLOGY in the newly established Faculty of Medicine, University of Malaya—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, August 31).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF PHARMACOLOGY—Clerk to the Council, School of Pharmacy (University of London), 29/39 Brunswick Square, London, W.C.1 (August 31).

LECTURER (with higher qualifications in physics or physical chemistry, and preferably research experience in the physics of solid/liquid, liquid/liquid systems or of powders) IN THE DEPARTMENT OF PHARMACEUTICS—The Clerk to the Council, School of Pharmacy (University of London) 29/39 Brunswick Square, London, W.C.1 (August 31).

SENIOR ASSISTANT (with experience of the application of electron microscopy to plant material) IN RESEARCH IN CASUAL MORPHOLOGY AND FINE STRUCTURE IN THE DEPARTMENT OF BOTANY—Dr. H. L. K. Whitehouse, Botany School, The University, Downing Street, Cambridge (August 31).

READER (with general experience in the various branches of microbiology) IN MICROBIOLOGY—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (September 1).

SENIOR LECTURER (with a special interest in physical-organic chemistry, and preferably experience in teaching and capable of initiating independent research; some industrial experience would be an advantage) IN THE DEPARTMENT OF CHEMISTRY—Clerk to the Governing Body, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (September 2).

LECTURER IN MICROBIOLOGY at the University of Western Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 7).

MYCOLOGIST (male, with postgraduate research experience) in the Chamber's Biological and Chemical Research Laboratory, Transvaal and Orange Free State Chamber of Mines Research Laboratories, to lead a team in systematic and ecological studies of fungi occurring in the mines, and in the gold and uranium recovery processes, including a study of fungi growing on timber and other organic materials in the mines,with particular reference to basidiomycetes and possible pathogens—The Officer-in-Charge, South African Scientific Liaison Office, Chichester House, 278 High Holborn, London, W.C.1 (September 7).

CHAIR OF FIBRE SCIENCE IN THE DEPARTMENT OF TEXTILE INDUSTRIES—The Registrar, The University, Leeds 2 (September 16).

READER IN MATHEMATICS (Applied) at Birkbeck College—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 16).

CHAIR OF MECHANICAL ENGINEERING—The Registrar, The University, Sheffield (September 30).

PROFESSOR OF MATHEMATICS (either Pure or Applied)—The Registrar, The University, Leeds 2 (September 30).

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in agriculture and an interest in applied research), for work of an essentially practical nature on the storage and handling of potatoes—The Secretary, Ditton Laboratory, Agricultural Research Council, Larkfield, near Maidstone, Kent.

BACTERIOLOGIST (non-medical, basic grade, with a B.Sc. qualification in biological subjects) IN THE BACTERIOLOGY DEPARTMENT—The Superintendent, Royal Infirmary, Infirmary Road, Sheffield 6.

BIOCHEMIST (basic grade) (with a degree in chemistry or biochemistry, preferably with honours, or an equivalent qualification) IN THE BIOCHEMISTRY DEPARTMENT—Mr. H. Varley, Biochemistry Department, Manchester Royal Infirmary, Manchester, 13.

BIOCHEMIST (with an honours degree in chemistry, an Associate of the Royal Institute of Chemistry, at least two years postgraduate experience in medical biochemistry, and national of, and permanently resident in, Great Britain or the Republic of Ireland) with the Government of Uganda, to be in charge of the Biochemistry Division of the Laboratory services located at Kampala and serving all parts of the country and all races—The Director of Recruitment, Department of Technical Co-operation, 3 Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. RC.205/153/026.

CHAIR OF PHYSICS (other than nuclear physics)—The Secretary, University of Lancaster, P. O. Box 78, County Hall, Preston.

CHAIR OF PURE MATHEMATICS—The Registrar and Secretary, The University, Bristol.

EXPERIMENTAL OFFICER (with distinct skill in experimental organic chemistry and a sound knowledge of chemical theory) IN THE DEPARTMENT OF CHEMISTRY, to carry out research and development work on the controlled oxidation of aromatic compounds and of more complex materials—The Registrar, The Manchester College of Science and Technology, Sackville Street, Manchester 1.

HEAD OF THE BIOCHEMISTRY AND BIOPHYSICS DIVISION—The Director, Arthur D. Little Research Institute, Inivereskgate, Musselburgh, Midlothian.

GEOLOGIST (with a university degree including geology as a major subject, at least six years practical experience since graduation, and preferably experience in engineering geology) IN THE DEPARTMENT OF MINES, Tasmania, for geological investigations in the exploration and development of mineral resources, regional geological mapping, and engineering geology—The Agent-General for Tasmania, 457 Strand, London, W.C.2.

JUNIOR BACTERIOLOGIST (qualified experienced male or female technician, or graduate), for duties involving the bacteriological analyses of frozen foods—The Director, Fruit and Vegetable Canning and Quick Freezing Research Association, The Research Station, Chipping Campden, Glos.

LECTURER (with a good honours degree in biology or botany and preferably experience on the mode of action of herbicides) IN BIOLOGY; and a LECTURER with a good honours degree in genetics, biology, zoology or microbiology) IN GENETICS—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1.

PRINCIPAL LECTURERS IN MECHANICAL ENGINEERING (two vacancies); and LECTURERS OR ASSISTANT LECTURERS IN MECHANICAL ENGINEERING (several vacancies)—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 21/G.

PRINCIPAL PHYSICIST at the Cardiff Radiotherapy Hospital—The Group Secretary, Whitchurch and Ely H.M.C., Whitchurch Hospital, Whitchurch, Cardiff.

RESEARCH ASSISTANT IN THE DEPARTMENT OF MUNICIPAL ENGINEERING, for work concerned with the performance of circular sedimentation tanks—The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester 1.

RESEARCH ASSISTANT IN ZOOLOGY—The Registrar, University College of Wales, Aberystwyth.

RESEARCH ASSISTANT (with a good honours degree in physics, but candidates graduating this summer may apply) IN PHYSICS, for research in the field of radiation physics—Head, Department of Mathematics and Physics, The Polytechnic, 309 Regent Street, London, W.1.

TECHNICIAN, Grade II (with some microbiological experience) IN THE DEPARTMENT OF GENETICS, to assist with research in microbial and bacterial genetics—The Registrar, The University, Liverpool 3, quoting Ref. 365.

TEMPORARY LECTURER (medically or non-medically qualified, with a special interest in endocrinology) IN THERAPEUTICS IN THE DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS—The Secretary, University of St. Andrews, Queen's College, Dundee.

REPORTS and other PUBLICATIONS

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Great Britain and Ireland

Department of Scientific and Industrial Research. Report of the Research Council for the year 1962. Pp. 62. (Cmnd. 2027). (London : H.M. Stationery Office, 1963.) 4*s*. net. [295

Royal Observatory, Edinburgh. Report of the Astronomer Royal for Scotland for the year ending 31st March, 1963. Pp. 15. (Edinburgh : Royal Observatory, 1963). [46

Ambassade de France : Service de Presse et d'Information. Trade Unionism in France. Pp. 20. (London : Ambassade de France, Service de Presse et d'Information, 1963). [46

Admiralty, London. Report by the Hydrographer of the Navy for the year 1962. Pp. 35+2 plates. (London : Admiralty, 1963). [46

Department of Scientific and Industrial Research, and Fire Offices' Committee Joint Fire Research Organization. Fire Note No. 3 : Roofs and Fire. Pp. v+10. (London : H.M. Stationery Office, 1963.) 2*s*. 6*d*. net. [46

Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1961–62. Northern Nigeria. Pp. 19. 2*s*. 6*d*. Republic of the Sudan. Pp. 44. 2*s*. 6*d*. (London : Empire Cotton Growing Corporation, 1963.) [46

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The British Food Manufacturing Industries Research Association. Annual Report, 1962. Pp. 60. (Leatherhead : The British Food Manufacturing Research Association, 1963.) [56

Department of Scientific and Industrial Research. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country around Kettering, Corby and Oundle, (Explanation of Sheet 171). By Dr. J. H. Taylor, with contributions by Dr. J. E. Prentice, Dr. P. A. Sabine, Dr. F. B. A. Welch and Dr. V. Wilson. Pp. ix+149+6 plates. (London : H.M. Stationery Office, 1963.) 30*s*. net. [56

Commonwealth Mycological Institute, Kew. Mycological Papers, No. 87: Dematiaceous Hyphomycetes, IV. By M. B. Ellis. Pp. 42. 15*s*. net. Mycological Papers, No. 88: Coelomycetes. 11: Neobarclaya, Mycophypallage, Bleptosporium, and Cryptosticitis. By B. C. Sutton. Pp. 50. 17*s*. 6*d*. net. (Kew: Commonwealth Mycological Institute, 1963.) [56

British Drug Houses, Ltd. Benzylidene-Azine (Benzal-Azine). Pp. 3. Sodium Diphenyl—Reagent for Determining Bromine and Chlorine in Organic Compounds. Pp. 7. Hydroxy-Ammonium Chloride. Pp. 8. (Poole: British Drug Houses, Ltd., 1963.) [56

The Edinburgh School of Agriculture. Experimental Work, 1962. Pp. vii+96. (Edinburgh : The Edinburgh School of Agriculture, 1963.) [56

Department of Scientific and Industrial Research. Report of the National Chemical Laboratory 1962 : Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1962. Pp. iv+91+plates. (London : H.M. Stationery Office, 1963.) 6*s*. 6*d*. net. ; 1.30 dollars. [56

Bulletin of the British Museum (Natural History). Zoology. Vol. 10, No. 5 : Observations on the Chaetotaxy of the Legs in the Free-Living Gamasina (Acari : Mesostigmata). By G. Owen Evans. Pp. 275–303. (London : British Museum (Natural History), 1963.) 13*s*. [56

Government of Northern Ireland : Ministry of Agriculture. Leaflet No. 82 : The Production of Clean Milk. Pp. 18. (Belfast : Ministry of Agriculture, 1963.) [116

Metropolitan Borough of Lewisham Library Service. Scientific and Technical Information Service Publication No. 1 : Abstracts and Indexes in Science and Technology—an Annotated Guide to the Abstracts, Indexes and Journals containing Abstracts provided by the Lewisham Library Service. Pp. 12. (Lewisham, London : Scientific and Technical Library, 1963.) [116

Other Countries

Deutsche Akademie des Wissenschaften zu Berlin. Veröffentlichungen des Geodätischen Instituts in Potsdam. Nr. 14 : Korrelaten-Tabellen für Trigonometrische Netze aus 10 Dreiecken. Von Werner Jenne. Pp. 85. 16 D.M. Nr. 15 : Formeln und Tafeln zur Lösung der 1. Geodätischen Hauptaufgabe auf dem Ellipsoid von Krassowski. Von Dietrich Schoeps. Pp. 64. 15 D.M. Nr. 16 : Numerische Beispiele zur Strengen Theorie der Figur der Erde. Von Kurt Arnold. Pp. 66. 18 D.M. Nr. 17 : Die Lösung der Neumannschen Randwertaufgabe für das Erdellipsoid mittels Abbildung auf eine Soldersche Schmiegungskugel. Satellitenbeobachtungen als mittel zur Geodätischen Punktbestimmung. Die Elements des Marussi-Tensors als Funktionen von Geodätischen Messungen. Die Operatoren grad, div, div grad in Gauss-Krüger-Koordinaten. Von Kurt Arnold. Pp. 36. 9 D.M. Nr. 18 : Ein Graphisches Hilfsmittel zur Gravimetrischen Verbesserung Astronomischer Nivellements. Von Kurt Arnold. Pp. 21. 11 D.M. Nr. 19 : Korrelaten-Tabellen für Trigonometrische Netze aus 11 Dreiecken. Von Werner Jenne. Pp. 154. 23.50 D.M. Nr. 20 : Tafeln zur Berechnung von Gauss-Krüger-Koordinaten auf dem Ellipsoid von Krassowski. Von Dietrich Schoeps. Pp. 85. 10.80 D.M. Nr. 21 : Bestimmung der Schwerediffernez Zwischen Potsdam und Sofia mit Pendelapparaten. Von I. Petkoff und C. Elstner. Pp .139. 24 D.M. Nr. 22 : Die Bestimmung der Lotkrümmung. Von Dietrich Schoeps. Pp. 87. 2. 50 D.M. Nr. 23 : Laplace-Azimute Zwischen Weit Entfernten Stationen aus Satellitenbeobachtungen. Zur Statistischen Analyse der Fehler im Feinnivellement. Von Kurt Arnold. Pp 48. 15 D.M. (Berlin : Akademie-Verlag, 1961, 1962 und 1963). [46

Australia : Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Applied Physics for year ending June 30, 1962. Pp. vi+41+7 plates. The Radiophysics Laboratory, 1961–1962. Pp. 32. (Sydney : Commonwealth Scientific and Industrial Research Organisation, 1962 and 1963.) [56

Institutt for Atomenergi. Kjeller Research Establishment. Kjeller Report No. 38 : A Chamber for Welding of Reactive Metals Under Controlled Atmosphere. By Ragnar Hagemann Søvik. Pp. v+11+5 figures. (Kjeller : Institutt for Atomenergi, Kjeller Research Establishment, 1963.) [56

United States Department of the Interior : Geoligical Survey. Water-Supply Paper 1539–G : Ground Water Geology of Karnes County, Texas. By R. B. Anders. Pp. v+40+plates 1–10. Water-Supply Paper 1596 : Geology and Ground-Water Resources of the Greybull River–Dry Creek Area, Wyoming. By Charles J. Robinove and Russell H. Langford. Pp. v+88+plates 1–4. Water-Supply Paper 1605 : Geology and Hydrology of Agua Caliente Spring, Palm Springs, California. By L. C. Dutcher and J. S. Bader. Pp. iv+43+plates 1 and 2. Water-Supply Paper 1619–AA : Ground-Water Conditions in the Fernley–Wadsworth Area, Churchill, Lyon, Storey and Washoe Counties, Nevada. By W. C. Sinclair and O. J. Loeltz. Pp. iii+22+plate 1. Water-Supply Paper 1619–H : Reconnaissance of Headwater Springs in the Gila River Drainage Basin, Arizona. By J. H Feth and J. D. Hem. Pp. iv+54+plates 1–4. Water-Supply Paper 1619–L : Ground Water in the Alluvium of Kings River Valley, Humboldt County, Nevada. By C. P. Zones. Pp. iv+38+plate 1. Water-Supply Paper 1619–Z : Water Resources Reconnaissance in Southeastern Part of Honey Lake Valley, Lassen County, California. By G. S. Hilton. Pp. iii+8+plates 1 and 2. Water-Supply Paper 1655 : Ground Water in the Pullman Area, Whitman County, Washington. By B. L. Foxworthy and R. L. Washburn. Pp. iv+71+plates 1–3. Water-Supply Paper 1669–K : Correlation of Water-Level Fluctuations with Climatic Cycles in the Oklahoma Panhandle. By I. Wendell Marine. Pp. iii+10+plate 1. (Washington, D.C. : Government Printing Office, 1963.) [126

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Indian Forest Bulletin No. 220 (New Series—Entomology) : Studies on the Natural Resistance of Timbers to Termites. By P. K. Sen-Sharma. Pp. 10+1 plate. (Delhi : Manager of Publications, 1963.) Rs. 0.50nP. ; 1*s*. 2*d*. [126

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"THE SHAPE OF THINGS TO COME"

A BROADSHEET issued by Political and Economic Planning on January 1, 1951, *Prospects of a Decade*, sought to forecast some of the dominant trends of the nineteen-fifties. This document was used as the basis in preparing a provocative and wide-ranging broadsheet which has now been issued under the title *The Shape of the Sixties**. The new broadsheet discusses with sense and insight the possibility of a scientific approach to forecasting in the 'sixties, the world population explosion, changes in population structure and Britain's part in world affairs. Scientists especially will find the sections on the emergence of the technocracy and on discarding political junk stimulating as well as interesting. Taken with the statement on principles and aims of the Marlow Conference on social and industrial relations, issued last April and known as the Marlow Declaration, and with such books as Prof. K. Denbigh's *Science, Industry and Social Policy* (see p. 418 of this issue of *Nature*), it provides encouraging evidence of a new approach to industrial well as to social problems. It signposts the road along ich Britain must undoubtedly travel if she is to achieve ner economic growth, or the leadership in political and ial advances or in science and technology that is stil hin her capacity.

The broadsheet begins by insisting on an ecological proach to attempts at planning or forecasting whether the industrial or the economic or political field. It ticizes the neglect of the contribution which biology can ten make. In advocating the wider and more effective se of modern techniques, such as the computer and operational research, it points out that too often these have been dominated by the particular outlook and techniques of physicists and chemists and their engineering counterparts. Admittedly, the critical and detailed application of such methods would require much greater resources than are at present available as well as tackling numerous technical problems which are still unsolved. Nevertheless, the advances recently made in ecology point the way, and what is written in this broadsheet is strongly underlined by Prof. Denbigh's comments on the industrial order and the rise of what he terms "economicism"—the acceptance of economic criteria as the only ones of significance in society.

The broadsheet forecasts that as in the 'fifties the evermore rapidly expanding human race must content itself with crowding into the 3 per cent or so of the Earth's surface which is by nature reasonably habitable, the net effect of man's activities being still rather to diminish than to expand the total habitable area. The only technological advance possible during the 'sixties which would permit, even locally, a substantial increase in culturable acreage is considered to be a really low-cost and efficient means of mass-processing saline into fresh water. The United States has already invested heavily in the necessary research and development programme, and, apart from its bearing on the water situation in Britain, the mere possibility that by this means during the late 'sixties spectacular improvements may be effected in the situation of tropical and sub-tropical territories with potentially good soil and growing conditions and plants of salt or saline water—but at present limited by lack of fresh-water supplies—warrants much greater attention to desalination than it has yet received in Britain.

* *Planning*, 29, No. 471 (29 April, 1963) *The Shape of the Sixties*. Pp. 143–174. (London: Political and Economic Planning, 1963). 4*s*.

Although some civil engineering works involving major interference with geography may come close to practicability by the late 'sixties, such projects as damming the Behring Straits or the Straits of Gibraltar are regarded as belonging to a later period and so very unlikely to modify appreciably, during the 'sixties, the alarming impact of the world population explosion. The seriousness of the situation is brought out briefly but vividly in the broadsheet, which emphasizes the implications in terms of investment in industry, agriculture and services, in education, health and local government, and of threats to world peace of the existence from now on in India and China, for example, of multitudes of new paupers. This is especially so, at a time when economic progress and higher labour productivity imply high labour redundancies, especially in agriculture, new entrants to the labour force and some 5 million additional jobs per year cumulatively—in China the number of extra jobs required up to 1975 is put at seven times the present British population.

The broadsheet does not suggest that it is impossible to avert catastrophic breakdowns in meeting elementary needs and in preserving law and order in the countries thus affected. However, here it points out that the breakdown witnessed in the Congo may well be repeated elsewhere and that at best we must anticipate on a vastly greater scale problems of relief akin to those of the Depressed Areas of the 'twenties and 'thirties in Britain. It suggests that a distinction must even be drawn between two types of 'underdeveloped territories'. On one hand, there are those which are genuinely so named and are probably capable of achieving acceptable and worth-while rates of improvement in standards of living by means of loans and grants of manageable scale and character. On the other hand, there are the much larger problem areas where decades of international relief will be needed while efforts are made to bring the population into some kind of equilibrium with the means of supporting it with some human dignity, education and prospects in life. This may well imply greatly increased contributions from advanced countries through international agencies, restrictions on international movement, tighter international health regulations and a vast educational effort. The broadsheet also instances the need for greater resources for research into birth control, and foresees the possibility during the 'sixties of a mass supply of economically efficient and widely acceptable methods which by the 'seventies could effect a spectacular drop in the excess of births over deaths. The formidable problem of feeding the 500 million additional population expected must be tackled along well-tried lines, especially by the more general use of the best agricultural practices already developed in advanced countries.

It is perhaps significant that Prof. Denbigh, too, looks at this international background in considering the future shape of industry in Britain, and that both recognize that this huge army of uneducated, untrained, underfed, underprivileged recruits to humanity is being bred at the very moment when science and technology are rapidly undermining the need for and the status of the unskilled. The warning is to be read as plainly in the broadsheet and Prof. Denbigh's book as in Dr. S. Lilley's recent lecture on the scientific revolution and industrial processes (see *Nature*, 198, 1132; 1963). What stands out most emphatically is in fact the insufficiency of economic factors or

motives if the challenge of the 'sixties, let alone the 'seventies, is to be met. Prof. Denbigh goes so far as to suggest that the economic side may be expected to look after itself if we find the right spirit and guiding idea of how we wish to progress, and he suggests that our aim should be to build up a balanced culture in modern terms fostering interests and values complementary to the commercial and economic interests which have dominated the past two centuries.

In particular, Prof. Denbigh condemns the primitive character of industrialism as seen in the spendthrift use of natural resources, the chaotic and haphazard character of the industrial environment, and the general sense of temporariness and instability. He urges that an important function of industry in society should be that the employment it offers enables a man to put into his work much of himself, and the importance of this aspect of satisfaction is all the greater with the dilemma of unemployment that the inevitable advance of automation involves as Dr. Lilley so clearly shows. Both Dr. Lilley and Prof. Denbigh recognize the educational issue that is involved, and undoubtedly the leadership that is required to foster this new outlook and practice in industry depends a good deal on the ability of our universities and colleges of technology to supply men and women with the comprehensive outlook required and the capacity for leadership.

One great value of the Marlow Declaration is that it is calculated to stimulate further thought and enquiry into the nature of economics itself and particularly into this question of satisfaction in work and what the job contributes to the workman himself as well as to the consumer. The Marlow Declaration in fact places the employee back where he was in the social theory of the Middle Ages before, as Prof. Denbigh points out, the mutual obligations of worker and employer had been disrupted by the joint stock company which recognized only the obligations of shareholders. The purpose of industry as the fulfilment of human needs imposes on industry specific responsibilities to the employee, the shareholder, the consumer, the local community and to the nation. These are explicitly set forth in the Declaration which equally recognizes the need for self-discipline and a profound regard for human values and that the purposes of industry cannot be achieved without technical and economic efficiency, in which co-operation and internal discipline are important factors.

There is thus substantial support for Prof. Denbigh's contention that our real problem to-day is one of work, not one of leisure, and, in particular, of transforming the working-day into something that is satisfying in itself as well as productive. Moreover, the vital importance of a new outlook—and not only in industry—could scarcely be more emphatically illustrated than in Dr. Lilley's remark that automation is now beginning to reverse the trend, which has persisted for two hundred years, for the proportion of skilled workers in industry to decrease and the proportion of semi-skilled operators, and particularly of those doing repetitive work, to increase. Further, Political and Economic Planning envisages the early crumbling of the legendary of the cultured, liberal-minded, arts-educated administrator and business leader and of the recently unchallenged power of mass Trade Unions, particularly the industrial as distinct from the craft Unions.

The passing of these two dominant influences over modern Britain is seen as a consequence of the country's growing dependence on science and technology, and therefore on the professions and groups associated with them. This is contrasted with the declining capacity of arts-trained elements in the Establishment to obtain the necessary results and to maintain prestige and leadership in an increasingly technological civilization, as well as to the disenchantment of professional, clerical and skilled workers with often absurdly stupid use of industrial weapons by the unskilled workers Unions. Whether or not this forecast is sound—and it could be challenged—there are large problems of power and influence in Government involved here, but the broadsheet does not attempt to consider the relations between science and Government. Prof. Denbigh shows himself fully aware of the existence and nature of that problem, but is content to indicate that it needs study. Political and Economic Planning inclines to the view that scientists and technologists will play a larger part in the task of Government without suggesting precisely how. However, the broadsheet ventures to predict that the use by unskilled organized workers of the cruder types of industrial pressure for their own advantage is likely to be terminated by public resentment against past abuse and the existence of much larger numbers of available and reasonably fit retired persons, the competition of immigrants and of redundant workers from automated industries.

The broadsheet sees no reason in principle why during the 'sixties all parts of Britain should not share adequately in a growing prosperity. If this does not happen it will be due rather to failure of foresight, of local and national leadership and adaptability, and of competent planning rather than to any inherent difficulties and the disappearance of chronically different levels of regional unemployment would be a reliable index of health and successful adjustment in Britain during the 'sixties. Here, of course, education is an important factor and stressing particularly the need to foster adaptability. The broadsheet comments on the importance of a great and rapid development in postgraduate instruction and research, and in institutes to fill such alarming gaps as the lack of hybrid studies in technology and economics, or regional planning and technology. Research into the processes and problems of education itself is also likely to expand quickly and to influence the rate and direction of change, while university expansion itself may well determine many changes in social patterns.

The significance as well as the importance of this influence of a large increase in the graduate population emerges further when, in a subsequent section, the broadsheet discusses the discarding of obsolete political ideas and structure. The heart of the planning problem is now seen as to create the essential highly trained post-graduate cadre able to combine the necessary breadth of view with real professional expertise in asking and obtaining prompt answers to the right questions with the aid of mass data processing and sophisticated forecasting techniques. It is not, of course, suggested that these qualities are the necessary outcome of a university career, though, as both Prof. Denbigh and Dr. Lilley recognize, universities and colleges of technology, by modifying their courses, could go far to provide men and women with the qualities required. This is unquestionably of fundamental importance if we are to find planners who create harmony through diversity and opening new choices, rather than imposing uniformity and making the state the master instead of the servant of the nation.

What is required is that from mistrusting we learn to trust the planners and accordingly to evolve a sufficiently sophisticated type of politics to give them ample support,

from some of the best teaching centres in the U.S.—the Massachusetts Institute of Technology, Princeton, Swarthmore, Dartmouth and Bryn Mawr are examples only.

An error in the design of the mass spectrometer was noted: the slideways employ two vee-grooves, and impose too many constraints; one vee-groove and one flat should be used. Oddly, too, the drawings are full of redundant dimensions leaving the amateur mechanic too many checks to do and the professional a problem in deciding which to use as his reference planes. The shop equipment needed for each construction is listed (lathe, mill, air-gas torch, hand solder, etc.), and so are the materials and American sources of supply of some of them. One difficulty to be anticipated is that British standard fittings (screws, pipes, couplings) seldom match the American counterparts. It seems likely, however, that constructional kits for some of the apparatus are now available.

The letterpress occupies about the same number of pages as the drawings. In it, there are hints, warnings and advice about use, evidently prepared for the college lecturer (the art of making vacuum-tight soldered joints; field homogeneity and many tricks in the nuclear magnetic resonance apparatus).

The average educational level of the work seems to be about that of the college of advanced technology in Britain. Probably such colleges will benefit most of all from it, but other technical colleges and traditional universities will find it helpful too. Simply to open it is like a child's first visit to a Christmas toyshop; the brain seethes with phantom adventures and ideas.

After sober consideration, many of our teaching institutions are certain to benefit from this original but sensible work.

A. R. MEETHAM

BOOKS ON NUCLEAR GRAPHITE

Nuclear Graphite

OEEC Dragon Project Symposium, Bournemouth, November, 1959. Pp. 269. (Paris: Organization for European Economic Co-operation; European Nuclear Energy Agency, 1961.) n.p.

THE Organization for European Co-operation and Development (OECD) *Dragon* high-temperature gas-cooled, nuclear reactor project has now been in operation since 1959, and indeed the 25 MW experimental reactor is due to start up at the Atomic Energy Establishment, Winfrith, during next year. The project held its first symposium at Bournemouth in November 1959, and it was very appropriate that this first technical meeting should have been devoted entirely to graphite because, as the chief executive, C. A. Rennie, pointed out in his opening remarks, the whole concept of the *Dragon* reactor is based on the use of this material. Although the European Nuclear Energy Agency did not publish the proceedings of this conference until July 1961, the mass of information given on graphite manufacturing technology was, and still is, worthy of the attention of a much wider audience than the small one which met at Bournemouth in 1959. The publication in the United States of a more recent work with the same title, *Nuclear Graphite* (*Nature*, 128, 420, 1962) prompts a further glance at the earlier book from the European Organization.

The introductory paper by Dr. L. R. Shepherd outlined the design philosophy behind the *Dragon* reactor system, concluding that fuel element components would have to be developed to stand temperatures of 1,000–1,700° C, in an intense neutron field. Since graphite had been selected as the moderator, the main structural member and the fission product containment barrier in the core, the real purpose of the symposium was to assess what processes were currently available or capable of development for producing a suitable form of graphite.

It appeared that a fine-grained graphite of very low permeability and nuclear purity was required, and at the session on manufacture and properties of fine-grain graphite, papers from Morgan Crucible Co., Atomic Energy Research Establishment Chemical Engineering Division, Siemens-Planiawerke (Germany), Société Le Carbone Lorraine (France) and Société Pechiney (France) were presented to show the possibilities in this direction.

Impregnation techniques, as a means of lowering the permeability of fine-grained graphite, were also described, and finally, the various methods used in the laboratory for assessing the more important properties of the final graphite were described.

The title *Nuclear Graphite* for this collection of papers is somewhat misleading, since the book does not give any experience of the behaviour of graphite in-pile, and is solely devoted to considering the special form of graphite required for the *Dragon* high temperature gas-cooled reactor. Nevertheless, the information published on graphite-making is a valuable contribution to the literature, since so few papers appearing, prior to, or since this date have disclosed recipes for making special graphitic materials on a commercial scale.

F. ROBERTS

DESCRIPTIVE OCEANOGRAPHY

The Sea: Ideas and Observations on Progress in the Study of the Seas

Vol. 2: The Composition of Sea-Water, Comparative and Descriptive Oceanography. General Editor M. N. Hill. Pp. xv+554. (New York and London: Interscience Publishers, a division of John Wiley and Sons, 1963.) 150*s*.

THIS is the second of a series of three volumes surveying the present position in physical, chemical and geological oceanography. For reasons of space, marine biology has only been included where it has a direct bearing on these three fields of study.

Following the first volume on physical oceanography, Volume 2 contains a series of review articles on the chemistry and fertility of sea-water, the biological problems related to the various water masses—productivity, fishery dynamics and the distribution of organisms—and largely descriptive accounts of some of the principal current systems. Also included are some fascinating miscellanea such as the largely unexplained seasonal changes in sea-level, a topical discussion of bathyscaphs and deep submersibles, and the navigational and observational uses of the gyropendulum.

Some subjects which appear in this volume, chemical sampling instruments for example, are as old as oceanography itself, while others such as estuary dynamics or the use of neutral buoyant floats are of such recent development as to appear in a collected volume for the first time. It is most noticeable how in recent years a gradual dissolution of the traditional subject barriers has occurred. Chemistry and biology are now largely inseparable in oceanography, and both contribute to our knowledge of physical and dynamical processes. The geographical distribution of both organic and inorganic matter is closely related to the current systems, and a knowledge of these distributions provides information which would otherwise require extensive series of current measurements. The very extent of the oceans makes the task of gathering information most formidable, and in this context the present international programme of observations in the Indian Ocean will prove of great value. It is gratifying to read that even radioactive fall-out yields some useful information on oceanic mixing and the general circulation.

The experts contributing to this volume continue the extremely high standard set in Volume 1, both in comprehensiveness and in the stimulating presentation of their special topics. Not only oceanographers but also all

who are interested in the sea will find themselves sharing their enthusiasm.

One criticism to be levelled at this volume results from the neglect of certain important topics such as western boundary currents. The editors apparently hope to rectify these omissions in a possible fourth volume. The printing and editorial work are again excellent. J. N. Hunt

SILICATES

L'analyses des silicates

By I. A. Voinovitch, J. Debras-Guedon and J. Louvrier. Pp. 510. (Paris: Hermann, 1962). 48 NF.

THE experience of the authors of this book is derived from several recent years in control of analytical operations at the Centre National d'Etudes et de Recherches Céramiques, and silicates here include a wide range of industrial products and raw materials associated with the manufacture of ceramics and with the building industry. Now that the analyst may have at his disposal a variety of physical techniques to supplement or replace chemical methods of analysis, some guidance in the scope and application of these techniques is highly relevant, and it is appropriate that in Part 1, comprising the first four chapters, some physical methods of analysis are discussed at sufficient length to be of real value to the non-specialist. These are flame photometry, absorption spectrophotometry including atomic absorption, emission spectroscopy, and X-ray methods by direct emission and by fluorescence.

Part 2 is concerned with analytical techniques, and begins with a chapter on dry and wet methods of chemical attack appropriate to various classes of materials. This very comprehensive and useful section is followed by a series of schemes for the analysis of aluminous, silico-aluminous and siliceous materials, carbonates, enamels, zircon refractories, chrome refractories, slags, mortars, and soluble clay extracts. Individual spectrographic methods covering approximately the same range of items, occupy the next 52 pages, and there is a short section on some applications of flame photometry by the authors' special 'oxine' technique which is claimed to give increased sensitivity and a reduction of mutual interferences for a number of common elements in the oxy-acetylene flame.

The remaining 200 pages are devoted to chemical methods, varying in number for each of the 28 elements dealt with according to the importance and difficulty of determination of the element; thus aluminium is given twelve methods, gravimetric, volumetric and colorimetric, some of which are, however, variations on a few basic methods, while silicon is alloted five methods, manganese three, and molybdenum one. The layout allows such items as reagents, concentration ranges, interfering ions, and the preparation of standards to be clearly and concisely presented. 'Classical' methods are freely supplemented by colorimetric and compleximetric procedures. Silicon is, for example, determined gravimetrically following an alkaline fusion, by fixation either with hydrochloric acid in the presence of gelatine, or by evaporation with sulphuric acid; or volumetrically by precipitation as potassium fluosilicate with an acidimetric finish; or colorimetrically as silicomolybdic acid with or without reduction to molybdenum blue. Separations by extraction with organic solvents seem to be avoided even, for example, in the colorimetric determination of copper by diethyldithiocarbamate, the coloured compound being here measured in aqueous suspension stabilized by a gum solution. The colorimetric determination of iron by *o*-phenanthroline or dipyridyl is now generally preferred to the one given using sulphosalicylic acid, and no method for ferrous iron has been included. At least one colorimetric method for phosphate would be expected in addition to the two gravimetric methods described. The absence of an index may also be noted in criticism; but the arrangement of the book makes this omission only occasionally a source of inconvenience.

These criticisms on points of detail need not be assumed to detract seriously from the value of a book which contains such a large amount of useful material, clearly and systematically presented, and which is certain to become widely consulted in industrial and other laboratories where siliceous materials are analysed. G. A. Sergeant

THE MIND AT REST

Sleeping and Waking: Physiology and Psychology

By Ian Oswald. Pp. ix+232. (Amsterdam and New York: Elsevier Publishing Co., 1962.) 45*s*. net.

DESPITE the large proportion of human life which is spent asleep, our knowledge of the mechanism of that state or of the biological function which it serves remains unsatisfactory. There is a dearth of good books on the subject, and, as Dr. Oswald says in his preface, most students have relied for nearly 25 years on the book of Kleitman. This has been joined by one of the symposia of the Ciba Foundation, but there remained a need for an account of the work which has been done on sleep since the thirties. It must be said at once that Dr. Oswald's book fills this need, and that it will undoubtedly become an essential part of the equipment of anybody working in this area.

The book starts with a review of the various physiological mechanisms which have in recent years been shown to relate to sleep. These largely concern of course the reticular formation. The particular physiological changes occurring in human sleep are then outlined; and this leads into an account of the changes in the electroencephalogram found during sleep. Using these changes as an indicator of the presence of sleep, the book then proceeds to a discussion of the changes in reaction, memory, and so on which occur at the borders of sleep. A discussion of the physiology and psychology of directed attention and of imagery then leads into an account of some of the curious subjective experiences and hallucinations which may occur when falling asleep or awakening, and so to dreams and the physiological changes which are now known to accompany them. The remaining chapters provide brief discussion of other functions similar to sleep which may be assimilated to it, such as animal hypnosis, experimental neurosis, the occurrence of deaths from emotional shock, fatigue in skilled tasks, brainwashing, and so on. There is a brief mention of diurnal rhythm, a chapter on the effects which may follow deprivation of sleep, and an account of some clinical conditions connected with sleep.

Any attempt to cover this wide topic in a reasonably short space must of course have some biases and limitations. It might perhaps be fair to characterize those which actually appear by saying that the book is much stronger on sleeping than it is on waking. In part this is, of course, because the physiology of sleep has been better studied than has that of alertness during the waking state. Unfortunately, however, Dr. Oswald supplements the real physiology of sleep with some hypothetical suggestions about the role of imagery in human thinking and about the underlying physiological mechanisms which organize and control it, and these can only be described as highly controversial. In general, the thesis is that behaviour becomes increasingly organized as one passes further from sleep towards a high state of cerebral "vigilance"; no consideration whatever is given to the work of psychologists such as D. O. Hebb, K. W. Spence, or the later publications of D. E. Berlyne, who have tried to grapple with the difficulty that thought and behaviour may become inefficient under conditions widely different from those of sleep. Indeed, Dr. Oswald himself describes the hallucinations occurring on the verge of sleep

as similar to those of schizophrenia; and yet also notes elsewhere that amphetamine in large doses will produce the same symptoms. One is therefore somewhat suspicious of Dr. Oswald's repeated suggestion that an inefficiency in behaviour is an indication of incipient sleep.

An engaging feature of the book is the author's willingness to state bluntly his own views on matters which are in dispute. In some such cases he is, from my point of view, on the side of the angels: for example, in questioning the suggestion by Dement that deprivation of dreams rather than deprivation of sleep produces disturbances of behaviour. Another example is Dr. Oswald's opposition to the suggestion of Malmo and Surwillo that deprivation of sleep produces a state of increased arousal. Nevertheless, and no matter how much one agrees with Dr. Oswald, there are a few cases where one feels that the other side of the controversy might have had a little more representation.

In summary therefore, this is an excellent review of the phenomena of sleep and at least a stimulating introduction to those of returning consciousness. It is written with attack and confidence, and liberally illustrated with incidents and cases known to the author. While it has the defects of its good qualities, it is bound to command the field for years to come. D. E. BROADBENT

MYCOLOGY FROM AN UNUSUAL ANGLE

Fungal Genetics.

By Dr. J. R. S. Fincham and Dr. P. R. Day. (Botanical Monographs, Vol. 4.) Pp. x+300. (Oxford : Blackwell Scientific Publications, 1963.) 50*s*. net.

THE present appalling dichotomy in biology, and particularly in the way it is taught and investigated in Britain, comes to mind at once in reading this useful book. On one hand there is 'classical' biology, with its sub-divisions either as to groups of organisms—botany, zoology, microbiology—or as to form and function: comparative anatomy, comparative physiology, ecology, ethology and evolution. On the other hand, there are the recent approaches represented by genetics, molecular biology, cell biology and cellular biochemistry. These have produced in the past twenty-five years a revolution at least as illuminating and far-reaching as that of the nineteenth century linked to Darwin's name.

Some of the practitioners of classical biology ignore, fear, affect contempt for, or, at best, pay lip service to the new ideas and techniques instead of being glad to absorb them in their own daily way of thinking and working. So far the chasm can still be bridged, because the older practitioners of the new biology, by virtue of age and training, have their roots in classical biology: their aim is still that of looking at the same problems in a new light. But the coming generation of molecular biologists, biochemical geneticists and cell biologists may not have any roots at all. If the dichotomy is allowed to go on we shall have two kinds of biologists: those who label migratory birds and those who label ribonucleic acids, neither knowing of the other, and each considering himself as the 'true' biologist.

In this respect, the book under review has a very refreshing quality. Though the authors state that their own research work "has not been instigated primarily by an interest in fungi for their own sake", in fact, out of a total of eleven chapters, at least three—one, introductory, on the biology of those fungi on which genetic research has been substantial, one on sexual development and one on pathogenicity—do reveal such interest, which also appears sporadically throughout. In other words, this is a book in which the dichotomy is not apparent. Since it is a very readable and good account of fungal genetics, which is, after all, an aspect of the biology of fungi, every mycologist should read it and assimilate it. For the geneticist—microbial or macrobial—it will be an indispensable reference book. The authors have made a real and successful effort not to limit their account to their own research interests, important as they are. There could perhaps be a little more about yeasts, but the mixed nature of the literature in this field makes the task very difficult.

The genetics of fungi has contributed decisively to some of the basic problems of genetics: recombination, complementation and alternatives to sex. The parts of the book dealing with these problems make stimulating reading. In addition, the detailed treatment of tetrad analysis is a useful teaching tool. There is throughout the crop of misprints which has become a familiar feature of present printing standards. There are also here and there a few minor factual mistakes and wrong or omitted references: these are minor flaws. The authors can be congratulated on having filled a gap very well.

G. PONTECORVO

BETWEEN THE TIDE-MARKS

British Seaweeds

(The Kew Series). By Carola I. Dickinson. Pp. 232+12 plates. (London: Eyre and Spottiswoode, 1963.) 25*s*.

DURING the nineteenth century when scrap-books, often very beautiful in character, occupied the leisure hours of ladies of fashion, a number of popular books were written, illustrating and describing the commoner seaweeds of British coasts. With the passage of time and life's increased pace these books have ceased to be available and they have not hitherto been replaced. Large numbers of people spend holidays near the shore, but comparatively few of them are aware of the fascinating occupants, both plant and animal, of the area between the tide-marks; modern books, with rare notable exceptions, mostly biological, have not been designed to help them. It is particularly appropriate therefore that Miss Dickinson's book should have been published just now when a determined effort is being made by both national and local bodies to revive the general interest in natural history.

The book is of convenient size and is illustrated by twelve colour plates, mostly of outstanding merit. There are also 92 text figures which vary very much in quality, some, such as those illustrating the species of *Laminaria*, being very beautiful, though others are much less pleasing.

Following a short introduction, the author gives a short account of the structure of seaweeds, their reproduction and their life-cycles. Brief sections follow on environment, collecting and mounting specimens and on the uses of seaweeds. A key to genera precedes an account of some 150 of the commoner British species. The descriptions of the species are written in a semi-popular style so that they can be used by an amateur and a glossary helps to elucidate the terminology. The book is indexed and there is a brief bibliography.

The problem of writing for the layman on such an unfamiliar subject must be recognized, and the author has obviously striven to make the descriptions both lucid and interesting. It is a pity, nevertheless, that in the introduction, words like 'rhizome' and 'stolon' are used without qualification. One hopes that some amateurs will be fired with sufficient enthusiasm to become formal students, so it is a pity to teach them to use words that are only applicable strictly to higher plants. In the same section a statement occurs that "holdfast indicates the sole function of this organ". That is certainly not true, for it may also be an organ of perennation and regeneration in both brown and red algae. These, however, are small points, and the book as a whole is a welcome addition to a series which is making a great deal of valuable material available to British naturalists, in a most attractive form.

LILY NEWTON

Science, Industry and Social Policy

By Kenneth Denbigh. Pp. vii+103. (Edinburgh: Oliver and Boyd, Ltd., 1963.) 16*s*.

THIS brief social criticism of applied science and industry is a notable tract for the times in the true lineage of Patrick Geddes and Lewis Mumford. Written with a refreshing freedom from jargon and giving ample and well-chosen references for further reading, it is a most stimulating challenge to thought as to what new forms of social progress we can achieve in our modern industrial societies. In particular, it challenges the over-emphasis on the economic factor, contending that the overdue orientation of the industrial order needs to be achieved in a social much more than in a purely economic sense. Moreover, while Prof. Denbigh pleads cogently for the individual and the need to preserve freedom of choice and opportunity in his conception of planning he displays the imagination and vision which mark Geddes and Mumford at their best.

The seven chapters of this book in which, successively, Prof. Denbigh discusses trenchantly the short-comings and primitiveness of industrialism as it moves forward, as he believes, into a new phase, the rise of 'economicism', as he describes the practice of accepting economic factors as if they were the only criterion, the relations of consumers versus producers, in which he pleads that work should be so planned and organized as to provide satisfaction to the producer; the paradox of resources, in which our failures in planning industry, towns, transport, etc., are overwhelmingly pilloried, on transcending the machine, on the public conscience and private uncertainty, and on science and world conditions abroad in stimulating suggestions. Questions are raised, implicitly or explicitly, and in a context of realism and vision, which should set the reader searching for an answer. Here is no blue-print for a new social or industrial order but some very shrewd pointers to the way in which that order may be created. The scientist will find some questions about the control of research expenditure or the relations between the scientist and Government which are the more suggestive because they are so unexpectedly raised. The book is short but never superficial, well written and full of ideas, many of which have long since been ventilated but are now formulated with fresh meaning in the context of to-day's needs and potentialities. R. Brightman

Enzymes and Drug Action

Edited by Dr. J. L. Mongar and A. V. S. de Reuck. (Ciba Foundation Symposium, jointly with the Co-ordinating Committee for Symposia on Drug Action.) Pp. xv+556. (London: J. and A. Churchill, Ltd., 1962.) 65*s*. net.

THE scope of this symposium is not as wide as its title would suggest. On the enzymatic side, it includes a paper on carbonic anhydrase and some discussion of microsomal enzymes involved in drug metabolism, but it is weighted disproportionately towards acetyl choline esterase. The drugs considered are mainly those affecting muscular contraction or acting on the central nervous system; the action of some hormones is also discussed.

The symposium, which was held in March 1961, was in two parts. The first part was a large gathering at which nineteen formal papers were presented. A report of this meeting occupies the first 400 pages of the book. The papers are preponderantly pharmacological in outlook, variable in standard, and many are repetitions of work published elsewhere. The discussions in this part are scrappy and irritating to read; they contribute little of scientific value, though in places they show a lively atmosphere of controversy. The second part, reported in the last 120 pages of the book, is much more valuable. It records the informal discussions of a selected group of about thirty well-known physiologists, pharmacologists, biochemists and physical chemists on such debatable and elusive problems as active transport, the nature of receptor sites and the mechanism of insulin action. The meetings were in four sessions devoted to the general topics of enzymes, receptors, and interactions at sub-cellular and cellular levels. These discussions reveal starkly the lack of common ground between the participants in their basic assumptions and in their methods. A lack of agreement in the interpretation of their results is scarcely surprising, and the outcome of most of the arguments is inconclusive, but the conflict is fascinating and illuminating to the reader. This is a big, untidy book plentifully sprinkled with misprints. It is not a book to keep for reference, but parts of it should provide stimulating thoughts for anyone interested in the way in which drugs work. G. A. Snow

Annual Review of Nuclear Science

Vol. 12. Edited by Emilio Segre. Pp. 633. (Palo Alto, California: Annual Reviews, Inc., 1962.) 8.50 dollars.

ONCE again the editors of the *Annual Review of Nuclear Science* present a series of review articles by authorities in a wide range of specialist topics in nuclear physics. The technical articles cover the subjects of the preparation of thin films for sources and targets, semiconductor particle detectors and research reactors, and theoretical articles cover the subjects of compound statistical features in nuclear reactions, electron exchange reactions, dispersion relation methods in strong interactions, and nuclear astrophysics. In between these extremes are articles on inelastic electron scattering, polarization measurements on β- and γ-rays, and an article on the Mössbauer effect. With a more chemical bias are two articles on isotopic exchange reactions and free radicals in irradiated biological materials.

It would be a wide-ranging physicist indeed who found more than half these articles of more than passing interest, but all active physicists will find this volume a valuable work of reference. T. E. Cranshaw

A New Mesozoic Flora from Tico, Santa Cruz Province, Argentina

By S. Archangelsky. *Bulletin of the British Museum (Natural History): Geology*, 8, No. 2. Pp. 47, 12 plates. (London: British Museum (Natural History), 1963). 30*s*.

SELDOM now does a fossil flora prove almost entirely new, but this one from the Upper Mesozoic of the Argentine does. The present paper deals with Pteridosperm-like leaves and conifer shoots (with a few associated fructifications). They have well-preserved cuticles which are described in detail. The stomata in some are remarkably like those of *Cycas revoluta*, though the finely divided leaf looks like a fern. They are placed in five new genera, and, while the number of genera of such leaves is already inconveniently large, it is hard to see what else could have been done with leaves as odd as these. A little two-seeded fruit is associated with one of them; it is quite unlike anything produced by a cycad, but more like *Caytonia*.

Most of the conifers are new species of *Brachyphyllum;* one is of interest in being associated with *Araucaria*-like male cones. One of the most interesting species in the flora and the only one previously known is *Athrotaxis ungeri*, previously described by Halle on material with no cuticles but with female cones. The cuticle of this fossil now proves very close to that of *A. cupressoides*, which still survives in Tasmania; it is thus a gymnosperm of greatly reduced range.

We may hope that further collection and study will add considerably to knowledge of these plants, which may represent some entirely unfamiliar families.

Tom M. Harris

TECHNICAL DEVELOPMENTS IN THE ELECTRICITY SUPPLY INDUSTRY*

By Dr. HARRY TAYLOR
Director of the Electrical Research Association, Leatherhead

Generation

PROPERTIES of Steam. In the early days of electricity supply, when almost every town had its 'electric light works', the generation of steam as a preliminary to the generation of electricity was a simple matter: simple, but not at all economical. Boilers operated at low pressures and were often fired by hand; engines were of the reciprocating type, with belt-drive to the dynamos.

Steam engine builders were then dependent on very primitive steam tables. With the coming of the turbine, however, and large power stations which supplied not single towns but extensive areas, came the need for increase in steam pressure and temperature to improve turbine efficiency, also an urgent demand for fresh data; and about 1900 Prof. H. L. Callendar began his life-long investigation of the properties of steam which provided the data for the higher pressures and temperatures so essential to turbine designers.

The Electrical Research Association supported, almost from the date of its formation, the work of Prof. Callendar. His investigation of the properties of steam led the Association to arrange international co-operation through the International Steam Tables Conferences held during 1929, 1930 and 1934, in London, Berlin and New York, respectively, and the final result was the publication of the 1939 *Callendar Steam Tables* giving the properties of steam up to pressures of 3,200 lb./in.^{2} and temperatures of 1,000° F.

Prof. Callendar's son and Sir Alfred Egerton carried on these researches, supported by the Association, and the researches have in recent years been under the control of Prof. D. M. Newitt and Mr. S. Angus, who have extended the work up to 14,000 lb./in.^{2} at 1,100° F and to somewhat lower pressures at higher temperatures.

The Association has continued to play a full part in international collaboration, conferences having been held in Moscow and Munich and planned for New York. As a result, *British Steam Tables* will shortly be available up to a range of temperature and pressure well in advance of anything needed to-day. Having achieved this position, and having paid for most of the British research over a period of forty years, the Association feels that it has achieved something well worth while, and can now safely leave it to others to carry on in a range which is full of theoretical interest but little practical importance to-day.

Properties of Steel. The efficiency of power generation has improved over the years. In the 'twenties, anything above 20 per cent was a figure to be proud of; to-day, all modern stations exceed 30 per cent, and the highest efficiency recorded by the Central Electricity Generating Board during 1961–62 was 34·5 per cent. Many factors contribute to improved efficiency, such as increased unit size, increased combustion temperature, reduced flue gas temperature, better thermal insulation, regenerative feed heating, combustion air pre-heating and hydrogen cooling of the alternator, but none of these single features is so important as higher working temperatures and pressures of the steam. These set a limit to the efficiency theoretically obtainable. The story cannot be continued better than by reproducing the words of Dr. N. P. Allen of the National Physical Laboratory (*Co-operative Electrical Research*, 13, July 1961):

"But there is a limit to the temperatures and pressures that the materials of construction can withstand, and if too severely stressed at high temperatures, superheater tubes and steampipes will swell gradually, and perhaps burst, turbine rotors and blades will deform, to the detriment of the dimensional accuracy upon which the working of the turbine depends, and bolted joints will relax, and no longer be able to prevent the escape of steam.

"It has therefore been necessary to determine very accurately the maximum stresses that the materials of construction can sustain at each temperature, and this has proved to be complicated, since steels at high temperature undergo slow changes which generally reduce their strength. Electricity generating stations operate as a rule for upwards of twenty years, and to be sure that no difficulties will arise in this period, very prolonged tests of several years' duration have to be made. If, in the interests of increased thermal efficiency, steam temperatures and pressures are raised, new steels, stronger and more heat-resistant than the old, have to be developed, and these must be subjected to an extensive and tedious process of testing before their capabilities can be properly assessed.

"When, about forty years ago, it was first realised that the high temperature properties of steel would limit the efficiency of electricity generation, it was not uncommon for steam to be raised at 600 psi and 680° F (360° C). Today, steam conditions of 2,000 psi and 1050° F (565° C) are becoming commonplace. The value of the saving of fuel, due to improved efficiency is immense, amounting to many millions of pounds every year. But it is known that heat-resisting steels and other materials exist which make it possible to do even better. Efficiencies of over 40 per cent are in sight, and 45 per cent appears to be worth working for.

"The Electrical Research Association first came into this work in 1930. At that time there was great controversy about the methods to be employed in the testing of steel at high temperature, and still more controversy about the way in which the results should be used in design. The Electrical Research Association formed a committee, upon which steel makers and turbine makers and users were represented, and this committee sponsored the accurate investigation at the National Physical Laboratory of the high temperature behaviour of certain typical steels. The mechanical testing was done in the Engineering Division of the National Physical Laboratory and the associated changes of metallurgical structure were examined in the Metallurgy Division. Very satisfactory methods of testing were developed, and in due course a creep testing laboratory was built which contained upwards of forty high-accuracy creep-testing machines. This was at that time considered a very large and up-to-date equipment, upon which the equipment of several other laboratories was modelled. Mild steel was examined first, and then 0·5 per cent molybdenum steel, and a variety of other low alloy steels, in various forms.

"As knowledge of the properties of steels increased, and steam-raising temperatures rose, the number of special creep resisting alloys that it became desirable to examine also rose. The combined effect of these three influences was that the testing programme expanded to such an extent that the creep testing laboratory at the National Physical Laboratory was quite unable to cope with it. It

* Substance of the Fifteenth W. M. Thornton Lecture delivered on June 14 during the Convention of the Association Mining Electrical and Mechanical Engineers at Newcastle upon Tyne.

was therefore decided to build a new and larger creep testing laboratory at the Electrical Research Association, Leatherhead, which would be devoted principally to long time tests."

This laboratory, which is one of the finest in the country, solely devoted to work on the creep of steel, has no less than 600 test points. Of these, 50 are devoted to high precision creep testing such as is required for steel used for turbines, and the remainder are used for 'time-to-rupture' tests, which is the information chiefly needed for boilers, steam pipes and superheater tubes.

The Electricity Council's Annual Report for 1957–58 states that—"The consumption of coal at the power stations in England and Wales in the ten years since vesting day would have been some 41·4 million tons greater and would have cost some £147 m. more, if average thermal efficiency had remained at the 1947–48 level."

Through its work on the creep of steel, the Electrical Research Association claims credit for at least a small part of this saving.

Steam Pipe Welds. It has now become standard practice in modern power stations using steam at the higher temperatures to eliminate bolted pipe joints wherever possible by welding. This practice has introduced the need for intensive investigation of the service life to be expected from welded joints. In view of the absence of adequate service experience of welded pipe joints in the materials they proposed to use for the new high temperature stations, the Central Electricity Authority expressed a wish for some full-scale testing of pipe welds made on specimen pieces of finished steampipe. The Authority through its Eastern Division, made available to the Association a site at Brimsdown 'A' Power Station (where steam is used at a pressure of 1,900 lb./in.^{2} and 930° F) where such tests could be carried out in electric furnaces so that the temperature could be raised to 1,100° F in order to accelerate the tests.

Briefly, the purpose of the test at Brimsdown Power Station was to simulate so far as possible conditions to be met with in the projected high-pressure high-temperature plants, and under these conditions to test the behaviour of sample butt-welded steampipes supplied by various manufacturers both under continuous high temperature and under cyclic temperature variation to represent operation when plant is started up daily or after week-end stoppage.

It is hoped to have available after a period of testing, material from which information can be obtained as to the conditions of steam-pipe welds in actual service. The test may also be regarded as a useful warning of anything untoward happening in welds that are in service, however unlikely this may be.

The test gear consists of two bell-type air circulating metal tempering furnaces, each containing a group of five pressure vessels fed with a small supply of steam at approximately 1,900 lb./in.^{2} pressure, the extra heat to maintain the vessels at the required temperature being provided by electrical heating. This test rig has in recent years been taken over and completely operated by the Central Electricity Generating Board, which is the organization primarily interested in the results.

Condenser Research. In the steam-generating field other investigations have been undertaken, in co-operation with the Institute of Metals and with the British Non-Ferrous Metals Research Association, on the corrosion of condenser tubes, the Electrical Research Association contributing towards the expense. New alloys were tested, and investigations made on the effect of the presence of impurities both in the tubes and in the cooling water.

An investigation of heat transmission coefficients was also carried out in the University of Manchester for the Association, to furnish a sound theoretical basis and provide new data for condenser designers.

Steam Nozzles. In the early days of its interest in power generation, the Association supported research on the design of turbine steam nozzles. This research, which extended over a period of eight years, was conducted in the Manchester Corporation Electricity Department and made a substantial contribution to the improvement of turbine efficiency.

Miscellaneous Researches. The Association has been responsible for organizing, supporting or co-ordinating a wide range of investigations in the field of power generation. The principal ones have already been mentioned, but others relate to microscopic and mechanical tests of turbine blading material, aquatic growths in condenser systems, effect of biological action on corrosion of condenser tubes, vibration of power station pipework, joint tightness of pipe flanges, grindability of coal and dust sampling of power station flues.

In the field of steam power generation of electric energy the Association has issued no less than 152 reports to its members, and many of these have since been published.

Transmission Lines

Lightning and Surges. Thunderstorms are less frequent in Great Britain than in many countries where transmission and distribution of electricity are effected largely by means of overhead lines, but even in Britain lightning is the most frequent single cause of supply interruptions. Statistics show that there are about 5,000 such interruptions each year, but the situation is not as serious as this criterion might imply. On 11 kV systems, for example, supply can be restored merely by replacing fuses or reclosing circuit breakers in about 80 per cent of cases. In a further 5 per cent damage is only slight and supply can be restored immediately, repair being deferred until convenient; only in the remaining 15 per cent of cases is it necessary to repair damage before the supply can be restored.

Sufficient is known about lightning and its effects to render it possible to secure complete lightning protection in nearly all cases, but to do so for even the majority of lines would add considerably to the cost of electricity. An occasional interruption may be inconvenient, but a big increase in the cost of electricity would be more so. An attempt at complete protection would be justified only for the most important lines; but there are relatively inexpensive palliative measures which can be applied to most lines.

The Association has for a number of years received records of all interruptions to supply which occur in Britain, and an analysis of these will lead in due course to general guidance on the means of providing the appropriate degree of protection to a line consistent with its importance.

Research on surge phenomena was started by the Association in 1930. Until then knowledge of the subject had been gained principally by researches elsewhere, particularly in the United States, and it was important to ascertain the bearing of this on British conditions and methods, also to fill many gaps in the data available. A certain amount of experimental work was done, and in 1933 financial assistance from the Central Electricity Board made it possible to expand the programme considerably, with the collaboration of the National Physical Laboratory and of manufacturers possessing the requisite experience, and to extend research to actual working conditions in the field rather than confining it to the laboratory.

In the autumn of 1935, the Central Electricity Board placed at the disposal of the Association an eight-mile length of overhead line, designed to operate at 33,000 V, which had been erected but was not urgently required for service, and a little later two more lines, each normally operating at 132,000 V, one fifty miles and the other twenty miles in length. Tests on these lines provided

information on the behaviour of different types of surge and the efficacy of the several types of protective device.

As transmission voltages increase to 400 kV and above, a stage is reached when the insulation-levels required against external overvoltages caused by lightning may be equalled or even exceeded by those needed to withstand the so-called internal overvoltages, due to deliberate or involuntary switching operations. A great amount of statistical and experimental data is required to provide information for the design of high-voltage equipment and for a determination of the operational conditions under which the various types of overvoltage are liable to arise.

As the first stage of this work a fully automatic cathode-ray oscillograph was designed several years ago. This equipment, known as the 'E.R.A. Surge Recorder', is housed in a trailer for installation in a sub-station. Connexion is made to the high-voltage system by means of specially designed voltage dividers, utilizing tapped bushings on a transformer as the high-voltage arms, when these are available. The equipment consists essentially of a three-element high-speed oscillograph combined with a rotating drum camera and an electronic circuit which continuously monitors the system voltages. The function of the monitoring circuit is to initiate the beams of the cathode-ray tubes when the system voltage, on any one phase, is subject to a disturbance exceeding in severity a pre-determined level. Severity is judged broadly in terms of rate of rise of voltage.

Each record shows the three-phase voltages for the duration of the disturbance. The recording material is a strip of film one metre long carried on the rotating drum, one revolution corresponding to about three cycles at power frequency. After completion of a record by extinction of the cathode-ray beams, the camera lens is shifted to bring another similar strip of film into recording position in readiness for the next disturbance. The drum rotates continuously and the cathode-ray tubes remain continuously energized (except that the beams are normally extinguished) until three disturbances have been recorded. Recording then ceases but can be restored by exchanging the camera drum for another similar drum loaded with unexposed film, an operation not requiring any special skill. At the instant at which any record is taken an automatic camera photographs a clock face in order to establish the time of the event.

This recorder, which is unequalled anywhere in the world, has been in operation for several years, mostly on the British high-voltage system. A large amount of statistical information has been assembled and many of the results obtained in automatic operations or during specific staged test series have been reported. In addition, evidence has been collected on the wave shapes of different types of switching surge. This information provides a valuable contribution to present-day discussions on standardization of what may be regarded as a typical switching surge.

As already indicated, the importance of switching overvoltages and their effects increase with increasing transmission voltage. In the British Commonwealth, 500 kV and 700 kV systems are being actively discussed. In many of these cases long lines and comparatively small sub-stations will be involved, thus increasing the risk of the occurrence of much more dangerous switching overvoltages than those to be expected in a highly interconnected system with large sub-stations as in Britain. The Association hopes to assist in the solution of at least some of the many outstanding problems to which answers are urgently needed.

Transformers: Short-circuit Forces. It has long been recognized that if a transformer is to give satisfactory service it must be capable of withstanding an occasional short-circuit. Specifications usually stipulate that this requirement shall be met with full voltage maintained across the primary terminals.

Prior to 1940 there was no reason to suppose that all was not well. Transformer designers used accepted methods for calculating the stresses produced by short-circuit currents and designed accordingly.

The introduction of barrage balloons during the Second World War produced conditions which had never previously been encountered. These balloons sometimes broke loose, drifted across country and their cables short-circuited overhead lines. Some transformers failed to withstand these abnormal conditions and interest in short-circuit forces was re-awakened. It was recognized that mechanical failure could be a cumulative process. The greater the asymmetry of the windings, the greater are the forces imposed by a short-circuit. Insulating materials are not perfectly elastic, and compressive forces can effect a permanent reduction in thickness. Consequently one short-circuit can increase the forces imposed by its successor.

The problem was submitted to the Electrical Research Association and a comprehensive investigation was undertaken. This comprised two main parts: (1) investigation of the forces involved; (2) examination of the effects of these on typical insulating materials.

Many reports have been issued on the subject of forces in transformers, and to-day there is little that is not known about the subject. A mathematical investigation has indicated the possibility of a simplified digital computer programme for the calculation of axial magnetic forces and interest is now centring on the dynamic behaviour of the windings under axial short-circuit forces and the determination of the strength of windings to resist these forces.

That the problem is a very real one still is shown by the fact that short-circuit forces were the principal cause of failure on the multiple-unit electric train in the Scottish Region. The Ministry of Transport report in its conclusions reads as follows:

"The damage was caused principally by the frequent application of severe electromagnetic forces, sufficient gradually to distort the outer layers of the secondary windings. This led to the over-riding of turns, high inter-turn pressure, the scuffing of paper insulation and its eventual breakdown."

Transformer Oils. The Electrical Research Association is responsible for various kinds of co-operative research, and this is one where its staff exercises only a co-ordinating function. The contending parties—if such is the right term—are the users of switchgear and transformers on one hand and the suppliers of oil, on the other. As early as 1910 the two parties were talking about sludging of oil and means of avoiding it. The Insulating Oils Sub-Committee played a useful part in the early days, and the work was taken over by the Association on its formation.

Investigations have been made of sludging, acidity tests, electric strength tests, maintenance of oils in service, international standards, inhibitors, and many other aspects of the use of oil in electrical apparatus. All this work has largely been done by the oil supply industry, the laboratories of the electrical supply and manufacturing industry, and the universities. In the last case the Association has provided the money.

Unfortunately, it cannot be said, even to-day, that all the problems are solved, but there is very much less trouble with such oils to-day than there was when the inquiries first started. During this year there will be completed ten years of testing inhibited oils in transformers under practical use in the London area.

Transformers: Ventilation of Sub-stations. Prior to the investigation by the Association no satisfactory method, confirmed by tests, had been produced for determining the performance of the ventilating inlets and outlets of sub-stations. As a beginning of the Association's work, equations were derived theoretically, and were found by independent workers to give closer agreement with experiment than any earlier work. It was still considered,

however, that a comprehensive investigation was essential to cover the detailed effects of ventilator sizes, types and positions on air and transformer temperatures. A small sub-station housing a 100 kV amp 50 c/s transformer has been erected in the laboratory; removable panels in walls and roof allow ventilators of a wide range of sizes to be inserted in practically any position. Transformer, air, and wall surface temperatures are measured by an array of thermocouples. To minimize the effects of changes of laboratory temperature, two sets of lagged thermometers are used to give reference temperatures.

From the experimental results, expressions have been obtained to link the transformer temperature and losses with the ventilation parameters of the sub-station. The discharge coefficients of various types of ventilators are calculated from the air temperature measurements. Inlet ducts, outlet chimneys and fans in ventilators or on the transformer are among other aspects receiving attention.

Overhead Transmission Lines: Tower Footings. Clearly, maximum economy is achieved in the design of an overhead line tower if the strength of the tower in bending is equal to the strength of the foundations in resisting overturning. If the tower bends before it pulls out then it is too weak; conversely, if it pulls over without permanent deformation of the structure itself, then the foundations are too weak. Obviously with varying soil types, it is impossible always to balance one against the other, but experiments were required to determine whether the two modes of failure were approximately the same. These showed that in certain conditions the foundations failed with only half the load required to buckle the tower. If this is a fair generalization—and further experiments are necessary to find out whether it is or not—then the economy possible in tower construction costs over the whole country is very considerable. On the other hand, it could be said that because of inadequate knowledge, the factor of safety on the foundations was inadequate and that these failed when, in fact, neither foundations nor tower should fail.

Overhead Transmission Lines: Wood Poles and Wind Loading. The Association made a comprehensive investigation in the period 1920–30 on the materials and methods used for the construction of overhead lines.

This concerned mainly the strength of single and compound wooden poles. Six designs of compound pole were used—two being 'A' poles, one an 'H' pole, another a French design, another the Rutter pole and finally, one known as the Wedmore pole, after its inventor, the first director of the Electrical Research Association, Mr. E. B. Wedmore. This was a very scientifically designed pole since two separate poles were bolted together with thicker spacers in the middle length than at the ends. In this way the outer fibres of the double poles were pre-stressed in tension and the inner fibres in compression. Since wood is stronger in tension than compression, the pre-stressed pole can be shown to be stronger than a simple Rutter pole.

In concluding his paper to the Institute of Electrical Engineers on this pole research, the late Mr. W. B. Woodhouse said: "The Association's work has for the first time placed the design of compound poles on a sound basis, has condemned as unsound and uneconomical the types in common use, and has evolved new designs in which the materials are used to greatly increased advantage."

It seems remarkable that at this date it was still necessary to make wind-tunnel tests to find the pressure exerted on poles, stranded conductors and lattice towers, but such was in fact the case, and the Electrical Research Association arranged for this basic work done at the National Physical Laboratory. Of the three problems, that of greatest practical importance was the wind loading on stranded conductors, which were shown to react appreciably differently from smooth conductors.

In the discussion following his paper, Mr. Wedmore complained of the shortage of funds for research. He said " . . . we find nothing in it [the paper] which could not have been done ten years ago if the Association had then been given the money to spend which it has been given since. . . . It is obvious to everyone that the paper represents a very big present to the whole industry, showing a return of probably 100 to 1 on the expenditure." This was not the last time that Mr. Wedmore or his successors complained about the shortage of funds, or was able to point to great advantage accruing from research work.

Overhead Transmission Lines: Bond Strength of Reinforcements. This is a good example of a problem clearly defined and restricted in scope which was experimentally investigated and completely solved. So often problems are far from clear, and their elucidation only reveals more unknowns. 'The more one learns, the more there is to learn' is so often the case.

The problem was simple: was the bond-strength between the galvanized leg of a steel tower and the surrounding concrete adequate to prevent slipping without the use of bolts, cleats, or deformations, before the tower buckled? The answer was 'Yes', and the bolts, cleats and deformations have since been abandoned, with a small saving in time and cost for every erected tower.

It should be explained that when the concrete is broken away from a galvanized leg, it comes away quite easily and cleanly, so that there was ample reason to doubt the shear strength.

Overhead Transmission Lines: Strength and Vibration of Conductors. Extensive tests were made on hard drawn copper and aluminium wire and conductors, as a result of which the strength of conductors was expressed in terms of the strength of individual wires, the limit of proportionality and its variation with loading was determined, and new figures were given for the elastic moduli. The properties of composite conductors consisting of steel and aluminium wires were also determined, and the load sharing between steel and aluminium was ascertained, to the great advantage of overhead line designers.

Æolian vibration of wires is very well known, and many of us as children must have put an ear to a telegraph pole to hear the singing of the wires. The vibrations are caused when the frequency with which eddies are thrown off from opposite sides of the line corresponds with the natural frequency, or a multiple of the natural frequency, of the span.

With stranded conductors the continual vibration, which is generally associated with low wind speeds, gives rise to strand failure at or near the clamps. Between layers it is described as 'nicking', and between clamp and outer layer it is called 'hammering'. The former appears to be a case of fretting corrosion such as commonly arises when the parts are firmly clamped together but where microscopic movements can still take place.

The explanation for the vibration, with examples of mitigative measures, was given by Whitehead and Morgan in an early report of the Association, and experiments and further inquiries were reported in 1940 by Double and Tuck, also of the Association. The importance of nicking and hammering was clearly shown in the latter work, and these authors examined various types of damper and a German vibration-free conductor. They showed that lines most prone to the trouble were those having a small ratio of weight per unit length to diameter, for example, steel-cored aluminium conductors and hollow copper conductors—but that vibration does not always cause failure. They advocated the use of dampers and showed that trouble was reduced with lowering of tension and reinforcing the conductor at clamps.

In recent years the matter has come to the fore again with the object of finding whether the working tension of low power rural lines could be increased to allow a corresponding increase of span length. It was anticipated that fatigue failure at points of support might arise with the increased tension, but all the evidence goes to show that the principal failures were those of the binding, not the

conductor, and that the advantages of increased tension did not justify the additional risk of failure.

Overhead Transmission Lines: Current Ratings. The current which a conductor can carry depends on the permissible temperature rise and the rate at which heat produced is dissipated. In the case of overhead lines the dissipation is by radiation and convection—the former being affected by the absorption due to solar radiation, and the latter being affected by the wind velocity. Other complicating factors are the stranding, and the emissivity of the conductor surface, which depends very much on its age and the weather conditions in the locality.

In order that the maximum safe current for any overhead line in any given condition could be ascertained, it was necessary to make experiments to determine the coefficients of convection heat loss in still air and in wind of various speeds, the emissivity of the conductor surface, and the effect of stranding on the electrical resistance of the conductor (and hence the heating) and on the loss of heat by convection. All these factors were unknown when the Electrical Research Association started its researches before the War; but by the time the work was completed and reported in 1949, it could be said that data were available which made it possible to determine the safe current for any practicable conductor in any reasonable condition of use. This work still stands to-day, and, in fact, is now probably more greatly valued than ever before, as it is becoming increasingly important, for economic reasons, to avoid over-design.

Interesting features which came out of these investigations were that: (1) Smooth conductors cool by convection better than do stranded ones. (2) A wind as low as 1 m.p.h. (which is the minimum sustained value generally encountered in Great Britain) increases the permissible current by as much as 50 per cent for a large power line and by even greater amounts for small lines. (3) Aged conductors can carry appreciably more current than new ones for a given temperature rise.

Circuit Breaking. Circuit breakers are the key pieces of apparatus protecting electrical systems, and their contacts form part of the electrical circuits which they are designed to break when they separate. The insulating gap formed between these separating contacts is the final barrier in the protection of the generating plant and equipment and, if the initial arc between them fails to clear, great damage, amounting at times to disaster, may result.

The first director of the Electrical Research Association was a switchgear designer, so it is scarcely surprising that its first experimental work was in this field. In those days a heavy circuit breaker was one capable of breaking 150–250 MV amp. To-day, the largest breakers will cope with about one hundred times this amount of energy, and the system generally used, namely, the air-blast extinction, is the one invented by Wedmore and Whitney within five years of starting research on circuit breaking.

To-day the breaking capacity of circuit breakers has become so large that it is impossible to test them at full capacity and there is great interest in what is called 'synthetic testing'. This is a system in which the current and voltage at break are supplied from different sources and the energy used is small, although the circuit breaker is submitted to what it is hoped are identical conditions to those occurring in a full power test. The Electrical Research Association produced the circuit which makes this kind of test possible, and extensive investigations are in hand to ascertain on relatively small circuit breakers whether in fact their behaviour under power breaks is identical with their behaviour under synthetic tests. If it is, and the position is thoroughly verified, then it will be possible to accept synthetic tests as a means of measuring the rupturing capacity of the largest breakers—an achievement which would be of very great value to the power supply industry and to the manufacturers.

Network Analysis. The complexity of a problem does not have to be very great before pencil-and-paper calculation becomes formidable. In some of these cases the results will best be obtained with the aid of a digital computer, but often the necessary calculations may be greatly assisted by using a model. Such a technique is well established for investigating the behaviour of ships in tanks or aircraft in wind tunnels.

It is even easier to construct a model to represent the behaviour of an electrical network, since the electrical quantities can be modelled independently of the physical size of the system. Furthermore, in most circuits the quantities may be considered to be linear, that is, doubling the voltage doubles the current.

Electrical network models—or 'network analysers'—are like other models in that their various parts represent parts of the original system to a definite scale and measurements on the model can be referred to the actual system values by means of a known scale factor.

When the Association was considering building a network analyser, various aspects of its use had to be borne in mind. It was recognized that internal research would not occupy the analyser full time and that it would be available for use by individual members, especially the supply industry and consultants.

As is well known the Association has, over a long period, been actively concerned with investigating the flow of harmonics in power systems and rates-of-rise of restriking voltage. The analyser had, therefore, to be well suited to these investigations. This was achieved by designing an analyser for operation not at a single frequency only but over a frequency-range of 100 : 1.

The Electrical Research Association analyser can be used to study, for normal operating conditions, the flow of active and reactive power, busbar voltages, the effect of changing transformer taps, and the effect of varying the source of power. These are what are called steady-state conditions, but the analyser can equally well be used for transient conditions such as increasing the peak power which a circuit breaker may have to clear under fault conditions and the effect of the location of the fault.

There are many network analysers in the country, but few have the accuracy and versatility of that at the Electrical Research Association, which was entirely designed and made by the staff and is now in constant use.

Fire Fighting. The dangers of fire need no emphasis, even in ordinary conditions; but when a fire occurs in or near an electrical installation, very special risks become prominent which need expert knowledge and an entirely revised technique if they are to be quickly and effectively countered.

One of the most serious situations, where electrical plant is concerned, occurs when fire threatens a power station, or a large sub-station; inflammable insulating materials, such as oil in quantity for switchgear and transformers, rubber and other substances are here part of the normal equipment. Even a comparatively small fire then becomes an imminent danger to the continuity of supply. Experience of such fires indicated that they were difficult to deal with, not only on account of the presence of inflammable materials, but also because fire officers feared risk to the lives of their men owing to the live electrical gear. Instances are on record where fires which could have been extinguished at an early stage have become dangerous through delays due to this quite natural hesitation. The Association therefore put in hand a thorough investigation of methods suitable for combating fires in electrical installations, with particular reference to oil fires.

The knowledge thus gained proved most valuable. Permanent records were obtained by the use of colour cinematography of the behaviour of fires and of fire extinguishing. After analysis, the results of the whole series were placed at the disposal of the electrical industry, the fire plant industry, the London Fire Brigade, and the Fire Officers' Committee, and through this enterprise a complete set of recommendations was drawn up which

now serve as a basis of instruction on the handling of electrical fires.

High-Voltage d.c. Transmission. This subject was considered so important in Germany that a high-voltage d.c. Berlin-Elbe trial line project was started during the Second World War (in 1941) and persisted in despite heavy air-raid damage to the Berlin terminal station.

The system was ready for tests when the Russians occupied their sector, dismantled the plant, and sent it east without allowing any tests to be made.

The late Dr. F. Busemann, who joined the staff of the Electrical Research Association in 1947, was an outstanding expert on the subject and, while with the Association, wrote twenty-three technical reports, filed some half-dozen patents, and made check tests, using the Association's model transmission system.

Section B: Transmission of Power at High Voltages, first met in May 1949, and prepared a detailed high-voltage d.c. transmission trial scheme giving particulars of equipment required, costs involved, and a time schedule for construction. The cost was £1½ million spread over seven years.

At a meeting of Section B in the summer of 1951, this scheme was referred to in a lengthy report to Council, who set up a panel to consider the matters raised. In 1955 the panel recommended that the future need for high-voltage d.c. transmission in Great Britain, and especially abroad, called for immediate inception of a programme of development and trial, and that manufacturers should be urged at high level to initiate valve development for a rating of 50–150 kV 250 amp, and assurance should be sought that one or more manufacturers were prepared to commence such development forthwith. In the meantime, Section B should proceed with its investigations to provide all the information possible.

These recommendations came to nothing; they were in advance of their time. Industry, both manufacturing and supply, was too concerned with nuclear power development to devote staff or money to high-voltage d.c., but within a very few years, the success of the Swedish Gotland scheme directed attention to the possibility of a d.c. cross-Channel link and this, as is well known, was eventually carried out, the necessary valve research having been done in Sweden. Once again Electrical Research Association had pointed the way; others had followed.

Distribution

Cables. One of the Association's first tasks was to find a more scientific basis for cable loading. The co-operative nature of the Association's structure enabled the manufacturers on one hand to supply information on the maximum permissible temperature at which the cables could operate, the supply authorities to provide the means for carrying out field tests, and the staff of the Association to compute the corresponding ratings. The report on the first investigation furnished for the first time reliable information on the heating of buried cables in relation to cable design, properties of the soil and atmospheric conditions.

Research on cables has continued from that day till this, and a summary of the Association's achievements is given here. Most of the easier and more rewarding jobs have now been done; but investigations in hand at present are of very great importance, though undoubtedly difficult. Examples are the determination of the effect of the grain size distribution of backfill sand on the movement of moisture away from a loaded cable, and the assessment of cable deterioration and the reduction of life arising from overloading.

Summary of Achievements. (*a*) Considerable economies in the use of cables already installed and in expenditure on new cables have resulted from investigations of cable ratings. This would have been brought to the stage at which continuous ratings for practically any type of cable and mode of installation are available or can readily be computed. The Electrical Research Association is the recognized standardizing body in Britain in this connexion.

(*b*) Similarly, cyclic and intermittent ratings are available or can be calculated by methods laid down by the Association for any load conditions specified.

(*c*) Short-circuit effects have been investigated and appropriate ratings are available for many types of cable.

(*d*) Recommendations have been made regarding the design and layout of joints and terminations for ducted cable runs so that failures in such runs, which have in the past resulted from movement of the cable, may be avoided.

(*e*) Methods of laying and bonding have been recommended which will avoid interference with communication systems, damage to cable sheaths by corrosion and hazards arising from excessive sheath voltages.

(*f*) Methods of test have been developed for the measurement of dielectric loss, impulse withstand and quality of insulation in cables and of thermal properties of soils.

(*g*) Considerable knowledge has been gained of the behaviour of soil under conditions of temperature gradient existing in the neighbourhood of buried cables, and the conditions are known in which the loading of a cable is such that the thermal resistance gradually increases to a very high value due to drying out.

Overhead Distribution Lines. In 1946 the Electrical Research Association issued a review of the work which it had done in the previous twenty-six years on overhead lines. This work, much of which applied to distribution lines, was covered by 44 reports dealing with properties of conductors, conductor clamps, insulators, wind pressure on lines and supports, shock and fire risk with wood poles, tower and pole foundations, concrete poles and line design.

Of all this work none was more important than that which culminated in the production of British Standards 1320: "High-Voltage Overhead Lines on Wood Poles for Lines up to and including 11 kV." This applies to both three-phase and single-phase distribution lines where the normal voltage to earth does not exceed 6·6 kV. The design is specified in detail and is based on an original design evolved by the Association. The specification describes the types and sizes of conductors, minimum height, length of span, details of supports, cross arms and insulators, earthing and loading. Conductor sags and tensions, and wind loading tables are also given.

In 1956 Mr. H. W. Grimmitt wrote of this work in *Co-operative Electrical Research*: "It is obvious that there is a substantial saving in money when overhead lines are erected according to B.S. 1320. . . . Last year about 3,490 miles of overhead lines were erected in England and Wales, to B.S. 1320. Of these, 3,118 miles were of 0·025 sq. in. copper or copper equivalent and 372 miles of 0·05 sq. in. This shows a saving of £423,400 . . . the above figures do not include the lines in Scotland or Northern Ireland. It would therefore not be unreasonable to suggest that at the present rate of construction, the E.R.A. has been the means of saving the Supply Industry about £500,000 a year."

Earthing and Safety. When the Electrical Research Association started to look into the subject of earthing in 1930, virtually nothing scientific was known about it by British electrical engineers. Practice was largely based on experience with lightning conductor earthing. The only earth electrode known was an iron casting set in coke and, whatever its shape, it was called an 'earthplate'. There was no knowledge as to how its resistance could be calculated, and instruments for measuring its resistance had only just appeared. No one measured soil resistivity; chemicals were never used to reduce the resistance; nothing was known about current loading capacity; cattle sometimes got shocks near earth electrodes but no systematic investigations had been made to account for the phenomenon; people got shocks in domestic installations but if an earthplate was provided the reason was regarded as a mystery. The whole situation was

thoroughly unsatisfactory and ripe for investigation, and since the supply industry, the contractors and the public were all involved, it was an appropriate subject for co-operative research.

Steady investigation through the 'thirties solved most of the problems and put the subject of protection by earthing on a sound basis, but it also revealed that satisfactory protection by earthing was not always economically feasible. Alternative methods were examined and practice in other countries studied. As a result, protective multiple earthing was introduced in Great Britain and found entirely satisfactory in areas of high soil resistivity. At first, as a precautionary measure, the conditions of application were somewhat onerous but, on the advice of the Association, these were later relaxed by the Ministry of Fuel and Power, and the system has since been more widely used with complete success.

Utilization

Space Heating. Most of the information so far given relates to improved methods of supplying electricity; but the Association has also made substantial contributions to what might be called the commercial or consumers' side of the industry, and one of the most important deals with the problem of floor heating.

One of the drawbacks of floor heating is that it cannot be turned off if the day should turn unexpectedly warm. The only solution for overheating in such circumstances is to open the windows. If, however, the heat extraction could be controlled, this would add greatly to the merits of off-peak underfloor heating, and research on means for doing this is now in progress. Also, investigations are being made to find out whether or not it is possible to control the heat input overnight (by controlling the time of switching on) to match the conditions forecast for the following day by the Meteorological Office.

There is no doubt that, with the continually rising standard of living, there is increasing use of central heating, and considerable research is still necessary if the electrical industry is to secure a good share of the potential business. Moreover, unless the energy is supplied "off-peak", such a load could well be an embarrassment rather than a blessing. Another move to prevent this is an investigation of chemical methods of thermal storage.

Rural Load Building. The Rural Electrification Department was formed by the Council of the Electrical Research Association in 1937 to encourage the extension of electricity supplies in rural areas by investigating methods by which the use of electricity in agriculture and horticulture could be increased with advantage to both consumer and supplier. The achievements of the supply industry since that time in fulfilling its rural electrification expansion programme have been greatly facilitated by the subsequent work of this Department.

During the twenty-five years of its existence, the Department has built up a valuable combination of experience, facilities, and contacts, enabling it to carry out research in the various aspects of the use of electricity in agriculture and horticulture. Since its inception the Department has produced 48 reports, dealing principally with the following subjects: soil heating, hay and grain drying, electrically driven farm machinery, soil sterilization, greenhouse lighting and heating for tomato and flower cultivation, effect of heat and light on poultry raising and egg laying.

This work has had a significant influence on the use of electricity on farms and in nurseries.

Integrating Meters. As a means of extending the life of the footstep-type bearing used in most electricity meters, extensive research has been carried out over a number of years by Mr. G. F. Shotter and Dr. G. F. Tagg which has led to a considerable knowledge of the phenomena associated with such bearings. Prior to this research very little was known of the many factors governing the performance of bearings of this type, but it is now possible, from the accumulated data obtained from this work, to design bearings with some certainty as to the performance that can be obtained. This knowledge has already been applied in practice and has led to a considerable saving of money to the supply undertakings in so far as the period of 'servicing' of meters has been extended. Whereas periods of the order of seven years were common some 15–20 years ago, the period can now be extended to twenty years when the principles laid down in the various reports of the Electrical Research Association are applied.

Radio Interference. From the earliest days of broadcasting, the Association has been involved in the problem of preventing radio interference. The power supply industry is affected in two ways: first, the low-voltage distribution systems are often the carriers of the interfering voltages, and second, at transmission voltage interference is created by corona discharge on lines and insulators, which is particularly noticeable during rain, fog, or snow.

The chief part which the Electrical Research Association has played has been to develop apparatus and methods for measuring the degree of interference, and to collaborate in arranging international standards of measurement. It has also been responsible for remarkable work on screening of radio waves so as to confine them to, or restrict them from, a given region. The application of this particular work made it possible to save about £10,000 in the type of screening used for the converter station for the cross-Channel power link.

Inductive Interference. When power and communication circuits run parallel to one another, currents in the former induce voltages in the latter which interfere with the satisfactory operation of telephone lines. From information collected by the Electrical Research Association, with the assistance of the Post Office, two main problems emerged: the danger of excessive induction in telephone circuits due to short circuits on power systems, and interference caused by unbalanced harmonic currents in power installations during normal operation. As a result of co-operation between the Post Office and the power authorities under guidance of the Association, and with the aid of the technical resources of the Association, these problems were analysed and finally overcome.

The work of the Electrical Research Association in this field has culminated in the production of a book which is a classic on the subject (H. R. J. Klewe, *Interference between Power Systems and Telecommunication Lines* (Edward Arnold, 1958)).

Load Research. To conceive electrical research as concerned only with the strength, electrical qualities, and suitability of materials to their purpose would be a mistake; there are many problems of direct interest to supply undertakings coming under what may be termed the human or personal aspect. After all, such undertakings serve a community, and a community is composed of individuals. What is their reaction to the electrical service offered? To what extent and in what manner do domestic consumers use electric cookers, water-heaters and other appliances; and in industrial areas is any special type of apparatus providing an exceptional load which requires special consideration?

The answers to these and other allied questions mean a great deal to the supply engineer; from them he can gather information which will enable him to determine the capacity of distribution plant, to estimate the cost of supply and, in brief, to plan his supply efficiently.

A section of the Association was formed during the Second World War to investigate such problems. Its activities fell under two general headings: "Load Research", and "Cost Research". Under the first, the characteristics of the various kinds and classes of loads on supply systems were investigated. The domestic field, owing to its potentialities, received particular attention, and extensive load records were obtained in a number of places; in addition, already existing material was

collected and analysed. In particular, tests were conducted in the systems of two undertakings with a view to deriving collective load curves of large groups of water-heaters.

Research into cost of electricity supply was conducted with the object of applying the scientific method to a field which, while of vital importance to the progress of electrification, had hitherto lacked systematic scientific treatment. As a basis for such treatment, a scientifically arranged system of cost classification was devised, with a view to being developed into a standard for the whole supply industry. A further step was the development of an improved method for allocating to classes of consumers the fixed portion of the cost of supply.

The work of this Section proved so successful that, by arrangement, it was taken over entirely by the Electricity Council.

Conclusion

The Electrical Research Association has many functions: it serves the manufacturers and users of electrical apparatus, the electricity supply industry and the general public. Its contribution to the supply industry has been selected for special attention here because it is a field in which its contribution is diminishing with the changing situation, and it seemed worthwhile to place on record the very significant part which the Association has played, particularly in the early days.

The nationalization of the electricity supply industry and its great increase in research activity has reduced its dependence on co-operative research at the Electrical Research Association. There is still, however, work to be done; the Association still makes a worthwhile contribution, and by adapting itself to the new situation, hopes to continue so to do for many years to come.

A MAGNETIZED ARTIFICIAL METEORITE

By Prof. CHARLES M. CARMICHAEL
Department of Geophysics, University of Western Ontario, London, Canada

On October 21, 1962, an object was found embedded in sod-covered ground which sloped at about 20° to the west, in Moore Township of Lambton County, Ontario, Canada, at about 42° 50′ latitude, 82° 17′ longitude. The sod was bulged up on the down-slope side, but the direction of entry appeared to be vertical. The broken sod was very fresh showing it had been disturbed since the last rain, which fell a week previously. In February 1963 I went to the site, cleared away the ice and snow and fitted the object into the hole to obtain a precise orientation relative to the Earth's magnetic field at the point of impact. Fig. 1 is a photograph of the object on the ground beside the impact hole, which is partly filled with water. The bottom of the hole is about 1 ft. below the mean surface.

The object weighs 15 kg and consists of two blocks of dark grey to black porous ceramic material. The blocks are fused together and are badly distorted by expansion on one side, but appear to have been originally rectangular with dimensions roughly 10 cm × 15 cm × 30 cm. The blocks were originally covered by a brick-red coating a few mm thick on the side and up to 1 cm thick at the corners, but this coating has come off about one-third of the total area. Polished section analysis shows the material to be an impure glass containing small grains of magnetite, ilmenite, hæmatite, pyrite and pyrrhotite. X-ray analysis of the undistorted part shows quartz, but in the expanded portions there is also the mineral cordierite. Heating experiments and the presence of cordierite indicate the expanded portion has been heated to a temperature of 1,000° C.

Eight cores 1¼ in. in diameter were drilled with a diamond core drill from the top and sides so as to include both undistorted and expanded material. From these, 15 separate specimens were cut for measurement in an astatic magnetometer. The directions of the remanent magnetization of the specimens are shown on a Schmidt plot in Fig. 2. This shows the whole block to be consistently magnetized throughout. The average direction of magnetization has a declination of 84° relative to magnetic north at the impact site and an inclination of 13° above the horizontal. A.C. demagnetization and field cooling experiments show the natural remanence of the expanded portion to be a thermoremanence produced by cooling from above the curie temperature in a field of slightly less than 0·5 œrsteds. The magnetization of the undistorted portion is also a thermoremanence produced in the same field below a temperature of 400° C.

A comparison of the impact of the object with that of the Dresden meteorite which fell in the same area in 1939, indicates an impact velocity consistent with free fall from above the dense atmosphere. Since the object is obviously man-made it can be best described as an artificial meteorite.

The properties of the artificial meteorite suggest that it is a part of the thermal shield of a satellite vehicle. The time of fall makes it probable that it came from 1962 *NU*1 or 1962 *B* β1 which decayed on October 17, 1962, and October 14, 1962, respectively. Of these the former, which is *Cosmos* 3 launched by the U.S.S.R. on April 24, 1962, seems most probable because of the date of its decay and the 49° inclination of its orbit. Another possible source could be one of the satellites in the National Aeronautics and Space Administration listing of June 25, 1963, which had not previously been announced.

From the physical distortion and the magnetic and thermal properties a probable sequence of events can be deduced. This portion of the thermal shield must have separated from the vehicle while in orbit or during the early stages of its re-entry. Since one side only shows evidence of being heated, the object achieved a stable orientation in the Earth's gravitational field. Friction



Fig. 1. Photograph of artificial meteorite beside the impact hole, which is partly filled with water. The holes are those left by the core drill used to get specimens for magnetic measurements; these holes are 2 in. in diameter

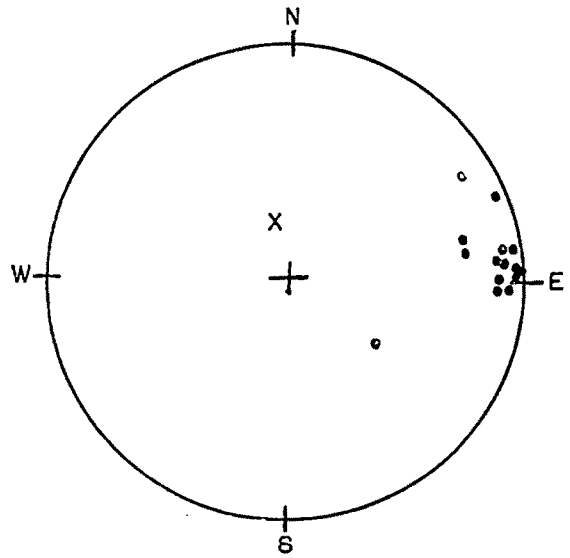


Fig. 2. Schmidt plot of the directions of the remanent magnetization of specimens cut from the artificial meteorite
O, Below horizontal; ●, above horizontal; ×, direction of Earth's magnetic field at the impact site

with the upper part of the atmosphere heated the forward portion at a rate balanced by conduction to maintain a surface temperature of 1,000°–1,200° C for a period of several hours. In the denser atmosphere the velocity would decrease more rapidly, an air cap would form and the temperature would fall. As the temperature fell below the curie temperature of the magnetic minerals they would receive their thermoremanence. Since the object was near the northerly limit of its orbit it would be travelling in a nearly west-to-east path so that the Earth's magnetic field would have an almost constant direction during cooling to produce a consistent magnetization. Near the end of its path the horizontal velocity would decrease rapidly, and it would go into a near-vertical free fall at a terminal velocity similar to that of meteorites. In this part of the flight the object would probably rotate slowly until it hit the Earth.

Whether one agrees with this sequence of events or not, these are several facts which are important for interpreting the magnetization of meteorites. An object has been heated in the atmosphere to a temperature of 1,000° C for a distance of 20 cm from its surface. It has since received a consistent thermoremanent magnetization which is not due to the Earth's field at the site of impact.

It would be most valuable if more information about the artificial meteorite could be obtained from the agency that launched it. A detailed report has been sent through Dr. D. C. Rose as chairman of COSPAR, to the Governments of the U.S.S.R. and the United States. This report will be published elsewhere.

MILSTEAD LABORATORY OF CHEMICAL ENZYMOLOGY, 'SHELL' RESEARCH, LTD.

By HUGH HARVEY

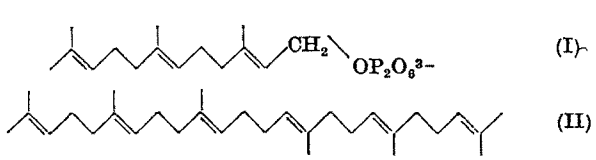
FOR the past thirty years or more the petroleum chemical industry has been supplying farmers with agricultural chemicals. These fall into two classes: fertilizers based on ammonia, and substances used for protecting plants, animals or man against harmful organisms. The second class includes insecticides, soil fumigants, fungicides, viricides, and weed-killers. Petroleum oils were originally used as insecticides in their own right; but the introduction during the Second World War of chlorinated hydrocarbons like DDT and BHC led to the use of chemicals from petroleum as raw materials for making synthetic pesticides. To-day the petroleum chemical companies manufacture and market not only chlorinated hydrocarbons but also organophosphorus and other compounds as well.

This relatively new field of endeavour has led to large-scale research programmes to find and test new chemicals, and to investigate the biochemical properties of the compounds under development. Serving the Royal Dutch/Shell Group of Companies are five laboratories concerned with various phases of research on agricultural chemicals. Two of these, Shell Development Company's Agricultural Research Division at Modesto in California and Woodstock Agricultural Research Centre at Sittingbourne, Kent, are concerned with many aspects of agricultural chemical research, all of which are directed towards increased efficiency in the production of food. In Germany, Shell Grundlagenforschung Gesellschaft at Schloss Birlinghoven works, *inter alia*, on the development of new synthetic routes and new compounds for biological screening at Woodstock and Modesto. Compounds emerging with promise from the screening process, which includes rigorous toxicological examination in the Tunstall Laboratory at Sittingbourne (*Nature*, 196, 809; 1962), are then synthesized on a larger scale to provide material for field trials and to explore manufacturing procedures.

This research effort has recently been supplemented by the new Milstead Laboratory of Chemical Enzymology, also at Sittingbourne, which came into operation in April this year. This Laboratory is under the joint direction of Dr. J. W. Cornforth and Dr. G. J. Popják, both of whom, before taking over the new laboratory, were with the Medical Research Council. Both are Fellows of the Royal Society. As a team, they are best known for their joint work on the biosynthesis of cholesterol.

Their scientific collaboration goes back to 1948 when Dr. Cornforth was working with Sir Robert Robinson (past-president of the Royal Society) on the total synthesis of cholesterol. At that time Dr. Popják was pioneering the use of isotopes to investigate biosynthesis, and he suggested to Dr. Cornforth that it might be profitable to degrade cholesterol synthesized by liver from isotopically labelled precursors.

The degradation of cholesterol, biosynthesized from either $^{14}CH_3.COOH$ or from $CH_3.^{14}COOH$, was duly carried out, and the origin of every carbon atom in the ring structure from either the methyl or carboxyl carbon of acetate established. This and other work, notably by Bloch and Lynen, has made the overall course of sterol biosynthesis better understood than any other biochemical process of comparable complexity. Some of the enzymatic reactions which advance the synthesis step by step are of unusual interest, and elaborate experiments were required to throw light on their mechanism. For example, farnesyl pyrophosphate (I) gives rise to squalene (II),

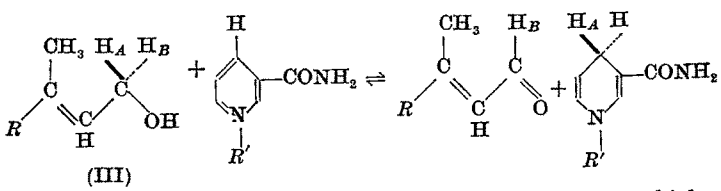


(I)

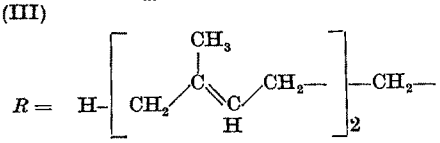
(II)

two molecules of precursor giving one of product in an apparently symmetrical manner. On detailed examination it turned out that the condensation of two molecules of farnesyl pyrophosphate to squalene was not a symmetrical process: one hydrogen atom attached to C-1 of one of the two farnesyl pyrophosphate molecules was lost in the reaction and was replaced by another supplied by reduced nicotinamide-adenine dinucleotide phosphate (NADPH). These experiments required chemical synthesis of isotopically labelled intermediates, biosynthesis by enzyme preparations, chemical degradation of enzymatic products on a micro-scale, crystallization of products in capillary tubes, and their final examination in a mass-spectrometer[1,2].

More recently, Cornforth, Popják *et al.* have shown that the synthesis of squalene from farnesyl pyrophosphate was an asymmetric process in the stereo-chemical sense: the hydrogen atom which is lost from (I) is that marked H_B in (III) and is epimeric with the hydrogen atom (H_A) removed by liver alcohol dehydrogenase from farnesol in the reaction:

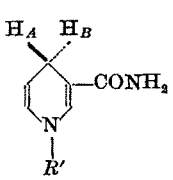


(III)



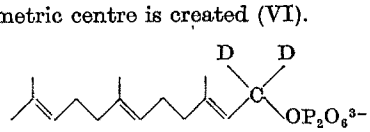
and R' = the remainder of the molecule of NADP or NADPH.

The external hydrogen atom replacing that removed from farnesyl pyrophosphate occupies also a sterically defined position in NADPH: H_B in formula (IV)[3,4]. Furthermore the insertion of hydrogen atom H_B from NADPH into squalene is likewise stereospecific; its absolute configuration was assigned recently. Thus, in squalene synthesized from 1-D_2-farnesyl pyrophosphate (V) with unlabelled NADPH:

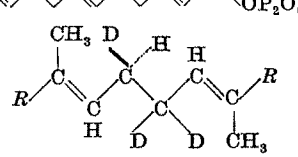


(IV)

an asymmetric centre is created (VI).

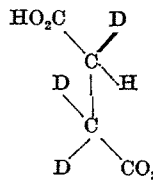


(V)



(VI)

It was shown that the succinic acid, derived by chemical degradation, from the four central carbon atoms of (VI) contained three atoms of deuterium, was optically active and had the absolute configuration *S* shown in formula (VII)[5].



(VII)

These examples serve to demonstrate the value of combining chemical and biological approach to one end, the understanding of the mechanism of enzyme action.

The work of Shell's new laboratory will be concerned mainly with similar investigations of enzymes; but research on topics of shorter-range interest will also be undertaken. The object of the basic research will be to contribute towards a complete understanding of the workings of the living cell, a goal which no longer seems impossible to achieve in view of recent advances. For example, the realization that enzymatic reactions do in general observe the ordinary laws of chemical reactivity *in vitro* opens the way for fruitful investigations in which organic chemistry and biology are blended. It is for this reason that chemists and biologists have been recruited in equal numbers to the staff of Milstead Laboratory.

The shorter-range projects will lie mainly, but not exclusively, in the application of biological chemistry to the development of pesticides and herbicides. It is known that the action of the newer agricultural chemicals, like that of the older arsenic and mercury compounds, is due to their inhibition of enzymes. By examining and observing the part played by particular enzymes in cell metabolism, and the mechanism of their inhibition, it is hoped to make easier the design of agricultural chemicals lethal to selected species and innocuous to other forms of animal life. There is also the possibility that research along these lines may ultimately point the way to biological control of pests.

The staff of the laboratory will initially number thirty, of whom 14 are graduates in physical or biological sciences. In addition to the permanent staff, a limited number of visiting workers from other establishments will be welcomed, and there will be opportunities for post-graduates to work in the laboratories for periods up to three years. Equipment covers a wide range of modern chemical and biochemical techniques, and includes a mass spectrometer, a nuclear magnetic resonance spectrometer, and facilities for radioactive tracer work.

[1] Popják, G., Goodman, DeW. S., Cornforth, J. W., Cornforth, R. H., and Ryhage, R., *J. Biol. Chem.*, **236**, 1934 (1961).

[2] Popják, G., Cornforth, J. W., Cornforth, R. H., Ryhage, R., and Goodman, DeW. S., *J. Biol. Chem.*, **237**, 56 (1962).

[3] Popják, G., Schroepfer, G., jun., and Cornforth, J. W., *Biochem. Biophys. Res. Comm.*, **6**, 438 (1961–62).

[4] Cornforth, J. W., Ryback, G., Popják, G., Donninger, C., and Schroepfer, G., jun., *Biochem. Biophys. Res. Comm.*, **9**, 371 (1962).

[5] Cornforth, J. W., Cornforth, R. H., Donninger, C., Popják, G., Ryback, G., and Schroepfer, G. J., jun., *Biochem. Biophys. Res. Comm.*, **11**, 129 (1963).

OBITUARIES

Prof. G. R. Goldsbrough, C.B.E., F.R.S.

Prof. George Ridsdale Goldsbrough, who died on May 26, 1963, at the age of eighty-two, had a long and distinguished association with King's College (formerly Armstrong College), Newcastle upon Tyne, in the University of Durham. He was a student at Armstrong College, and after graduating he held for fourteen years the post of senior mathematics master at Jarrow-on-Tyne County School. During this period he published papers in the *Proceedings of the London Mathematical Society* on the effect on ocean tides of variations in the depth of the sea and of coastal barriers. His interest in research led to his appointment to a lectureship in mathematics at Armstrong College in 1919, and he was very soon made

reader in dynamical astronomy and then, in 1928, professor of mathematics, which post he held until his retirement in 1948. He became head of the Department when Prof. T. H. Havelock (now Sir Thomas Havelock) retired, and as Sub-Rector of King's College during 1942–47, he played a most valuable part in seeing the College through difficult war-time years and those of the post-war expansion which was taking place. He was awarded the C.B.E. in 1948 for his work as chairman of the Durham University Joint Recruiting Board.

Although the dynamical theory of tides was his first and continuing interest in mathematical research and his papers on this subject appeared until 1954, mostly in the *Proceedings of the Royal Society*, the work that created most interest at the time was his theory of Saturn's rings. A theory of this unique ring system had been given by J. Clerk Maxwell in 1856, who concluded that it was composed of meteorites moving in circular orbits round the planet. The divisions in the ring system had to be accounted for, and the suggestion had been made that they were due to zones of instability around the planet set up by the satellites. In a fundamental paper in the *Philosophical Transactions of the Royal Society* (1922), which was followed by two papers in the *Proceedings*, Prof. Goldsbrough examined the influence of the satellites of Saturn on its ring system, on the basis of Maxwell's theory, and he was able to account for divisions in certain zones which were in good agreement with the salient observed features. For this work he was elected Fellow of the Royal Society in 1929. He returned to the subject again in 1941, when by a different method of solving the intricate perturbation problem, he clarified some points of criticism of his earlier work, and in 1951 he showed that, under conditions satisfied by the Saturnian rings, a pair of rings forms a stable system.

Prof. Goldsbrough had a clear, straightforward approach to any problem in hand, which, combined with his absolute integrity, led to his being much sought after as a counsellor in University affairs. He had an interest in all aspects of astronomy, and on many occasions gave lectures at meetings of the Newcastle upon Tyne Astronomical Society of which he was president during 1944–61. His wife died a few years ago, and he is survived by three daughters.

C. GILBERT

Prof. W. H. Lewis

WILLIAM HENRY LEWIS, who died on May 25 at the age of ninety-four, was educated at University College, Aberystwyth, and Jesus College, Oxford. After seven years (1894–1901) as science master at Exeter School, he joined the staff of the embryonic University College, Exeter, where he held the chair of chemistry from 1901 until 1935; he was vice-principal of the College from 1925 until 1935.

In the first few years of his tenure of the chair at Exeter, Lewis carried out research in organic chemistry and published a number of papers in collaboration with the late Dr. F. D. Chattaway, then at St. Bartholomew's Hospital Medical School. However, he soon found himself fully occupied with the affairs of the struggling young college and during the last thirty years of his occupancy of the chair devoted himself entirely to promoting the interests of the College and of his Department. During the First World War, Lewis, together with his colleagues, the late Prof. W. J. Harte and Prof. A. E. Morgan, was instrumental in the setting up of a Committee for the Furtherance of University Education in the South-west; there is no doubt that it was Lewis's devoted work as a secretary of this Committee which sowed the first seeds of growth which led, in due course, to the foundation, by Royal Charter in 1955, of the University of Exeter. Between the two World Wars much hard work was needed to get the University College fully accepted and once again Lewis was a leading member of a small group of devoted men, without whose efforts nothing lasting could have been achieved. The University College signalized its appreciation of his services by making Lewis professor emeritus on his retirement and the University by conferring on him, in 1957, the honorary degree of LL.D., a distinction which he greatly prized.

With all this, Lewis in no way neglected his Department. Starting from almost nothing, he built up a strong and active department of chemistry, with a striking record of academic successes in the external degree examination of the University of London and a sound reputation for research, which Lewis fostered by attracting good men to his staff and seeing to it that they had the facilities they needed for their work. He was responsible for the planning of new chemical laboratories, in the Washington Singer Building, opened in 1931; it would surely have pleased him to know that his old Department has now outgrown accommodation which must have seemed to many to be over-generous at the time.

Although so much involved in College affairs, Lewis found time to serve on the Council of the Royal Institute of Chemistry (1924–27) and on the External Council of the University of London (1930–35).

Lewis has rightly been called one of the founding fathers of the University of Exeter; as such he has an assured and honoured place in the history of English universities.

H. N. RYDON

Dr. G. Arnold, O.B.E.

WITH the death on August 9, 1962, of George Arnold, not only has the scientific world lost a great scientist but also we, in Africa, are deprived of a figure of outstanding merit, a redoubtable character.

Born in Hong Kong eighty-one years ago, his early education was undertaken, consecutively, in Devon, at Waldkirch in the Black Forest and at a senior school at Compiégne in France. He made full use of this early, varied education and, with his remarkably retentive memory, retained his knowledge of these languages throughout his days. After a spell of clerical work in London he commenced his scientific career at the Royal College of Science, and followed this with an appointment in the Department of Cytology and Cancer Research at the University of Liverpool.

In 1911, at the age of thirty, he was appointed to the curatorship of the ten-year-old Rhodesian Museum in Bulawayo, which eventually expanded, through his leadership, to become the National Museum of Southern Rhodesia and, largely through his investigations, an institute of international repute. Director of the Museum until 1947, he was for many years the only zoologist (in the wide sense) on the staff. Always outstandingly neat, a rare gift for a museum specialist, he was adept at casting reptiles and fish, adding all the intricate finishing touches himself.

Apart from building up vertebrate and insect collections his primary scientific interest was in the Aculeate Hymenoptera. Probably his earliest publication was a joint paper with J. E. S. Moore, director of cancer research. This was "On the Existence of Permanent Forms among the Chromosomes of the First Maiotic Division in Certain Animals", published in the *Proceedings of the Royal Society* (1905). This was followed by papers on chromosomes in *Hydrophilus*, on ovigenesis and on digestion in *Planaria*, on blood, epithelia and chondriosomes, the last of these cytological articles in 1912. Then, in 1912, Arnold read a paper to the Rhodesia Scientific Association on the "Foods of Ants" and afterwards his published works were mainly devoted to Hymenoptera. His first major entomological work was "A Monograph of the Formicidae of South Africa", published in the *Annals of the South African Museum* (1914–26). This was followed by "The Sphecidae of the Ethiopian Region" (1922–31) and "The Pompilidae of the Ethiopian Region" (1932–37) in the

Annals of the Transvaal Museum. A fourth book, *The Sphecidae of Madagascar*, was printed as a special memoir (No. 1) by the National Museum of Southern Rhodesia (1944). The last major work was entitled "Sphecidae and Pompilidae collected by Mr. K. M. Guichard in West Africa and Ethiopia", published as a bulletin by the British Museum (Natural History) (2, No. 3; 1951). Apart from these monographs many papers, chiefly on Hymenoptera, flowed from his pen; papers on the Formicidae and Methocidae of Africa south of the Zambezi, on the genera of African Apidae (1947), on the Mutillidae of Rhodesia and on the Sphecidae and Pompilidae of the Ethiopian Region; and a chapter on insects in the *Victoria Falls Handbook* (1952). The Hymenoptera papers continued after his retirement in 1947 and, in fact, right to the end of his days. He was the proud possessor of the honorary fellowship of the Royal Entomological Society of London, and he was elected president of the Rhodesia Scientific Association for the period 1917–19 and vice-president for several other terms of office.

Of small physical stature Arnold invariably made his presence felt. His alert brain was a formidable weapon in debate or argument, and his ready sense of humour earned him many an admirer even among those who had felt the biting lash of his criticism. He was severely dogmatic and a strong critic in his outlook on scientific workers in his own and other fields, particularly in economic entomology. I first came up against this aspect of his character about twenty years ago. Letters on official matters frequently contained some caustic comment directed at red tape or inefficiency. In his own systematic work he aspired to that point of view of taxonomy described as 'lumping'. To those who had only a passing acquaintance his acid comments sometimes caused resentment. But he always gave what he considered an honest opinion and on better acquaintance these traits were accepted as a matter of course, merely one of the characteristics of his active mind.

On any expedition, even into the roughest and wildest places, Arnold must have been almost unique for Africa in turning out immaculately dressed. This outfit would usually be a well-pressed tropical suit complete with waistcoat, tie, watch-chain and trilby. It did not matter what savage tribe or animals might be in the vicinity. In earlier days he was said to have worn spats, rather than leggings, against the attack of snakes, but in later years he evidently decided that long trousers were either adequate protection or at least more comfortable.

Arnold had enjoyed a last brief expedition not long before his death. He had completed his last paper, and I was able to show him the proofs of this—which he signed—on the night he died. Elliot Pinhey

NEWS and VIEWS

Director of the National Chemical Laboratory, Teddington : Prof. J. W. Mitchell, F.R.S.

Prof. John Wesley Mitchell, at present professor of physics in the University of Virginia, is to return to the United Kingdom following his appointment as director of the National Chemical Laboratory, Teddington. He succeeds Dr. J. S. Anderson, who has been appointed to the chair of inorganic chemistry in the University of Oxford (see *Nature*, 198, 135; 1963). The appointment takes effect from October 1. Prof. Mitchell was born and educated in New Zealand and is a graduate of the University of Canterbury. From 1935 until 1938 he was at Trinity College, Oxford, where he obtained his D.Phil. While at Oxford he worked with Sir Cyril Hinshelwood on a number of problems in chemical kinetics. During the Second World War he was with the Research Department of the Royal Arsenal, Woolwich, and later with the Ministry of Supply, where he developed high-speed photographic methods of studying the fragmentation of shells and the interaction of armour-piercing projectiles with targets. Prof. Mitchell was appointed lecturer in experimental physics in the University of Bristol in 1945, and reader in 1948. While at Bristol his main interests were in the surface properties of thin films of metals and in the nature and formation of the photographic latent image. Since 1959 he has been professor of physics at the University of Virginia, where he has built up a research group for work on the physical and chemical properties of single crystals of metals and other inorganic materials. His personal interests are, at present, principally in the study of the properties of the surfaces of single crystals of metals, in the synthesis of new inorganic materials and in the production of inorganic crystals of the highest possible chemical purity and physical perfection and the study of their properties. Prof. Mitchell was elected a Fellow of the Royal Society in 1956.

Chemistry at University College, London : Prof. A. Maccoll

Dr. Allan Maccoll, who was recently appointed by the University of London to a chair of chemistry tenable at University College as from October 1, 1963, arrived in England from the University of Sydney in 1945 to work on the mechanism of gas-phase reactions in Sir Christopher Ingold's Department at University College. As this work developed, it became apparent that there was a striking analogy between unimolecular gas-phase dehydrohalogenation and the reaction of substitution and elimination of the corresponding compounds occurring in a polar solvent. Further investigations led to the idea that there is a class of unimolecular gas phase reactions the mechanism of which is best interpreted as proceeding through a polarized ion pair transition state, and which is essentially heterolytic in character. These ideas received confirmation recently when rearrangements of the Wagner–Meerwein type, characteristic of carbonium ion mechanisms in a polar solvent, were reported for the gas-phase.

The recent installation of a high-resolution mass spectrometer in the Department of Chemistry at University College will greatly extend the scope of Dr. Maccoll's kinetic work, by making possible investigations of the energetics of carbonium-ion formation, and the effects of isotopic substitution on reaction rates. In addition, he hopes to tackle, by mass-spectrometry, problems of the structure of molecules of intermediate molecular weight. While Dr. Maccoll was at the University of Sydney he was awarded a 'blue' for swimming and was elected president of the Science Association, Chemical Society and the Sports Union. He has acted for many years as assistant tutor and adviser to chemistry students at University College, and during 1954–59 he served on the Council of the Chemical Society of London. Dr. Maccoll has long shown a special interest in the developing universities in the overseas territories which participated in the Special Relationship scheme of the University of London. He has from time to time visited them as London examiner in chemistry.

Electrical Engineering at Birmingham : Prof. H. A. Prime

Mr. H. A. Prime has been appointed to a new chair of electrical engineering in the University of Birmingham. The new chair is additional to those already held by Prof. D. G. Tucker (head of the Department) and Prof. J. T. Allanson. Its object is to enable much greater emphasis

to be placed on long-term research for industrial application. Mr. Prime's background is suited to this objective, as he is at present manager of the Application and Control Division of the Brush Electrical Engineering Co., Ltd., at Loughborough, having been during 1955–58 chief electronic engineer in the same firm. Mr. Prime, who is forty-two, was educated at the North Manchester Grammar School and the University of Manchester, from which he obtained an honours degree in physics in 1942 and later an M.Sc. He spent the years from 1942–46 in the Admiralty Signal and Radar Establishment, where he worked on radar and made a particular contribution to the work on displays. During 1946–50 he was a lecturer in electronics at the University of Liverpool and during 1950–55 was a senior lecturer in electronic engineering in the University of Adelaide, South Australia. During these years of academic work he did research in various fields of electronics including instrumentation, microwave measurements and ionized gases. In his eight years at the Brush Co., Mr. Prime has been responsible for some notable design and development work in control engineering. Perhaps the most spectacular has been his design of the control gear for both the Jodrell Bank radio telescope and the huge aerial installed at Goonhilly Down in connexion with satellite communication. But of more direct value to industry, no doubt, has been his work on the control of electrical machines. It is expected that Mr. Prime's appointment as the new professor at Birmingham will enable the Electrical Engineering Department eventually to make as big a contribution to industrial research as it is already making in various fields of communication and electronic engineering.

Controller of Aircraft, Ministry of Aviation : M. B. Morgan, C.B.

Mr. M. B. Morgan has been appointed to be the controller of aircraft, Ministry of Aviation, in succession to Sir George Gardner, who retired from the public service on April 29. Mr. Morgan was born in 1912 and educated at Rutlish School, Merton, and St. Catharine's College, Cambridge. He entered the Scientific Civil Service in 1935 at the Royal Aircraft Establishment and remained there until 1959. At the Royal Aircraft Establishment he was concerned, during 1935–39, with flight test work including aircrew tests, drag measurements, turbulence performance, stability and handling. During 1939–45 he was in charge of a group in the Aero Flight Section, dealing with handling stability and control, accident investigation liaison with firms, etc. From 1946 until 1948 he was superintendent (stability and control) and head of the Aero Flight Section concerned with all aspects of flight test work from helicopters to high-speed testing. In 1948 he was appointed head of Guided Weapons Department, and in 1959 became deputy director. In 1959, Mr. Morgan was appointed Scientific Adviser, Air Ministry, a post which he filled until 1960 when he was appointed Deputy Controller of Aircraft (Research and Development) in the Ministry of Aviation.

Royal Ontario Museum: Dr. W. E. Swinton

Dr. William E. Swinton has been appointed director of the Royal Ontario Museum (University of Toronto). Dr. Swinton, head of the Life Sciences Division of the Museum since 1961, succeeds Dr. T. A. Heinrich, who resigned last year. An internationally known authority on dinosaurs, Dr. Swinton, who is sixty-two, is the author of sixteen books and more than two hundred scientific papers and reports. During 1925–61, he was on the staff of the British Museum (Natural History), rising to principal scientific officer in charge of fossil amphibians, reptiles and birds. He was also training officer for non-curatorial staff and, for a time, chairman of the scientific staff association. Dr. Swinton was educated in Scotland at Trinity College, Clenalmond, and the University of Glasgow. In 1932 he was elected a Fellow of the Royal Society of Edinburgh. He was president of the Museums Association (Great Britain) during 1958–60.

Earthquake in Jugoslavia

On July 26, 1963, at approximately 5.18 a.m. local time, an earthquake of intensity about 9 on the Mercalli Scale (maximum 10) occurred with epicentre near latitude 42° 1′ N., longitude 21° 26′ E., near the centre of the town of Skoplje (Uskub) near the northern borders of Macedonia, in Jugoslavia. This follows smaller tremors in southern France a week earlier, and between then and July 26 in central Italy (Terni, 46 miles north-east of Rome). There was a slight earth tremor in Skoplje on February 22, 1963.

About half the buildings in the centre of Skoplje are reported to have been destroyed, including the Makedonija Hotel, the Jugoslav Army Club, the City Hall, the National Bank and a Teachers Training College. The Post Office was badly damaged and the telephone lines cut. The ancient fortress and the railway station were partly damaged, but the central hospital was not badly affected. Sewer and water pipes were cracked, electricity supplies cut, and fires were started because embers were thrown out of the grates. The death-roll is tentatively estimated at 1,000 from a population of some 215,000. In Gnjilane, 40 miles north of Skoplje along the Morava Valley, 24 houses were damaged, and the tremors were felt 100 miles away from the epicentre. After-shocks are continuing. The ruins of the ancient town of Scuppi, near by, are considered to have been caused by an earthquake in the year A.D. 518. Skoplje is approximately along the median line of a wide belt of epicentres which stretches from Mid-Atlantic, through southern Spain, Italy, Albania, Jugoslavia, Greece, Bulgaria and Turkey to Georgia, Azerbaijan and the Himalayas. An earthquake with epicentre west of Skoplje (42° N., 21° E.) occurred on 1942, August 27d. 06h. 14m. 11s. G.M.T., which is nearer to Tetovo than to Skoplje. It had magnitude 5·6 ± 0·3 on the Richter logarithmic (instrumental) scale. Further news from the locality, from Belgrade and from seismological observatories throughout the world is awaited.

Electric Power in Scotland

In a statement in the House of Commons on July 10, the Secretary of State for Scotland, Mr. M. Noble, said that the Government had given very careful consideration to the findings of the Mackenzie Committee, including its central recommendation that the functions of the two electricity boards in Scotland should be transferred to a single authority. In making this recommendation, the Committee's purpose, which must also be the Government's, was to ensure that the whole operation of generating, transmitting and distributing electricity in Scotland was carried out on the most economic basis possible, and that judgments on questions arising, such as the erection of new generating stations, were not influenced by narrow financial considerations. It was clear, however, that the proposal was unwelcome to a wide range of interests, and Mr. Noble did not think that it had yet been established that the continued existence of the two boards was incompatible with the most economic provision of electricity. He intended to see what could be done by close consultation and co-operation between the two boards, and, in the expectation that their purpose could be achieved by this means, the Government had decided not to proceed at the present time with the legislation that would be necessary to implement this recommendation of the Committee. Discussions were proceeding on the comparative cost of providing electricity by the proposed hydro-electric generating schemes published by the North of Scotland Board, and by the installation of additional thermal generating capacity elsewhere in

Scotland, and in the light of these comparative costs, the North of Scotland Board would decide whether to make the necessary formal inquiries into the hydro-electric schemes. A subordinate recommendation of the Mackenzie Committee was that special assistance should be provided to enable rural electrification in the north of Scotland to be speeded up and to link the islands with the mainland by submarine cable. Mr. Noble said that the North of Scotland Board considered that most of these cables would be needed only when the existing Diesel generating stations on the main islands had to be renewed, but had drawn up a programme for accelerating the present rate at which consumers in the more remote areas could be given supplies.

The Deer Bill

In moving the Second Reading of the Deer Bill in the House of Lords on June 28, Viscount Massereene and Ferrard pointed out that the reasons for the Bill were not entirely the same as those which led to the Deer (Scotland) Act, 1959, which followed the report of the Scott-Henderson Committee on cruelty to wild animals, although the clauses dealing with the avoidance of unnecessary cruelty to deer were essentially the same as the corresponding sections of the Scottish Act, but confined to English as opposed to Scottish law. There had been a great increase in the deer population of England and Wales following the increased acreage of timber, now reaching 600,000 acres, and this had altered the pattern of wild-life, while there had also been an increase in arable land. Further, especially in the past fifteen years, the number of weapons in private hands had greatly increased, and while motor-cars had made the countryside more accessible there was still no proper legislation in force to protect deer. Among those who had contributed to the preparation of the Bill besides its sponsors, the British Field Sports Society, Viscount Massereene mentioned the Forestry Commission, the Nature Conservancy, the Council for Nature and the Fauna Preservation Society. Clause 1 dealt with close seasons and provided a close season for stags and bucks from May 1 until July 31, but no close season was provided for the roebuck, and Viscount Massereene explained that the shorter season than that provided under the Scottish Act was due to the difference in habitat. He also pointed out that under Sub-sections of this Clause the Secretary of State was authorized to amend this Schedule by Order by the addition of any species not mentioned in that Schedule. This would enable other species of wild deer, such as the muntjac and the Chinese water deer, which appeared to be becoming indigenous, to be included. This Clause was the subject of some discussion in a debate in which the Bill was generally welcomed, and Lord Dowding and other speakers advocated that the close season should be advanced to February. Clause 2 empowers the Nature Conservancy to license such prohibited articles as stupefying drugs or muscle-relaxing agents with the object of taking deer alive for scientific or educational purposes. Controversy centred chiefly on the weapons Clause and was concerned essentially with the technicalities of prohibited weapons, both rifles and shot guns. The Bill was committed to a Committee of the whole House.

The National Ports Council

In moving a resolution in the House of Commons on July 10, welcoming the Government's statement of March 6, 1963, on the report of the Rochdale Committee of Inquiry into the Major Ports of Great Britain, the Minister of Transport, Mr. E. Marples, said that the Government fully shared the view expressed in the report as to the need for more research, both technical and operational, but no final conclusion had been reached as to the kind of studies needed and how they should be made. The first need was reconnaissance to supply a complete picture of the technical and operational research already in progress and how it was being conducted. The Lord President of the Council, after consultation with Lord Rochdale and the Minister of Transport, had arranged for such an investigation to be made, and Mr. Marples said that he would expect to receive advice from the National Ports Council on the need for economic research on particular problems. On July 9, Mr. Marples, in a written answer, gave the following names of those who had agreed to serve under the chairmanship of Lord Rochdale on the National Ports Council: Mr. E. D. Arney, J. H. Joyce, Ian W. Macdonald, Sir Eric Millburn, D. M. Robinson, E. G. Whitaker and L. T. Wright.

Expenditure on the Commonwealth Education Scheme

In a written answer in the House of Commons on July 9, the Secretary for Technical Co-operation, Mr. R. Carr, stated that expenditure on the Commonwealth Education Scheme, for which the Government had undertaken to make available up to £6 million during the five years ending in 1965, included provision for the supply of teachers, for which purpose about £1 million would be used, and so far nearly £700,000 had been committed.

The Royal College of Science and Technology, Glasgow

The annual report of the Royal College of Science and Technology, Glasgow, for the one hundred and sixty-sixth session, 1961–62, records a total of 1,997 full-time students, together with 1,203 part-time day and 1,343 evening students (Pp. 89. Glasgow: The Royal College of Science and Technology, 1963). The associateship of the College was conferred on 294 students, of whom 28 were in chemistry, 19 in metallurgy, 48 in civil, 102 in mechanical, 11 in chemical and 41 in electrical engineering. The titles of theses approved for the award of the associateship are listed and there are also lists of recent publications by members of the College and of industrial research reports issued during the year. Twenty-five members of the College obtained the degree of Ph.D. of the University of Glasgow and three the degree of M.Sc. 31 diplomas of membership for postgraduate study were awarded; the titles of theses for all these degrees and awards are also listed. Brief particulars of research in progress are included under the several departmental reports, but a full record is given in the *Research Report for the Session 1961–62*, which is published separately and also includes lists of the more important publications and of the titles of theses approved for higher degrees; these lists of publications are annotated (Pp. 43+4 plates. Glasgow: The Royal College of Science and Technology, 1963). A research group in elasticity has been set up in the Department of Mathematics, and sponsored industrial research for the South of Scotland Electricity Board, the British Shipbuilding Research Association, the Ministry of Aviation and the Cold Rolled Sections Association continued in the Department of Mechanical, Civil and Chemical Engineering, while work also continued on traffic engineering with reference to the traffic problems in Glasgow.

The British Hydromechanics Research Association

The fifteenth annual report of the British Hydromechanics Research Association, covering the year October 1961–September 1962, refers to exploration of the possibility of a closer link with an academic or research institution in a related field, and plans for expanding the laboratory (Pp. 40. Harlow: The British Hydromechanics Research Association, 1963). Research on centrifugal pumps included a study of the axial thrusts that act on some pump impellers and the behaviour of balancing vanes attached to the impeller or to the front-side wall of the pump casing. Low-pressure work on seals and glands is concentrated on elucidating the nature of the interfacial fluid film and the mechanisms involved in pressure generation within this film. Considerable pro-

gress is reported on the study of the power losses of various components of axial piston pumps and motors, and methods of designing hydrostatic slipper pads, pistons and valve plates; means have been devised to minimize the sum of the viscous-drag and flow-leakage losses. A large-scale model study has commenced involving the flow of water, ash and air, of an open channel installation designed for the rapid discharge of a railway train of ash wagons. The work on ejectors has been extended to include those in which both the driving and entrained fluids are compressible, and a programme has been written for the digital computation of initial downsurge at pump shutdown. Problems in the design of power stations which have been investigated relate to cooling-water systems and the ventilation of boiler-houses. A list of publications during the year is included, and application in advance for invitation to visit the Laboratory on the afternoons of September 11 and 12, when exhibits illustrating most phases of the Association's work will be shown, is invited from those interested.

The Lace Research Association

THE fourteenth annual report of the Lace Research Association for 1962 records that sixteen machines fitted with the beam thread-break indicator are now in regular use (Pp. 30. Bilborough, Nottingham: Lace Research Association, 1963). Moreover, although the prototype of a new device traversing the facing bar of the Leavers machine to detect holes caused through broken threads worked well, improvements made or in hand include a better driving system for the scanning head, facilities for increasing the number of scanning heads, and a means for pre-selecting the length of hole which will trip the warning signal. Measurements have been made of the various features relating to gap widths and packing density in brass-bobbin winding, and a technique of 'potting' bobbins with synthetic resin has been evolved which allows the bobbin to be radially sectioned without disturbing the lie of the yarns. Photographic techniques have been examined which are aimed at producing points at about 200 times magnification, so that individual filaments in the yarn can be counted, and thus the packing density in the bobbins checked. Considerable advance is reported in the project to mechanize the 'clipping' process in lace finishing and several different types of scalloping machine have been examined as to their suitability for scalloping Leavers lace. Small amounts of triallyl phosphate have been attached to cellulose by graft polymerization, but the flame-proofing achieved was insufficient to bring cotton net into the 'fabric of low-flammability' category. Trials of sodium borohydride as a bleaching agent showed that when used on cotton after hypochlorite bleaching improved fluidity figures are obtained, and it was also useful in cleaning heat-discoloured nylon. A report has been presented to the Council on recent work on lubrication.

Petroleum Consumption in the United Kingdom, 1962

FIGURES released by the Petroleum Information Bureau on behalf of the United Kingdom Petroleum Industry Advisory Committee in May 1963 reveal that demand for petroleum products in the United Kingdom in 1962 was 10·6 per cent higher than in 1961 (*U.K. Petroleum Industry Statistics: Consumption and Refinery Production, 1961 and 1962*. Pp. 8. London: Petroleum Information Bureau, 1963). If bunkering for ships engaged in foreign trade be excluded, total deliveries into consumption of all petroleum products amounted to 50,900,433 tons as against 46,014,950 tons in 1961. Consumption of motor spirit increased by 5·2 per cent, deliveries to dealers being 7 per cent up while those to commercial consumers dropped by 3·5 per cent, reflecting the continual change-over from petrol to diesel-engined commercial vehicles. Deliveries of DERV (diesel-engined road vehicle) fuel actually increased during the year by 7·1 per cent. The average temperature in 1962 was some 2° F below that of 1961 and to this fact is ascribed the considerable increase in demand for the 'black oils', gas/Diesel oil deliveries rising by 17 per cent, fuel oil by 13·4 per cent compared with the previous year. This weather factor also led to an increase in consumption of burning oil, which showed an increase of 16·3 per cent, due largely to the growing popularity of oil-fired central heating, which represented more than 50 per cent compared with 1961, also of free-standing space heaters the demands of which rose by 12·7 per cent. In agricultural operations, Diesel-engined tractors are steadily replacing the less efficient vaporizing-oil type, and this process is reflected in the decline of vaporizing-oil deliveries by 18·7 per cent. Regarding aviation fuel consumption, there was a slight decrease of 2·2 per cent compared with 1961; of a total of 2,108,348 tons, approximately nine-tenths of this comprised aviation turbine fuels, demand for which in 1962 amounted to 1,886,420 tons as compared with 221,928 tons for aviation spirit. Recession in industry and shipping, which account for nearly 60 per cent of all lubricants sold, was reflected in the lower demand for lubricating oils and greases, a decrease of 1·2 per cent compared with the 1961 figure. The use of liquid gases, propane and butane, by Gas Boards and industry is shown by the remarkable growth-rate of 42·6 per cent, deliveries being 294,163 tons compared with 206,285 tons in 1961. Finally, as might well be expected having regard to the phenomenal expansion of the petrochemicals industry, petroleum chemical feedstock deliveries were up by 23·5 per cent, 1,948,861 tons, compared with 1961.

Stable Isotopes of Oxygen

HIGH enrichments of both stable isotopes of oxygen have recently been achieved. Up to 99 atomic per cent of oxygen-18 has been available for some time in various chemical forms and has already been widely used in chemistry and biology as a tracer and as both target and projectile in nuclear studies. Oxygen-18 labelled compounds also have great potentialities in studies of isotope effects and spectral isotopic shifts. Oxygen-17 with a spin of 5/2 is the only isotope of oxygen which can be used for nuclear magnetic and electron spin resonance investigations. Enrichments of more than 10 atomic per cent oxygen-17 in water and 50 atomic per cent oxygen-17 in oxygen gas have recently been reached on a production scale in the Isotope Separation Plant of the Weizmann Institute of Science. These concentrations represent a 250-fold enrichment of oxygen-17 in water and 1,350-fold enrichment in oxygen gas. These materials are now available through YEDA Research and Development Co., Rehovoth, Israel. A booklet containing some pertinent references and a catalogue of materials enriched by oxygen-18 and oxygen-17 can be obtained from that Company.

Organ Pipes

CENTURIES of organ building have firmly established a preference for alloys of tin and lead as the best material for organ pipes, though in recent years to save cost zinc has been used with a sacrifice of some tonal quality. Acoustic research in the Physikalisch-Technische Bundesanstalt at Brunswick, by Drs. W. Lottermoser and J. Meyer, using pipes of 'lead' (lead 70 per cent, tin 30 per cent), 'tin' (tin 70 per cent, lead 30 per cent), copper, both electrolytic and fire-refined zinc and pine wood, has confirmed this distinctive acoustic characteristic of the different pipe materials, determined mainly by their damping properties. From the musical point of view, organ pipes should radiate harmonic tones uniformly in all directions and emit a minimum of discordant sounds due to eddies set up by the jet of air issuing between the tongue and lip. These cause not only strong whistling sounds—especially noticeable at the instant that the

pipe begins to sound—but also set up vibrations in the metal of the pipe which may bear no relation to the fundamental note. The more rigid the material the stronger are these effects; weaker ones, such as the tin–lead alloys, damping them out. The results show clearly the sharper tones due to the use of copper and particularly the hardness and musically disturbing notes produced by zinc. Wooden pipes yield softer notes and are specially suited to some special stopped pipes and flutes, while lead–tin alloy is best for those stops required to give a full, soft note. Of the materials examined, the 70 per cent tin + 30 per cent lead alloy is outstanding as the nearest approach to the ideal material for such use. Drs. Lottermoser and Meyer's results are summarized in No. 58 of *Tin and Its Alloys* (1963), published by the Tin Research Institute, and also in full in the February 1962 issue of *Metall* (p. 108).

Chemical Analysis of Fruit Juices: Standardization of Methods

The Scientific and Technical Commission of the International Federation of Fruit Juice Producers have set up a Sub-commission for the Standardization of the Chemical Analysis of Fruit Juices. Several European countries are represented on the Sub-commission and their recommendations have now been published (International Federation of Fruit Juice Producers. *Analyses*. Pp. 38. Wädenswil, Switzerland: Dr. Hans Lüthi, Sektion Getränkechemie und Biologie, Eidg. Versuchsanstalt für Obst-, Wien- und Gartenbau. 1962). Methods are described for the determination of specific gravity, alcohol, titratable acid, sugar, volatile and non-volatile acids and sulphur dioxide. In each case the details of the necessary apparatus, reagents and determination procedure are described in separate English, German and French sections. A useful feature is the inclusion of the degree of accuracy to which each result should be reported. In addition the general principle of each of the procedures is briefly indicated, but, as usual with standard methods, the interpretation of the results is not discussed. The publication is in loose-leaf form so that supplements can be added when necessary. The introduction states: "This standardization of our chemical methods is very urgent, especially with regard to international negotiations".

Survey of Hospital In-Patients in Britain

The full report on the *Hospital In-Patient Enquiry for the Year 1959* has recently been published by the Registrar General (H.M.S.O. £1 7*s*. 6*d*.). The inquiry is based on forms completed by hospitals for every tenth patient discharged or died, the selection of patients being at random. The inquiry was originally begun in 1949; but until 1957 it was based on hospitals whose co-operation was voluntary. Since 1957 all non-psychiatric hospitals in England and Wales have been included, though some cases, mainly private and staff patients, have been excluded from the analysis. This consists of tables, showing the number of deaths and discharges (spells) and the associated rates by various characteristics, such as sex, age, region, diagnostic group, and department of hospital in which the treatment took place. There is also a special and detailed analysis of maternity cases. The figures are extremely detailed, and defy any attempt at summarizing within a short space. They do, of course, relate to spells in hospital, rather than to individual cases, and no attempt has been made to use the information about the occupation of the patients or the industry in which they worked which is collected on the forms. Many other possible cross-tabulations will no doubt occur to users of the material, and the foreword invites interested persons to inquire at the General Register Office for additional information which may be available.

Tenth International Botanical Congress, Edinburgh

The tenth International Botanical Congress is to be held in Edinburgh during August 3–12, 1964. Some ten thousand preliminary circulars have been dispatched to botanists all over the world. The second Congress circular, which gives details of the scientific programme and the full programme of botanical excursions, is due to be dispatched in August or early September. Copies of the circular can be obtained from the Secretary (Executive Committee), Tenth International Botanical Congress, 5 Hope Park Square, Edinburgh 8.

The Night Sky in September

Full moon occurs on Sept. 3d. 19h. 34m. U.T. and new moon on Sept. 17d. 20h. 51m. The following conjunctions with the Moon occur: Sept. 2d. 05h., Saturn 1° N.; Sept. 6d. 07h., Jupiter 4° N.; Sept. 21d. 03h., Mars 5° S.; Sept. 29d. 12h., Saturn 1° N. In addition to these conjunctions with the Moon, Mars is in conjunction with Spica on Sept. 1d. 00h., Mars being 2° N. Mercury, Venus and Mars are too close to the Sun for easy observation. Jupiter rises at 20h. 00m., 19h. 00m. and 18h. 00m. on September 1, 15 and 30, respectively. It is in Pisces, its stellar magnitude is −2·4 and its distance from the Earth on September 15 is 375 million miles. Saturn sets at 3h. 20m., 2h. 20m. and 1h. 20m. at the beginning, middle and end of the month, respectively. It is in Capricornus, its stellar magnitude is + 0·6 and its distance from the Earth on September 15 is 840 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Sept. 5d. 0h. 36·9m., 30 Psc. (*R*); Sept. 7d. 22h. 49·3m., μ Cet. (*D*); Sept. 7d. 23h. 48·7m., μ Cet. (*R*); Sept. 26d. 20h. 45·2m., 154 B. Sgr. (*D*). *D* and *R* refer to disappearance and reappearance, respectively. The autumnal equinox occurs on Sept. 23d. 18h.

University News : Belfast

The following appointments to lectureships have been announced: Dr. R. M. Hobson (physics); Dr. M. A. Leonard (inorganic and analytical chemistry); Dr. P. J. S. Boaden (zoology); Dr. J. M. Dunbar (bacteriology). Mr. C. D. Kemp has been appointed to a senior lectureship in statistics.

Bristol

Dr. F. G. A. Stone, reader in inorganic chemistry in Queen Mary College, London, has been appointed to the chair of inorganic chemistry. Dr. J. M. Ziman, lecturer in physics, University of Cambridge, has been appointed to the chair of theoretical physics. The following appointments to lectureships have been announced: J. F. Harper (mathematics); Dr. G. M. Jarman (zoology); A. Keller (physics); Dr. K. Lederis (pharmacology); D. Lovelock (mathematics); Dr. J. MacMillan (organic chemistry); Dr. A. Simpson (aeronautical engineering); Dr. M. A. Sleigh (zoology).

Cambridge

Dr. L. Weiskrantz, of King's College, assistant director of research in experimental psychology, has been elected to a fellowship (Title *A*) of Churchill College as from October 1.

Cardiff

Dr. L. Crombie, at present reader in organic chemistry at King's College, London, has been appointed to the chair of organic chemistry at University College of South Wales and Monmouthshire.

East Anglia

Dr. R. T. Joy has been appointed lecturer in the School of Biological Sciences.

Leeds

DR. F. A. GOLDSWORTHY, at present lecturer in mathematics at the University of Manchester, has been appointed to a second chair of applied mathematics. Dr. A. W. Goldie, at present reader in algebra at King's College, Newcastle upon Tyne, has been appointed to a second chair of pure mathematics.

London

PROF. F. K. HARE, professor of geography and meteorology at McGill University, has been appointed to the chair of geography tenable at King's College. Mr. H. D. Ritchie, reader in surgery at the London Hospital Medical College, has been appointed to the chair of surgery tenable at that College. Dr. D. H. Michael, lecturer at University College, has been appointed to the readership in applied mathematics tenable at that College. Titles of reader have been conferred on the following: Dr. E. M. Deeley (electrical engineering), in respect of his post at King's College; Miss Vera I. Evison (archæology of the Anglo-Saxon period), in respect of her post at Birkbeck College; Mr. A. R. Ilersic (economic and social statistics), in respect of his post at Bedford College; Dr. N. Waloff (animal ecology), in respect of her post at the Imperial College of Science and Technology; Dr. E. M. Yates (geography), in respect of his post at King's College.

THE annual report for 1961–62 of University College, London, includes the report of the Chairman of the College Committee and the Provost, lists of publications by members of College Departments, August 1, 1961–July 31, 1962, as well as particulars of staff, higher degrees awarded, and College awards to students (Pp. 105. London: University College, 1963). A Development Council set up to further a comprehensive long-term programme has recommended maximum utilization of the present site as a first step. Student accommodation remains a major concern, and of the 3,119 full-time students, 1,708 live in lodgings, often at a considerable distance, and only 610 in University halls of residence. The number of postgraduate students increased by 5 per cent to about 1,100. Expenditure on research increased by £92,424 to £408,224.

Rehovoth

THE report of the Weizmann Institute of Science, Rehovoth, for the two years 1960–61 contains brief accounts of the work in progress in some seventeen sections together with lists of publications, including patents, and lists of scientific staff (Pp. 151. Rehovoth, Israel: The Weizmann Institute of Science, 1963). Major building activity is at present centred in biology, but a new focus of collaborative research in the chemistry and physics of the solid state is being created. Besides plans for construction of a high-speed transistorized computer for applied mathematics, enlargement of the present Van de Graaff accelerator is contemplated.

Swansea

THE following appointments have been made for the session 1963–64: *Readers*, Dr. D. A. Long (chemistry), Dr. K. C. Rockey (civil engineering); *Senior Lecturers*, A. P. Greenough (metallurgy), Dr. E. Naylor (zoology), G. L. Rees (economics), Dr. J. A. Sandover (civil engineering); *Lecturers*, Dr. R. H. Jones (physics), Dr. D. Betteridge (chemistry), C. Tomlinson (geography), Dr. B. Atkinson (chemical engineering).

Announcements

THE following officers of the Canadian Biochemical Society have recently been elected: *President*, J. H. Quastel; *Vice-President*, L. Berlinguet; *Secretary*, H. D. Branion; *Treasurer*, R. M. Hochster; *Councillors*, J. M. R. Beveridge, S. H. Zbarsky and P. B. Hagen.

A MEETING on "The Rheology of Clays and Fine Particle Suspensions" will be held by the British Society of Rheology at the University of Keele during September 26–27. Further information can be obtained from J. F. Hutton, Shell Research, Ltd., P.O. Box 1, Chester.

A SYMPOSIUM on "The Ellipsometer and its Use in the Measurement of Surfaces and Thin Films" will be held at the National Bureau of Standards, Washington, during September 5–6. Further information can be obtained from Elio Passaglia, National Bureau of Standards, Washington 25, D.C.

A COURSE on "The Genetics and Physiology of Bacterial Viruses" will be held in the International Laboratory of Genetics and Biophysics, Naples, during September 18–October 12. Further information can be obtained from the Laboratorio Internazionale di Genetica e Biofisica, Casella Postale 104, Napoli.

THE fourth British Congress on the History of Medicine and Pharmacy entitled "The Evolution of Pharmacy in Britain" will be held in the University of Nottingham during September 20–23. Further information can be obtained from Dr. F. N. L. Poynter, Wellcome Historical Medical Library, the Wellcome Building, Euston Road, London, N.W.1.

THE Plant Phenolics Group is widening its scope to include other plant constituents. A symposium on "Comparative Biochemistry of the Leguminosae" will be held during September 10–11 at the John Innes Institute, Hertford, and will include nitrogenous compounds as well as phenolics. Further details can be obtained from the honorary secretary, A. H. Williams, Research Station, Long Ashton, Bristol.

THE third annual meeting of the Plant Phenolics Group of North America will be held in the University of Toronto during September 6–7. The programme will include a symposium on "Aspects of Plant Phenolic Chemistry". Further information can be obtained from Dr. V. C. Runeckles, Imperial Tobacco Company of Canada, Ltd., P.O. Box 6500, Montreal, Quebec.

A CONFERENCE on "Some Aspects of Surface Behaviour", arranged by the Materials and Testing Group of the Institute of Physics and the Physical Society, will be held at the University of Bristol during September 16–17. Subjects under discussion will include: surface adsorption, diffusion and energy; macroscopic effects involving surfaces, for example, friction, stress corrosion and fatigue. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A VACATION course in "The General Theory of Electrical Machines", arranged by the Electrical Engineering Department of the Imperial College of Science and Technology, London, will be held at the College during September 16–20. The course will provide an introduction to modern methods of calculating the performance of d.c. and a.c. machines under any conditions of operation. Further information can be obtained from B. J. Prigmore, Electrical Engineering Department, Imperial College of Science and Technology, London, S.W.7.

A CONFERENCE on "Cellular Control Mechanisms and Cancer", organized under the auspices of the Commission on Cancer Research of the International Union Against Cancer and the Netherlands Cancer Institute, will be held in Amsterdam during September 9–13. Subjects under discussion will include: genetic control of protein synthesis; mechanisms of cell differentiation; growth regulation; structure and function of tumour cells; carcinogenic agents. Further information can be obtained from Prof. O. Mühlbock, Netherlands Cancer Institute, Antoni van Leeuwenhoek-Huis, Sarphatistraat 108, Amsterdam *C*.

SPEEDING THE PACE OF TECHNOLOGICAL DEVELOPMENT IN THE PETROLEUM INDUSTRY

PERHAPS in no other branch of applied science has fundamental research, both in the field and in the laboratory, borne so much fruit in so short a time as that of petroleum technology. During the past thirty years, since the first World Petroleum Congress, the evolution of the industry has not only been underlined by its magnitude and spectacular accomplishments but also by the complexity of the problems, both human and technical, that such rapid advancement of our knowledge of this technology has engendered. It is, indeed, timely that attention has been directed to the more philosophical aspects of the trend of modern research in the industry and its possible limitations if certain human factors are not taken into account. In no more appropriate setting than that of the sixth World Petroleum Congress held in Frankfurt during June 19–26 could the facts and fears be proclaimed and few are better qualified than Mr. John Loudon, president of the Royal Dutch Petroleum Co. and senior managing director of the Royal Dutch/Shell Group of companies, to state the case as it really exists.

In his executive position in the industry and with all the experience he brings to bear, although he disclaimed at the outset of his address to the Congress: "any competence as an expert in science and technology", Mr. Loudon touched on some of the most sensitive aspects of planned research in the industry which have in recent years impressed themselves on the more far-seeing people concerned with guiding research teams and particularly with financing the adventures they are called on to undertake. For example, nobody could disagree with his contention ". . . that the limiting factor in science and technology is the number of creative individuals who provide the ideas from which all novelty comes". Again: "We have become so accustomed to thinking in terms of money and to assuming that more money will get us more of anything that is desired that we sometimes lose sight of other limitations. In research we may already have reached the state of diminishing returns for our investment —not only of industry but of society as a whole". Much has recently been heard of the lure of some of British top-grade scientists abroad, tempted by higher remuneration and alleged superior research facilities. But this competition is by no means confined to prospects overseas. The influence of Government Departments in Britain, quite apart from foreign interests, concerned with atomic energy, space research and national defence projects, for example, is felt in their ". . . competing for the limited available talent not only with industry and with universities but with each other". Mr. Loudon went on to say: "It is evident that much of the effort, both military and civilian, has been devoted not to really original concepts but to the rapid exploitation in many different laboratories of the few ideas that are occasionally produced by brilliant individuals".

The trend of policy of the Royal Dutch Petroleum Co. can be discerned from the statement that economically the profitability of industrial research, while still high, is decreasing due, apparently, to the increasing number of instances of simultaneous inventions; these complicate patent portfolios and render it difficult to decide whether money should be invested for projects based on technical novelty or superiority which could well turn out to be illusory. Much the same precepts, we suspect, are influential in the research policy of the other major oil companies at the present time. Thus, both the technical and economical difficulties ahead can, Mr. Loudon argued, only be solved by using available brainpower efficiently. "In our efforts to truly speed the pace of technology in the petroleum industry we must try to do two things: first, by supporting and undertaking basic research increase the number of new ideas; secondly, encourage our applied scientists and our commercial staff to be adventurous in their choice of new processes and products for development. And as citizens, we should ponder the question of whether the world can long afford an inefficient use of our previous asset of brainpower".

Only creative human talent can provide the science and technology needed to-day. That talent is limited, as is the case throughout the world. It is not enough to seek refuge in criticism of our present educational systems, on one hand deploring the fact that not enough scientists and technologists are being trained to meet the needs of our culture, or, alternatively, that science and technology are being over-stressed at the expense of the humanities, to the future detriment of that culture. Mr. Loudon concluded this most stimulating discourse with a statement of his belief ". . . that with a modicum of good sense and a minimum of dogma the paradox (in the above opposing views) vanishes. Science and the humanities are by no means incompatible. . . . In our business particularly, we need men trained in the sciences with an understanding of the humanities, men trained in the humanities with an understanding of science". Moreover, ". . . the rare individuals who can master both worlds are those who are contributing most to the effective use of technology. Such men are also continuing to aid the universities, giving lectures, serving on advisory committees, sometimes even leaving us to take up permanent posts. And by prompt publication of our basic research we are contributing to the body of scientific knowledge". H. B. Milner

THE INTERNATIONAL SCIENTIFIC RADIO UNION

FOURTEENTH GENERAL ASSEMBLY

By Dr. R. L. SMITH-ROSE, C.B.E. (President)

PREPARATIONS are now well in hand for the fourteenth general assembly of the International Scientific Radio Union (URSI) which will be held at the Takanawa Prince Hotel, Tokyo, during September 9–20. For the past two years a large committee, appointed by the Science Council of Japan, has been preparing the general arrangements under the guidance of a smaller Executive Board, of which Prof. I. Koga, vice-president of the International Scientific Radio Union, is the chairman, and Prof. S. Okamura the secretary. The detailed programme for the assembly is being organized by six small committees including two dealing with the scientific and ladies programmes respectvely.

The forthcoming general assembly is noteworthy in that it will mark the fiftieth anniversary of the formation of the International Scientific Radio Union at a small

preliminary meeting in Brussels attended by nine scientists during October 1913. A second meeting was held during April 1914, and was attended by fourteen scientists from six European countries. After the First World War the Union was one of the first to be organized in 1919 under the general supervision of the International Research Council, which in 1931 was replaced by the International Council of Scientific Unions. The other three Unions were the International Astronomical Union, the Union of Geodesy and Geophysics and the Union of Pure and Applied Physics.

The first General Assembly of the International Scientific Radio Union was held in Brussels in 1922, and was followed by others in Washington (1927), Brussels (1928), Copenhagen (1931), London (1934) and Venice (1938). After an interval during the Second World War, the activities of the Union were resumed at the seventh general assembly held in Paris in 1946; and this was followed at two-year intervals by meetings in Stockholm, Zurich, Sydney and The Hague until 1954. On the last occasion it was decided to change the interval between assemblies to three years; and the twelfth and thirteenth were held in Boulder and London in 1957 and 1960 respectively. The attendance on the latter occasion was about 830, of whom nearly 450 were official delegates, and 150 were observers from the host country interested in various aspects of the scientific programme. There were also a small number of invited guests, and some 200 members of the families of delegates and observers.

The International Scientific Radio Union is one of the fifteen scientific bodies which now adhere to the International Council of Scientific Unions and it is in effect a federation of the national scientific academies in 28 countries throughout the world. The objectives of the Union are to promote and organize radio research requiring international co-operation, to encourage the development of such agreed methods of measurements as may be required for the investigation of any radio phenomena with particular reference to those associated with the Earth, its atmosphere and outer space. The influence of the Sun and of general and geophysical conditions on the radio phenomena has naturally required close collaboration between the four Unions referred to here. In more recent years, this collaboration has been extended to include the International Committee on Space Research (COSPAR) which was formed in 1958 and has recently held its fourth international symposium in Warsaw (May 1963).

To return to the forthcoming general assembly of the International Scientific Radio Union, the scientific programme is being organized under the existing seven commissions, the titles and present chairmen of which are: (1) radio standards and measurements methods (Dr. U. Adelsberger); (2) radio and troposphere (Mr. J. Voge); (3) ionospheric radio (Mr. J. A. Ratcliffe); (4) radio noise of terrestrial origin (Prof. R. A. Helliwell); (5) radio astronomy (Sir Bernard Lovell); (6) radio waves and circuits (Mr. J. Loeb); (7) radioelectronics (Prof. W. G. Shepherd).

The wide range of scientific activities in various parts of the world in most, if not all, the aforementioned fields has made it necessary to restrict the scope of the items selected for discussion at each of the sessions which will be available to the commissions. Accordingly, each chairman has formulated the subjects to be discussed by his commission and has invited some well-known authorities to prepare introductory papers for presentation at Tokyo. These papers, together with a summary of the main features of the discussion thereon, will be published in the *Proceedings* of the general assembly. In accordance with past practice, no uninvited papers will be considered for acceptance. As some of the subjects selected for discussion are of common interest to more than one commission, arrangements have been made in the programme for joint meetings and also for meetings of the commissions with other committees of the Union such as that on space radio research or with the International Committee on Geophysics. The latter body, incidentally, has replaced the former committee which organized the co-operative scientific work during the International Geophysical Year of 1957–58 and afterwards, and it has recently been actively engaged in formulating the corresponding programme for IQSY, the International Year of the Quiet Sun (1964–65).

At the general assembly in London in 1960, representatives from the International Scientific Radio Union were appointed to its parent body—ICSU—as well as to various specialist committees in which the Union is associated with the interests of other unions or committees in such subjects as the ionosphere, radio meteorology, solar and terrestrial relationships, space research and the allocation of radio frequencies for radio astronomy and space science. In this way, full collaboration is maintained between scientists in all parts of the world engaged in research in adjacent fields concerned with the Earth and its atmosphere, the Sun, the planets and outer space. Meetings of these committees will be called as may be appropriate during the general assembly, taking advantage of the presence in Tokyo of the various interested scientists.

During the Assembly various formal receptions are being arranged by the Japanese Committee, and some visits of technical interest will be offered to the visitors. A special programme for ladies has also been distributed. After the assembly, a four-day tour is available to Kyoto and Nara, towns which are of historic interest because they were former capitals of Japan.

MULTIPLE MOLECULAR FORMS OF ENZYMES AND THEIR USE IN CLINICAL DIAGNOSIS

A SYMPOSIUM on "Multiple Molecular Forms of Enzymes" organized by the University of Ghent in collaboration with the Belgian Society for Clinical Chemistry, was held in Ghent on April 27. It was the first of its kind in Europe, and the interest it arose can be judged from the fact that several weeks before the deadline for registration, the limiting number of 150 participants had already been reached. Representatives from most west European countries were included among the speakers and also several from the United States.

The meeting was concerned with the structure of isoenzymes and with their clinical uses. As to the first, E. Appella (Centre de Chimie Macromoléculaire, Strasbourg) presented data defining the physical properties of lactic dehydrogenase 1 (LDH1) and LDH5 (beef tissue) and their amino-acid composition. All available evidence tends to support the hypothesis formulated by the author together with Markert that LDH1 is composed of four identical sub-units, while LDH5 is composed of four identical sub-units of another type; LDH2, LDH3 and LDH4 correspond to combinations of each of these sub-units into groups of four. These hybrid forms can be produced *in vitro*: following dissociation into sub-units of pure LDH1 and LDH5 molecules in a medium of high ionic strength, randomized re-association occurs on removal of the sodium chloride.

M. D. Poulik (Child Research Center, Detroit) took ceruloplasmin as an example of a second class of isoenzymes

—the polymer type. He advocated the use of electrophoretic media differentiating between size of the molecules; experiments with such dissociating substances as urea and SH-compounds also give useful information concerning the number and nature of the sub-units. K. Van Asperen (Laboratory for Insecticide Research, Wageningen) stressed the importance of genetic investigations in drawing up the isoenzyme concept. He pointed out that from his work on esterases in the house-fly it is clear that a third class of isoenzymes occurs where the difference is one in primary structure. In support of this view, M. Rabaey and H. Gysels (Department of Ophthalmology and Zoology, University of Ghent) described the multiplicity of myoglobins in vertebrates. Myoglobins can be considered isoenzymes since they all have peroxidase activity.

Several speakers surveyed the method for isoenzyme separation which they developed. H. J. Van der Helm (Provinciaal Ziekenhuis, Santpoort) discussed electrophoretic methods. He made interesting comments on specific problems that are encountered when a conventional histochemical technique is applied to the detection of enzyme activity in electrophoresis slabs. Here, one is more concerned with quantitative aspects than with precise localization. Eluting effects should be reduced by choosing a thick layer for electrophoresis, but rapid contact with the reagents is obtained if thin layers are used. Both requirements can be met in an agar medium. G. A. Whyley (University College, London) used cellulose acetate electrophoresis followed by elution of the segments. H. Barnett (South London Hospital for Women, London) favoured the tetrazolium procedure for direct staining of the cellulose acetate strips. She described two types of 'nothing' reactions. The first occurs in the presence of tetrazolium and phenazine methosulphate and results from reduction of the tetrazolium salt by —SH groups of proteins. The second requires in addition the presence of NAD and seems to be enzymatic, but occurs without any addition of substrate.

Column chromatography on DEAE cellulose was utilized by S.-I. Walter (Medizinische Universitäts-Klinik, Heidelberg) for separation of LDH isoenzymes and by B. Schobel (Medizinische Universitäts-Klinik, Vienna) for isolation of leucine aminopeptidase (LAP) isoenzymes. Walter also used a simple batch procedure, developed together with Hess for detecting LDH5 in human serum. The procedure is rapid but gives only a 'yes' or 'no' answer.

J. H. Wilkinson (Westminster Medical School, London) commented on a new procedure for assay of LDH isoenzymes. He found, together with Rosalki, that LDH1 has a much higher activity against α-ketobutyrate than LDH5. Thus an assay of LDH activity is made with each of these substrates and an index established which reflects changes in the isoenzyme pattern. The procedure proves especially valuable in the follow-up of myocardial infarction.

Another indirect procedure depending on selective thermal inactivation of LDH5 was described by H. Wust (Medizinische Universitäts-Klinik, Erlangen). The serum is heated for 30 min at 56° C. If more than 40 per cent of the activity disappears this indicates the presence of LDH5. However, several environmental factors, especially protein concentration and *p*H, tend to render this test less reliable.

Clinical applications were introduced by E. S. Vesell (Rockefeller Institute, New York), who warned against overestimating the specific diagnostic value of LDH isoenzyme determination. Similar patterns may be observed in sera from patients with different, unrelated disease. An increase in serum LDH5 is found not only in liver injury but also in some cases of acute arthritis and of dermatomyositis. In particular, shock, both hæmorrhagic and endotoxic, may liberate LDH from various tissues and thus distort the pattern from that expected for an uncomplicated disease.

H. A. Zondag (St. Elizabeth's Gasthuis, Haarlem) summarized the experience he collected from 4,000 determinations of the LDH isoenzyme pattern (agar-gel and 'Nitro-BT' method) in serum and other body fluids. He distinguished three main patterns: that of acute myocardial infarction (high LDH1), that of acute hepatic necrosis (high LDH5) and that of malignancy (high LDH2, 3, 4). The pattern of acute myocardial infarction has the highest clinical significance. Samples should not be stored in the frozen state since LDH5 may then be inactivated. U. C. Dubach (Medizinische Universitäts-Klinik, Basle) used thermal inactivation at two temperatures for differentiation between LDH isoenzymes. The method proved especially useful for follow-up of myocardial infarction, also for the diagnosis of pulmonary infarction and of primary myopathies. In the diagnosis of liver diseases results were less encouraging. L. J. Denis (Medical College of Virginia, Richmond) used agar-gel electrophoresis combined with the tetrazolium method for LDH analysis in malignancy. In cases of prostatic carcinoma in relapse an increase of serum LDH5 is regularly found. S. B. Rosalki (Kingston Hospital, Kingston upon Thames) investigated the isoenzymes of alkaline phosphatase, LDH and leucine aminopeptidase (LAP) in pregnancy. L. Pagliaro (Clinica Medica Generale, Palermo) used the Rosalki–Wilkinson test and obtained encouraging results. In muscular dystrophy the index pointed to an unexpected increase of LDH1. In the discussion it was made clear that this shift is to be explained by the modification in the LDH pattern of dystrophic muscle as described by Wieme and Herpol using agar-gel electrophoresis. G. Schapira (Hôpital des Enfants-Malades, Paris) reported on the changes in the LDH pattern of dystrophic muscle as revealed by starch-gel electrophoresis. The pattern recalls that of fœtal muscle. This suggests the occurrence of an arrest in the development of certain muscle proteins. J. Herpol (Kinderkliniek, University of Ghent) studied 150 normal children for the LDH serum pattern. The distribution was found to be similar to that in adults. In some cases a value higher than 75 per cent was found for LDH1; total LDH activity was then generally low.

Several other types of isoenzymes were also discussed for their clinical value. Malate dehydrogenase (MDH) was studied by M. Van Sande (Instituut voor Tropische Geneeskunde, Antwerpen) and proved of little diagnostic value. Despite separation into some nine fractions, all tissues tend to exhibit the same pattern. Leucine aminopeptidase was investigated by F. W. Wewalka (Medizinische Universitäts-Klinik, Vienna). Modifications in the serum pattern (starch-gel electrophoresis) mainly concern the appearance of new fractions. In liver disease modifications appear in the slow area. In pregnancy a new fraction of intermediate mobility is found. No direct relation with corresponding tissue fractions is manifest. This point was confirmed by R. Delcourt (Institut Médical, Ixelles), who also directed attention to the possible interference from inhibitors. A. J. Van Triet (Antoni van Leeuwenhoekhuis, Amsterdam) studied LAP isoenzymes in urine and found that in reticulosis a fraction is present that has a somewhat lower mobility than that usually found.

Alkaline phosphatases seem to constitute a promising field for isoenzyme analysis. B. N. Jensen (Psychiatric Institute, Aarhus) found three alkaline phosphatases in serum (electrophoresis on a polyvinyl chloride block). The ratio between the slowest and the intermediate form constitutes an index that serves to separate patients with liver disease (low index) from those with bone involvement (high index). W. G. Haije (Radiotherapeutisch Instituut, Rotterdam) used agar-gel electrophoresis for detecting physiological or pathological bone formation (increase of slowest) and for detecting liver disease (increase of most rapid fraction). The method seems especially interesting for detection of liver metastases.

Those present at the symposium agreed that: (1) The isoenzyme concept should be kept broad with a definition that could be given as follows: "different proteins with similar enzymatic activity". (2) Determination of LDH isoenzymes already now occupies a useful place in the clinical laboratory. (3) Direct histochemical procedures are preferable, but among indirect methods the Rosalki–Wilkinson test deserves special attention. R. J. WIEME

THE EAST AFRICAN ACADEMY

FIRST SYMPOSIUM AND INAUGURAL ANNUAL GENERAL MEETING

NEARLY three years since the idea was first put forward, the East African Academy came into being by the ratification of its constitution at Kampala in February 1963. Four months later, during June 14–17, the first symposium and annual general meeting was held at which more than 120 scholars, including eight delegates (representing the Royal Society of London, the Japanese Academy of Sciences, the Weizmann Institute of Science, and the U.S. National Academy of Sciences) attended. Messages of greetings and good wishes, apart from the aforementioned institutions, came from the U.S.S.R. Academy of Sciences, the Academia Sinica, the American Academy of Arts and Sciences, and from individuals and local organizations.

In many ways the East African Academy shows interesting characteristics. It is a regional, rather than a narrowly national, academy—covering the four East African countries of Kenya, Tanganyika, Uganda, and Zanzibar; it takes all departments of learning within its purview; apart from the usual aims of national academies, namely, the promotion of learning and the dissemination of knowledge, the Academy will take as its first-priority objective "to encourage scientific, technological and other advanced training within East Africa and to find ways and means for such training elsewhere"; and, because the Academy regards research by its members as one of its most important activities, it has already appointed a powerful four-man committee to be responsible for devising and taking immediate action on a policy for sponsoring research programmes, approving other research projects, selecting candidates for research fellowships, and handling research funds.

Two whole days were devoted to the symposium meetings, at which twenty-nine papers were read representing general themes (5), physical and chemical sciences (6), biological sciences (10), and arts and social sciences (8). The opening address was delivered by Dr. H. H. Storey, as a member of the Academy, on "East African Research on Plant Virus Diseases". After dealing with the history of virus research for the past seventy years briefly, he emphasized the contribution of East African work to virology by reviewing the investigation on cassava mosaic, groundnut rosette, and maize streak—all three being entirely African plant virus diseases. Finally, he demonstrated how the work on the obligate relationship between arthropod vector and virus has contributed to our fundamental knowledge of the vector–parasite association.

Prof. L. C. Beadle ("Inland Waters of East Africa as a Field for Fundamental and Applied Research") considered that East Africa presents particular problems because of its geological history, and the mode of origin of its lakes since the Miocene has provided a great stimulus for the evolution of new species of animals, for example, 400–500 species of fish are endemic. Factors which determine breeding seasons are more problematical in tropical environment: for example, there is a correlation with the onset of rain, and there seems to be an internal hormonal rhythm. The lake bottom is highly reducing, and it is suggested that it might be possible to pump up deep water in order to increase the circulation of organic matter (as the Belgians are already attempting in regard to Lake Kivu).

Glynn Isaac ("Olorgesailie: a Study of the Natural History of a Pleistocene Lake Basin and an Early Stage in Human Cultural History") affirmed that East Africa, particularly during the past thirty million years, is comparatively well endowed with basins containing deposits of the largest unbroken fossil record, later made available by tectonic disturbances followed by erosion. East Africa, therefore, appears to have been a centre of cultural and physical evolution. Olorgesailie Lake Basin in the Kenya section of the Rift Valley offers a good opportunity for such Quaternary research: he outlined the geological circumstances of the basin, and attempted a preliminary reconstruction of the environment of the basin 100,000–200,000 years ago, and of man's (supposed) activities in relation to it.

J. Gitau ("Importance of Soil Fertility and Fertilizer Use in Kenya, with Special Reference to African Farmers") stated that farmyard manure has been used from early times, but that this is increasingly becoming difficult in Kenya owing to the limitation of available land to peasant farmers. Hence, the use of artificial fertilizers offered a solution. This, however, raised the vital question of cost; and it was suggested that, as one of their important services, co-operatives should shoulder the responsibility of buying and distributing fertilizers.

Dr. Z. Subarsky ("Operation Ecology") described how a private committee was recently formed to rectify a state of affairs in biology teaching in East African schools in which the syllabuses, text-books, methods, and sometimes even type specimens for practical classes are imported from abroad. The committee is making an ecological classification in the form of a series of maps (physical, geological, climatic, and vegetational) on which schools will be marked. This procedure will facilitate selection of type specimens for particular topics in the syllabuses.

Physical and Chemical Sciences

L. A. J. Williams ("Geology of the Narok District of Masailand") described the mapping in reconnaissance style of this area, and included a summary of the exposed geological successions, maximum thicknesses, and some of the principal tectonic and physiographic events in the area. The talk was followed by that of Miss M. Lindley ("Notes on the Hydrology of the Mara–Narok Area"), who considered the relatively meagre hydrological records available of Masai areas. From these, she made the following tentative conclusions: the 49-year (1913–62) mean rainfall is 28·29 in., the 1933–49 period was one of generally below-average rainfall (24·0 in.), the 1950–53 period one of varying rainfall, and the 1954–62 period was a wet one (27 per cent above the mean). November–May is the wet period, but no month is free from the risk of drought; on the other hand, the pattern of evaporation through the year fluctuates much less than that of rainfall. She came to the conclusion that the average rainfall left no excess moisture for streamflow or replenishment of water stores. P. H. Temple ("Raised Features along the Southern Shoreline of Lake Victoria") noted the presence of extensive deposits of lacustrine sands and clays, now considerably above the present level of the Lake; and he offered speculations as to their significance.

Prof. M. Crawford ("Chemical Problems concerning Lemon Grass") stated that *Cymbopogon citratus* gives a 0·3 per cent yield of oil, and, by steam distillation, 80 per cent of the latter is found to be citral. The Uganda Development Corporation is taking this as an industrial proposition, extracting citral for flavouring, perfumery, and the manufacture of vitamin A. The biogenesis of citral is unknown, but the recent fall of citral content of lemon grass to 65 per cent is tentatively explained as related to age of the grass at the time of gathering.

Starting from the Appleton–Hartree formula, which gives the relationship between the refractive index of the ionosphere (itself essentially determined by the two factors of electron density and the Earth's magnetic field) and other quantities, S. Amer ("A Theoretical Method of Determining the Electron Density Profile of the Ionosphere") considered that the problem of determining the electron density profile is reduced to the determination of the refractive index as a function of altitude. Calculations, using data collected by measuring properties of radio waves transmitted from a satellite and received by a fixed observer on the Earth's surface, showed that the refractive index is a function of radial distance, that the Appleton–Hartree formula determines the electron density profile of the ionosphere, and that the Faraday rotation avoids any ambiguity due to birefringency of radio waves.

Biological Sciences

Dr. J. W. Kibuka-Musoke ("Antibodies and Low Temperatures") reported an important effect of exposure of the human body to low temperatures, in which antibodies with hæmagglutinating properties are found. In several recent cases in Uganda, the thermal limits of these antibodies were such as to produce venous thrombosis, often leading to gangrene. Genesis of these 'cold' antibodies is unknown, but they have been isolated. A vital aspect of the problem associated with the Miridae, a family of plant bugs containing extremely important crop pests, is that of the feeding process itself and of how this leads to the formation of characteristic lesions. Thomas R. Odhiambo ("Restatement of the Mirid Problem") gave a critique of the major researches on this subject. He suggested that it is the injection of salivary proteases into the feeding punctures which leads to the primary Mirid lesion. It was further proposed that "the salivary glands have the genetic potentiality of producing proteases (and other enzymes); but that whether or not this potentiality is translated into actual production of the enzymes depends on the kind of diet utilized". This proposal is thus linked to the wider problem of enzymatic adaptation. Prof. D. S. Kettle ("The Assessment of Biting Fly Population by Means of Biting Rates") listed the many sources of variation present when recourse is had to a direct measurement of the biting rate as a method of assessing the size of biting-fly population. Quasi-factorial experiments were described in which three Jamaican biting-fly species were caught simultaneously from an arm or leg by 4 individuals, in 4 positions, for 4 consecutive periods of 15 min each. It was found that all these factors influenced the catches, that the four weather factors investigated accounted for 88 per cent of the variation, wind speed and air temperature alone accounted for over 85 per cent, light intensity played merely a minor part, and saturation deficit played scarcely any part at all.

Some of the phenotypic and genetic parameters of East African zebu cattle and their crosses with the Indian Sahiwal zebu cattle, especially in relation to milk production, were discussed by E. B. Galukande ("The Genetics of Milk Production in East African Zebu Cattle"). The available genetic parameters suggest that up to 50 per cent of the total variation in milk yield and length of lactation period are attributable to additive genetic variation, but the rates of genetic progress due to selection have been very low—a result in agreement with what has been discovered elsewhere in the tropics. He considered that the small East African zebu animal has only about half the genetic potential of the Indian Sahiwal for milk production, and he associated the increased milk production in Sahiwal grades with an increased length of period of lactation, a shorter dry period, and a slightly longer calving interval than in the East African zebus. M. Harrison talked on "The Application of Population Genetics to the Improvement of Maize in Kenya". Pyrethrum breeding was discussed during two talks by R. B. Contant. In the first ("Present Concepts of Pyrethrum Breeding"), he considered the genetic nature of the main selection characters and some experimental and mathematical procedures for the assessment of variability in populations, heritability, and progress under selection. He then considered the content of pyrethrin in relation to complications arising from the negative correlation between flower yield and pyrethrin content, and made predictions of future progress in both. In the second paper ("Investigations on the use of *Chrysanthemum* species in the Improvement of Pyrethrum (*C. cinerariaefolium*)"), existing and desirable properties of pyrethrum were listed, and cytological investigations in regard to interspecific crossings were detailed. In this connexion, present work at Molo (Kenya), with special reference to *C. nivellei*, was described.

Malcolm J. Coe ("Primary Stages of Flowering Plant Colonization in the Nival Zone of Mount Kenya") described the disruptive distribution of this zone in relation to the peaks and glaciers of Mount Kenya. He observed that *Senecio keniophytum* is the first flowering plant to become established at the edge of retreating ice, followed by *Arabis alpina* and *Agrostis trachyphylla*. This investigation, supported by geological observations, indicated that the glaciers of Mount Kenya have undergone a period of advance in recent times.

Morphological features of a fungal pathogen of wheat were discussed by Dr. N. C. Otieno ("Ingress of *Cephalosporium gramineum* into Winter Wheat Seedlings and Subsequent Histological Relationships between the Pathogen and the Suscept"). He then described, for the first time, methods and avenues through which the pathogen enters the plant, the precise time of entry, and the subsequent course of the pathogen. The restriction of the pathogen to the vascular bundles was tentatively explained by regarding the pathogen as actually a saprophyte which can only attack the non-living tissues. What factor keeps it out of living tissues is so far unknown. Since it was first recorded in Kenya in 1940, bacterial wilt (*Pseudomonas solanacearum*) is one of the two most destructive potato diseases. It contaminates the soil, and R. A. Robinson and A. H. Ramos ("Bacterial Wilt of Potatoes in Kenya") have established that the Kenya strain is different from the more common brown rot. These authors amassed circumstantial evidence to show that the pathogen is exotic, and resistant varieties are as yet unknown.

Arts and Social Sciences

The papers under this heading will be treated more briefly, and are mentioned in this journal primarily to demonstrate the breadth of the meetings at the symposium.

The 1948 census revealed that the plateau areas of western Kenya had two main areas of population concentration. Dr. S. H. Ominde ("Some Aspects of Land and Population Problems in the Lake Districts of Western Kenya") detailed these, and then observed that the provisional figures of the 1962 census have shown that this concentration has become more marked since 1948. A detailed examination of the rate and source of economic expansion in three East African countries during the post-war period was made by D. P. Ghai ("Growth and Fluctuations of Money Incomes in East Africa: 1946–

1960"), and he derived statistical measures devised to quantify the relationship between changes in export and money incomes.

The concept of the African personality was presumed to have a social significance by Cleo Kumalo ("Toward a Sociological Approach to the Concept of the African Personality"), and he went on to raise the basic question of whether and how that reference can be discovered through the logico-experimental method of science. In his topical talk, Donald S. Rothchild ("Progress and the One-party State") concluded that the evidence for a correlation between political processes and economic progress is neither complete nor convincing. R. C. Bridges ("Are Explorers Redundant in East African History?") asserted that impressions given in Europe by explorers' exploits created a "frame of reference" within which missionaries and administrators worked, and in this way explorers' work is, indeed, a factor in the history of East Africa. Another topical talk was that given by A. K. Mayanja ("The Rule of Law in Emergent African States"), who recommended that both the formal and metalogical aspects of the rule of law need to be insured against legislative erosion.

Jonathan Silvey ("Formal and Informal Learning through the Medium of a Second Language") considered Bernstein's concept of public and formal (or individualized) language in relation to the language modes characteristic of rural and educational life in East Africa. These meetings were ended by J. Kariuki ("Poetic Works") reciting a number of his own poetic works.

The proceedings of the entire symposium are to be published. This is just one of the many decisions made at the annual general meeting and the meeting of the Executive Council. Others are the decision to hold the next symposium meeting in Nairobi next May; the appointment of several standing committees (Research and Studentships, Conferences and Liaison, Editorial and Symposium, Education, and Membership); collaboration with the Ghana Academy of Sciences in bringing to fruition the *Encyclopaedia Africana* project; decision on the principle of admitting societies operating within East Africa to institutional membership; and plans for bringing about the formal recognition of the Academy as their national academy by the East African Governments. Dr. N. C. Otieno, lecturer in botany, Royal College, Nairobi, was elected president of the Academy, Dr. J. Njoroge (plant pathologist, Scott Agricultural Laboratories, Kabete), secretary, and Mr. M. Alala (lecturer in mathematics, Royal College, Nairobi), treasurer.

THOMAS R. ODHIAMBO

THE DELHI PILLAR

THE mystery which has enveloped this quite remarkable piece of metal should be once and for all removed as a result of three papers published in the February issue of the *Journal of the National Metallurgical Laboratory of India* (5, 24; 1963). All that has been previously written is summarized and the theories propounded to explain its resistance to corrosion are collected. Despite efforts by M. K. Ghosh and by Lahiri, Banerjee and Nijhawan to support the view that there was something about the manufacture of the metal which endowed this remarkable forging with special resistance, there can be little doubt that Bardgett and Stanners's conclusions deserve acceptance.

Made by hammer-welding small balls of native iron of a fairly wide range of carbon contents, its resistance to corrosion during the 1,600 years or so of its existence is ascribed to the combined influence of a number of normal, favourable factors. Among these are included the climate and freedom from pollution of the atmosphere which exceeds Vernon's critical humidity of about 80 per cent for less than 5 per cent of the year. Secondly, to a progressively decreasing rate of attack due to the building up of a protective layer of oxide and scale during the early years of exposure.

That in the past on ceremonial occasions the pillar was anointed appears to be probable, while even to-day it is repeatedly handled. This is supported by the X-ray analysis of a minute sample of the surface coating about 5 ft. above the base which contained no iron and only quartz and chalk providing another source of protection. The authors conclude, and it is impossible to disagree with them, that "It is evident that a number of favourable factors have been acting simultaneously and the sum total of the information leads to the view that, although one may justly admire the skill and technology of the early Indian ironworkers in producing so large a monument, there is no reason for surprise at the longevity of the pillar. The composition and structure of the iron or its method of manufacture can only have contributed in a minor degree to its preservation and are in no way essential for this. The present authors, indeed, are as confident as one can be in such matters that, given the same circumstances and environment, a pillar forged from modern tonnage steel would, after 1,600 years, have been practically as preserved". Coming from men of such experience and in view of all the evidence here set out, it is impossible to disagree with this view.

The other two papers also discuss the technological aspects of ancient iron-making in India and confirm the account given in Percy's book on iron and steel of a hundred years ago. In particular, they refer in some detail to the iron beams at the Sun Temple of Konarak which show similar resistance to corrosion to the Delhi pillar despite the fact that they are situated on the sea coast. Lahiri, Banerjee and Nijhawan's insistence on the protective influence of envelopes of slag around the metal may here have exercised a decisive influence.

F. C. THOMPSON

RECOGNITION OF SEDIMENTARY ENVIRONMENTS

FOR more than a century geologists have striven to discover criteria by which the environment of deposition could be inferred from the characteristics of arenaceous rocks. Concentration on one or another aspect has changed over the years, providing a mass of data which have underlined the breadth of the problem without providing a simple solution.

Investigations of the particle size distribution of modern sediments have shown that many environments can share similar distributions, while work on the hydraulic aspects of particle transport and deposition has failed to explain the complex nature of many natural sediments. For some years there has consequently been a tendency to rely less on granulometric analyses and more on forms of bedding, such as ripple marks, laminations, and other small sedimentary structures. But here also there are hints that few, if any, of the supposedly diagnostic features are unique to any one environment, and the feeling is gaining ground that the environment in which a given sample was deposited can only be deduced reliably by considering the complete suite of associated sediments.

Some renewal of hope that at least wind-laid, water current-laid, and wave-laid sands and gravels can be differentiated by an examination of the size and shape relations of the particles in a small sample is offered by A. J. Moss ("The Physical Nature of Common Sandy and Pebbly Deposits. Part II": *American Journal of Science*, 261, 297; April, 1963). Because the stability of a particle on a bed depends largely on the effects of neighbouring particles, it is shown that there is a strong tendency for grains of similar size, shape, and density to accumulate together from a saltating traction load. The resulting population may be accompanied by a population of particles small enough to reside in its interstices, and one of particles large enough to be slid or rolled over its surface. The relative abundance of these populations, which can be recognized in modern and fossil sediments, forms the basis of interpreting the environment.

Extensions of the idea help to explain many peculiarities of natural deposits, such as the formation of heavy mineral concentrates, the abrupt changes in texture of adjacent laminæ, and the simultaneous accumulation of particles of widely differing size. They also explain the failure of granulometric analysis, as usually carried out, and provide a unifying link between the textural and structural approaches to the problem.

J. C. M. Taylor

ESTIMATION OF RUBY LASER OSCILLATION LOSS

By JOSEPH I. MASTERS

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LASER action in a pulsed system requires that during the pump cycle a population inversion be attained which is sufficient to provide enough gain per optical transit to overcome the various losses of the loaded laser enclosure. In addition to end-wall transmission losses, which are accurately known from end-reflectivity specifications or direct measurement, there are also internal oscillation losses in solid laser media caused primarily by scattering, non-resonant self-absorption, end-wall absorption and scatter, and crystal non-uniformities, none of which is directly measurable.

The two principal effects of internal losses are an increase in threshold pump power for onset of oscillation over predicted values and a decrease in output energy above threshold. The latter is dramatically indicated by the ruby laser output shown in Fig. 1 (ref. 1), where the Q or internal radiation lifetime of the laser enclosure is made greater by increasing end reflectivity r from a low value while the pump energy pulse remains unchanged. From energy considerations, we would expect to see no decrease in integrated output energy under these conditions in the absence of internal loss mechanisms. Therefore, the observed reduction in output is evidence of significant loss per transit, enhanced in this example by the effective increase in the number of transits as r is increased.

It was found practical to examine internal losses by investigating conditions at threshold, since this specialization eliminates from the problem well-known uncertainties in measurement and interpretation of output power. The estimate of the degree of internal loss is accomplished by comparison of experimental results with theoretical curves. From the point of view of a mathematical model, threshold is defined by use of a step function optical pump input to a population rate problem that is based on an idealized ruby energy level system. Threshold pump power, then, is a step function in time of photon flux density or of pump transition probability sufficient to produce a steady-state population inversion ΔN that satisfies an oscillation condition of the form $\Delta N\, l\, \sigma = \ln r$, where σ is the cross-section for the laser transition and l is the optical path-length between reflexions characterized by r. Similar mathematics with only slight variation in assumptions have appeared in detail previously[2-5]. Therefore, only the particular assumptions and solution employed here are stated.

A four-level ruby system is assumed in which atoms excited by pump photons from the ground-state to absorption bands rapidly decay non-radiatively to the metastable R_2 and R_1 levels ($\nu_{R_1R_2} = 29$ cm^{-1}), which, in turn, rapidly thermalize to a Boltzmann distribution. These two processes are so fast compared with the reciprocal of the pump transition probability or the R level lifetime that a pump photon absorption is assumed to excite a ground-state chromium atom directly to one of the R levels. For the low-temperature limit of interest in this article, ~150° C, the Boltzmann distribution of the closely spaced ground-state levels is taken as equal. Since small ΔN threshold conditions prevail, the only laser transition considered is from the level R_1 to the ground-state, and the only loss assumed from the excited state prior to laser action is by unamplified spontaneous emission. The variations of the laser transition line width $\Delta\lambda$ with temperature and R level Boltzmann distribution are the only temperature dependencies considered. In addition, we make the assumption that the laser medium is optically thin, or that the pump photon flux is uniform in the sample. These assumptions yield the following solution for ruby threshold power versus reflectivity and temperature T:

$$P_{\text{rel}} = A\,\{[1 + \exp(42/T)] - 2\ln r\}\,/(2A + 2\ln r) \qquad (1)$$

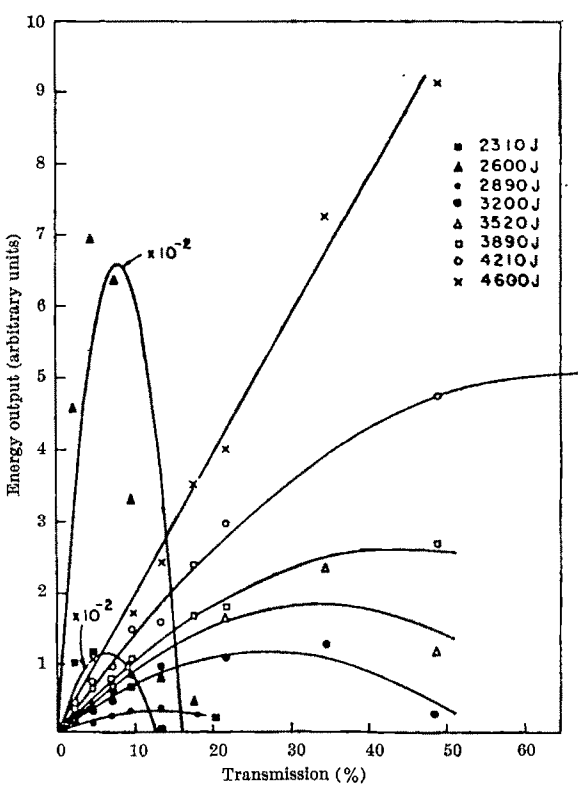


Fig. 1. Ruby laser output for various fixed values of input energy

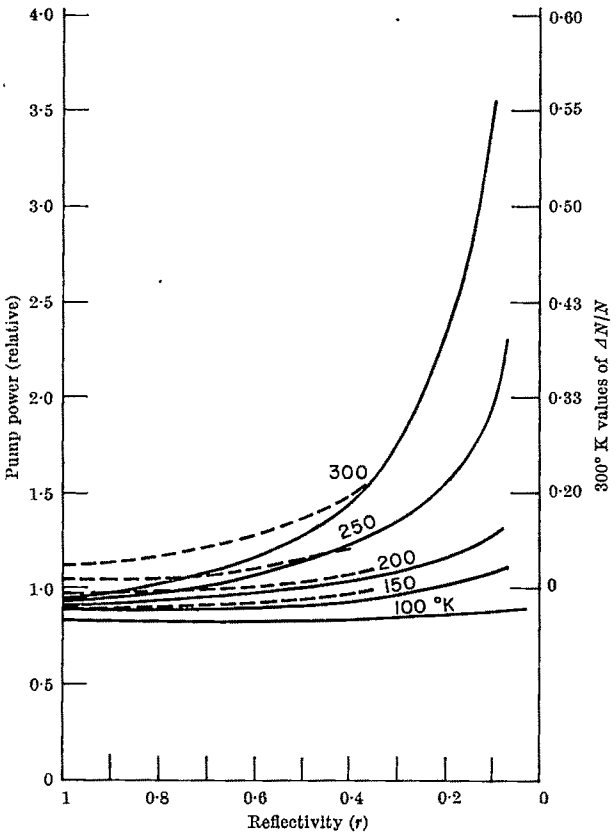


Fig. 2. Ruby laser threshold conditions. $l = 10$ cm

where $A = N\sigma\ l$ and $\sigma(T) = \sigma(300°)\ \Delta\lambda\ (300°)/\Delta\lambda(T)$. For the room temperature value of the laser transition cross-section, the value $\sigma(300°\ \mathrm{K}) = 2{\cdot}5 \times 10^{-20}$ cm^2 is used[2]. Values of $\Delta\lambda(T)$ are taken from Schawlow[6]. For comparison with laboratory data, values of constant pump power are given on a relative basis and are normalized so that $P_{rel} = 1$ is the pump power required for inversion ($\pm\ \Delta N = 0$) in a three-level system. Theoretical curves, however, will drop below unity for large r and low T because of the effect of the Boltzmann distribution of the R levels on the population of the R_1 laser transition level. As an example, equation (1) is plotted in Fig. 2 as a family of solid curves for various temperatures for the case $l = 10$ cm. Inversion values for 300° K only are given on the right scale.

Laboratory results were obtained using ruby rods of 90° orientation and ~ 0·05 per cent Cr^{+++} doping ($N \cong 1{\cdot}6 \times 10^{19}$ ions-cm^{-3}) that were nearly totally reflecting on one end. Variation of reflectivity at the output end was accomplished by using mirrors of several reflectivities employed as an external opposing reflector of the laser enclosure. External reflector displacement, however, was small enough to preclude far field effects[7]. A conventional helical xenon flashlamp was used for pumping. Because of near total reflexion at one end of the laser rod, the value of the transit distance l within the active medium between mirrors of reflectivity r is effectively twice the physical rod-length.

In actual practice, experimental threshold results versus reflectivity and temperature are not in a form suitable for direct comparison with theoretical curves. The comparison is made possible by the use of two first-order approximations. The first approximation accounts for the fact that only threshold electrical energy rather than threshold radiant pump power is measurable, and that the pump energy is not delivered at a constant rate, as assumed theoretically, but usually follows the discharge of a large inductive storage capacitance C. The second approximation must establish a criterion for normalization of experimental pump data, since values of this quantity are, at best, relative.

The pump power approximation is made by relating electrical stored energy E and average lamp power P and by assuming further that effective pump photon flux is proportional to P. We note from xenon flashlamp data that lamp resistance R is nearly constant, at least for the first half of the flash, and that the voltage decay is approximately exponential. Thus:

$$\bar{P}_\theta = \frac{1}{R\theta} \int_0^\theta \left(V_\theta e^{-t/RC} \right)^2 dt$$

where θ is the time to reach threshold as measured from the beginning of the pump pulse, or, when θ is used as a subscript, it denotes a threshold quantity. The integration yields:

$$\bar{P}_\theta = E_\theta\ (1 - e^{-2\theta/RC})\ /\theta = E_\theta F(\theta)$$

where $F(\theta)$ is a slowly varying function that decreases from the value $2/RC$ with increasing θ. We are particularly concerned with threshold data at various temperatures for values of r approaching unity, and under these conditions θ itself was found to vary only slightly. We assume, therefore, that $F(\theta)$ is essentially constant or that threshold electrical energy values and average pump power are proportional.

The second approximation, that of normalization, is handled by taking advantage of a property of the physical system apparent from the 100° K or 150° K theoretical curves shown in Fig. 2. Here we see that at low temperatures, when the gain is great, the pump power for threshold is only slightly larger than the pump power for attaining inversion even if losses are very large; that is, at 150° K, if r is as small as 0·3, less than 10 per cent increase in P_{rel} is required to attain threshold. The experimental value of E_θ for 150° K and $r \to 1$ for a given sample was thus co-plotted with some confidence on the theoretical curves for this temperature, and all other experimental points were normalized by dividing their E_θ values by $E_\theta\ (150°)/P_{rel}\ (150°)$. The experimental curves in Fig. 2 (dashed lines) result from this procedure.

An enhanced vertical spread of the data curves as $r \to 1$ results from various internal propagation losses. Evaluation of the internal loss factor is accomplished by generalizing the threshold condition in the form $\Delta N\, l\sigma = \ln r_{eff}$. In this notation $r_{eff} = r$ for the idealized model (solid-line curves), whereas $r_{eff} = rr'$ for real samples in which $1 - r'$ accounts for internal losses, that is, all losses other than transmission loss. The desired loss factor $1 - r'$ for a laser rod sample being examined may now be obtained simply by fitting the vertical spread in the experimental temperature curves at $r = 1$, for which $r_{eff} = r'$, with an equal spread in the solid theoretical curves. Thus the estimated loss factor is unity minus the abscissa value corresponding to the matching vertical spread in the solid-line curves.

By following this procedure, the data taken from a 2 in. × 0·25 in. ruby rod (Fig. 2) indicate an estimated loss factor of 35–40 per cent for a transit of twice the rod's length, which is typical of the best available ruby samples of this size that were tested.

I thank John H. Ward and Howard R. Huff for assistance and Sylvia W. Masters for performing calculations. This work was supported by the U.S. Air Force Cambridge Research Laboratory under Contract No. *A F*19(628)–405.

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JOULE HEATING OF THE IONOSPHERE OVER HALLEY BAY

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In earlier communications I reported investigations of joule dissipation in the ionosphere[1–3]. Some spot estimates of the heating were given previously. It is the purpose of this communication to derive a distribution (with magnetic disturbance) of such heating in the ionosphere over Halley Bay (75·5° S., 26·6° W. geographic) using information provided by the Royal Society International Geophysical Year Antarctic Expedition[4] to that place. An important correction to one source of information previously used by me in connexion with joule heating is also noted.

The Royal Society's expedition to Halley Bay made simultaneously observations of the geomagnetic field and soundings of the ionosphere with an ionosonde. These two sets of observations together provide estimates of the joule dissipation in the ionosphere which is given by the formula:

$$Q_j = j^2/\sigma_3 \qquad (1)$$

where j = ionospheric current density, σ_3 = Cowling conductivity[5].

Consider a polar situation in which the geomagnetic field is approximately vertical; and electro-static field is applied so that the geomagnetic field lines are equipotentials, and the ionosphere is assumed at rest. The heating due to an ion-electron pair and the current per ion-electron pair are distributed approximately as in Fig. 1. The peak in heating below 100 km is unimportant for changes of atmospheric structure because of the high density of atmosphere there. The peak at 150 km, however, has significant consequences. For present purposes it is assumed that the electron density is uniform with height. Two extreme models are investigated to place bounds to joule heating as a function of magnetic disturbance. Model A assumes that current flows in one direction in an infinite plane slab with a height distribution as in Fig. 1. Model B assumes that current flows above the observatory in one direction in a jet 100 km wide and distributed in height as in Fig. 1. In both models it is assumed that the value of the critical frequency f_c of sporadic E ionization can be used to define the electron density in the current region by:

$$n = mf_c^2/4\pi e^2$$

where m is the electron mass and e its charge.

If two-thirds[6] of the disturbance (ΔH) of the horizontal component of the geomagnetic field is due to ionospheric current then model A gives (in E.M.U.):

$$\left.\begin{aligned} j_{\text{av.}} &\approx 2 \times 10^{-8}\ \Delta H \\ \text{and model } B: \quad j_{\text{av.}} &\approx 5 \times 10^{-8}\ \Delta H \end{aligned}\right\} \qquad (2)$$

At the altitude where the heating peaks (about 150 km) $\sigma^3 \approx 2\sigma_1 \approx 3 \times 10^{-20}\ n$ (see model h of ref. 7) and $j \approx j_{\text{av.}}$ It should be noted in passing that these estimates of conductivity depend finally on collision cross-sections of low energy ions and electrons with neutral particles and so remain uncertain perhaps by a factor 2.

Thus, upper and lower limits to peak joule heating at 150 km altitude are suggested by the following equations:

$$\left.\begin{aligned} \text{Model } A: Q_j &\approx 10^{-10}\ \frac{(\Delta H)^2}{f_c^2}\ \text{ergs cm}^{-3}\ \text{sec}^{-1} \\ \text{Model } B: Q_j &\approx 6 \times 10^{-10}\ \frac{(\Delta H)^2}{f_c^2}\ \text{ergs cm}^{-3}\ \text{sec}^{-1} \end{aligned}\right\} \qquad (3)$$

where ΔH is now in gammas ($1\gamma = 10^{-5}$ gauss) and f_c is in Mc/s.

In their Fig. 27, Bellchambers *et al.*[8] show the distribution at Halley Bay of f_c with magnetic disturbance as measured by the magnetic disturbance index Q (not to be confused with Q_j). Q is a quarter-hourly measure of disturbance based on the same principles as the magnetic K index. It therefore does not strictly represent steady-state disturbance; however, it is here considered to give the best available representative estimate of the steady (or d.c.) disturbance at any one time. The bulk of their observations relate to magnetic disturbance in the range 10–500γ. Elsewhere[9] it was shown that at a station of similar distance from the auroral zone as Halley Bay (namely, Macquarie Island) magnetic bays of amplitude $> 200\gamma$ had durations generally > 50 min. Such bays can therefore be considered as virtually a d.c. phenomenon.

Using Fig. 27 of Bellchambers *et al.* and equations 3, a distribution of Q_j versus ΔH is delineated (Table 1). This suggests upper and lower limits for Q_j.

Table 1

ΔH (γ)	50	100	200	500	1,000
		Q_j (ergs cm^{-3} sec^{-1})			
Model A	6×10^{-8}	7×10^{-8}	3×10^{-7}	10^{-6}	3×10^{-6}
Model B	3×10^{-7}	4×10^{-7}	2×10^{-6}	6×10^{-6}	2×10^{-5}

It seems that only *in situ* simultaneous determinations of j and n can resolve the heating to better than the present estimates. The estimates given here confirm those obtained earlier[2], except for one previous estimate which is corrected below.

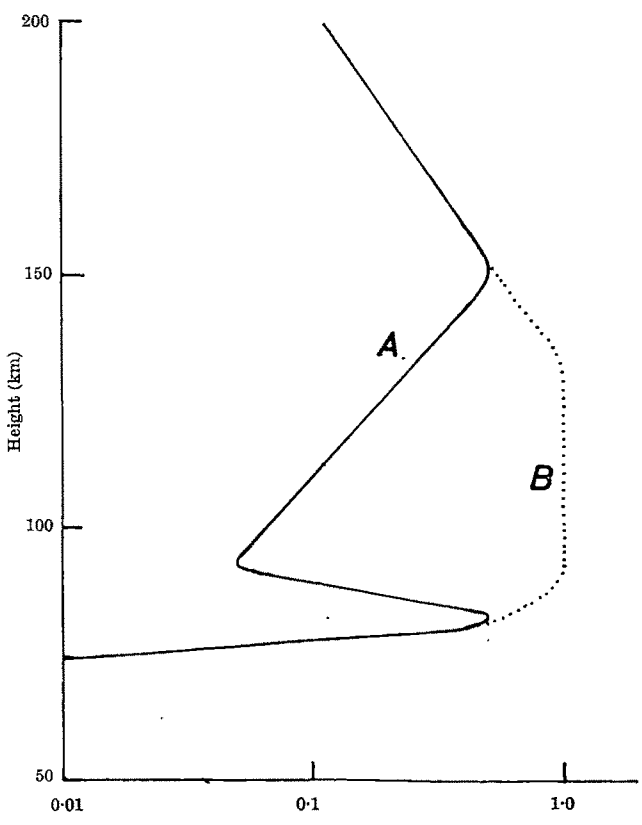


Fig. 1. Schematic representation of the distribution of: (A) Q_j per ion-electron pair (proportional to σ_3/n; (B) j per ion-electron pair (proportional to $(\sigma_1\ \sigma_2)^{1/2}/n$ under conditions in text. (σ_1, Pedersen conductivity)

Geomagnetic observations at Halley Bay during the same period are reported by McDowell and Blackie[11]. Their Fig. 32 shows that on the five most disturbed days of the months the disturbance was 200γ for about one-quarter of the time. The heating implied thereby could have important implications for the local ionosphere, such as modification of the scale height[2], changes of temperatures[2,10] and creation of winds[2]. It is suggested here that the observed increase of the heights of the tops of auroral rays at sunspot maximum (ref. 12, Fig. 76) is explainable by the bulging of auroral zone atmosphere due to joule heating.

The opportunity is now taken to correct an estimate of joule heating due to average *DS* currents in the ionosphere[1-3]. The intensity of these currents which I used had been given[13] as $1{\cdot}5 \times 10^5$ A between flow lines (about 1 degree apart near the auroral zone). It has since been pointed out[14] that this quantity was misprinted and should have been $1{\cdot}5 \times 10^4$ A. On the model adopted here this would cause a ΔH of about 100γ, and therefore a Q_J (*DS*) value in the range 7×10^{-8}–4×10^{-7} ergs cm^{-3} sec^{-1}.

Several mechanisms have been suggested for the apparent abnormal heating of the ionosphere during magnetic disturbance. First, corpuscular bombardment by energetic charged particles has been considered in various places. The observation of McIlwain[15] suggest that electrons in the energy range 1–100 keV are absorbed principally below 120 km altitude. *Bremstrahlung* from these electrons presumably is absorbed principally at lower heights. The energy flux of these electrons may be[15] commonly of order 20 ergs cm^{-3} sec^{-1} and rarely 2,000 ergs cm^{-3} sec^{-1}. Supposing their energy is absorbed in a 50-km thickness of atmosphere, then commonly their energy input into the atmosphere may have an upper limit of about 4×10^{-6} ergs cm^{-3} sec^{-1}. This energy absorbed by neutral particles would appear to have little effect on their temperature because of the high density of atmosphere below 120 km.

Secondly, Dessler[16] considered the damping of hydromagnetic waves in the ionosphere and came to the conclusion that waves of frequency about 1 c/s were most important in this regard and deduced a peak heating rate of about 3×10^{-9} ergs cm^{-3} sec^{-1} at an altitude of about 175 km. Francis and Karplus[17] subsequently examined the dissipation of hydromagnetic waves in the frequency range $\omega = 1 - 25$, and fully confirmed Dessler's estimates of heat input. Akasofu[18], however, found that the rise in atmospheric temperature due to the likely naturally occurring hydromagnetic waves in this frequency range was not greater than 5 degrees.

Thirdly, I[1-3] have investigated the joule dissipation of the quasi-steady electric currents which flow in the ionosphere to cause the basic geomagnetic disturbance variations, for example, the auroral electro-jets and the associated *DS* current system. The heat input due to this d.c. heating peaks at an altitude of about 150 km and is capable of causing significant changes in the temperature of gases there[1-3,10]. Table 1 shows estimates of such heating. Heat input at this height and of this magnitude is favourable for the production of significant changes in structure of the atmosphere at and above 150 km. It is suggested that the joule dissipation of these quasi-steady ionospheric currents is the most effective of the three heating methods mentioned here for the production of such atmospheric changes.

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PETROFABRIC ANALYSIS OF QUARTZ USING THE POINT-COUNTER

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AT the beginning of the century, people were toying with the idea of determining the orientation of quartz c-axis in a rock using the gypsum plate and statistical methods[1-3]. After the publication of Berek's book[4], the use of the universal stage for petrofabric determinations was realized. The gypsum plate method has since been completely neglected; quantitative estimation by this method was extremely inaccurate and had no time advantage over the universal stage.

The method described here was developed independently of the foregoing methods in response to a need to investigate systematically both the compositional variations and the fabric variations throughout a large gneiss body (the Antigorio nappe, the lowermost of the Alpine nappes). Owing to the length of time needed to analyse a single thin-section with the universal stage (measuring, plotting and contouring), systematic investigations cannot be carried out by this method. Using the point-counter and gypsum plate, the quartz orientation can be estimated at the same time as the mineral composition, and without any increase in the time required to estimate the mineral composition alone. Modal analysis of a stained thin-section of a normal gneiss, including the procedure for quartz about to be described, takes 0·5–0·75 h.

The procedure is exactly as for a normal estimation of mineral composition, with the following modifications:

(*a*) The thin-section must be stained (plagioclase—red, alkali felspar—yellow, quartz—uncoloured[5]); the gypsum plate is kept in continually and all minerals except quartz are counted with uncrossed nicols.

(*b*) The thin-section is always cut perpendicular to the foliation and the trace of the foliation is orientated always parallel to the same vibration direction of the gypsum plate (I place the foliation of every thin-section parallel to α (see Fig. 1)).

(*c*) Quartz is counted with crossed nicols on three separate keys, one for when the quartz is definitely blue, one for when definitely yellow and one for when red (that is, any shade of colour which cannot be called definitely blue or yellow), the numbers obtained being *B*, *Y* and *R* respectively.

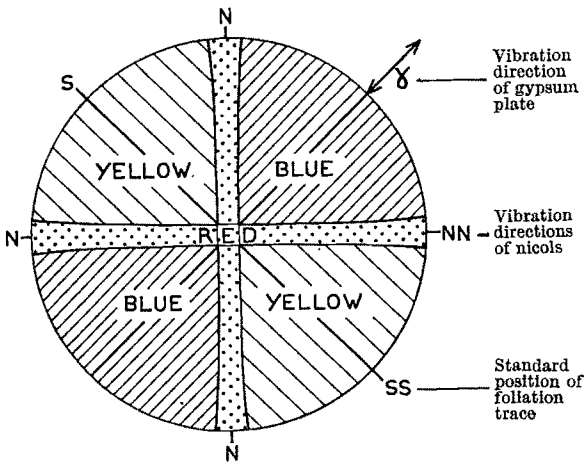


Fig. 1

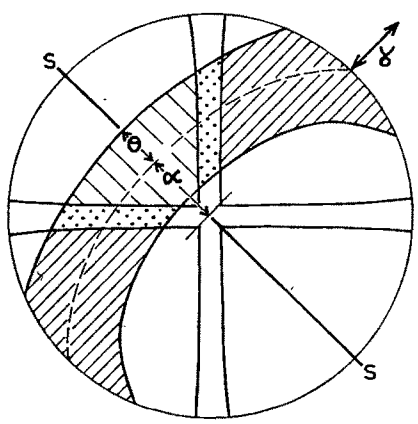


Fig. 2

(*d*) The BY-ratio and R-ratio are calculated and plotted as described below.

Determination of the red cross. Let us consider a thin-section of a quartz-rich sediment, for example, rounded quartz grains in a calcite matrix, the quartz having been subjected to no systematic movements with respect to the *c*-axis. If we measure the *c*-axes using the universal stage, and plot the measurements in the usual way on a Schmidt net, the result will be a homogeneous even-density pole distribution, corresponding to random axis orientation. Suppose also that we note the colour of each grain we measure when the gypsum plate is inserted and the thin-section is placed in one previously decided position. The colour scheme of the resulting diagram will be as in Fig. 1.

If we measured enough axes, we would be able to draw a line between the red and blue and yellow areas of the diagram and these lines would lie approximately along small circles, the four limbs of the red cross being symmetrical and of equal angular width.

Now let us take the same thin-section and, counting the quartz on three keys as above, use the point-counter to obtain the numbers B, Y and R. What is the relation between these? We would obviously expect that one relation should be: $B = Y$. Also, the relation between R and the others must be a measure of the angular width of the limbs of the red cross. Recognition of the truth of this last statement is the key to this method, since the angular width of the red cross is a constant, independent of the pattern of the quartz orientation. It should be noted that the numbers B, Y and R are proportional to the areas of the blue, yellow and red regions of the diagram we prepared using the universal stage.

In order to illuminate the significance of the relations between these three numbers, when they are obtained from actual thin-sections, I have worked out a number of idealized quartz orientation patterns. The first step is to determine the angular width of the red cross using thin-sections in which it is reasonably certain that there is random orientation of the quartz *c*-axes. I chose sections of sandstones with the quartz grains more or less separated by calcitic or chloritic matrix. Some sections were repeated with different positions of the stage. The following ratios and standard deviations were obtained, using the point-counter to determine the numbers B, Y and R as above.

$$BY\text{-ratio} = 1{\cdot}0 + 0{\cdot}1$$
$$R\text{-ratio} = 7{\cdot}7 \pm 0{\cdot}7$$

the ratios being defined as follows:

$$BY\text{-ratio} = B/Y \text{ or } Y/B \text{ (always greater than 1)}$$
$$R\text{-ratio} = (B + Y + R)/R$$

From these ratios the angular width of the red cross was obtained by a method of trial and error—crosses of varying width were drawn over a Schmidt net and the diagram covered with points placed at the intersections of an evenly spaced rectangular grid (20 cm net, axes of grid not parallel to axes of cross, lines of grid 0·5 cm apart). Hence the area of a given cross could be determined by counting the points within its boundaries, as could the total area of the circle. By this method it was found that an angular width of 8° gave:

$$R\text{-ratio} = \frac{\text{total area } (B + Y + R)}{\text{area of red cross } (R)} = 7{\cdot}54$$

Hence it was determined that I could not distinguish between blue and yellow if a quartz *c*-axis lay with an angle of less than 4° from two vertical planes representing the vibration directions of the upper and lower nicols. The cross is drawn with this angular width in Figs. 1 and 2.

Now we can go on to consider what ratios we are likely to obtain from certain simple idealized orientation patterns.

(1) *Girdle perpendicular to foliation.* This is a common case in nature; sometimes the girdle will be around a linear structure, but often no lineation will be observable in the hand-specimen. When no lineation is available, the thin-section will be cut at some unknown angle with respect to the plane of the girdle, the thin-section only being orientated with respect to the foliation. The angle between the plane of the girdle and the pole of the thin-section is designated α (Fig. 2).

In order to treat the shape of the girdle we must make the following simplifications: (*a*) the girdle is parallel-sided, that is, with small circle boundaries; (*b*) the pole distribution within the girdle is homogeneous. Hence we can designate the angular half-width of such a girdle, θ (Fig. 2), a quantity which is a measure of the intensity of development of the girdle.

From the second simplification, we see that the areas of blue, yellow and red of the girdle, as in Fig. 2, are proportional to the number of grains falling in each area (as would be determined using the point-counter). Different values of α and θ will produce different pairs of BY- and R-ratios.

The areas of such girdles have been calculated systematically (by the same method as described above for determining the width of the red cross), that is, values of θ were taken 10° apart from 0° to 90°, and for each θ the areas of blue, yellow and red (=numbers B, Y and R) calculated for α = 0°, 30°, 60°, 90°. From these areas the BY- and R-ratios were worked out and plotted as in the lower half of Fig. 3. Each point lies at the intersection of two lines, an α-line (thick dashed) and a θ-line (thin continuous).

In order to test these curves with a real thin-section, a hand-specimen was chosen which was suspected to show a

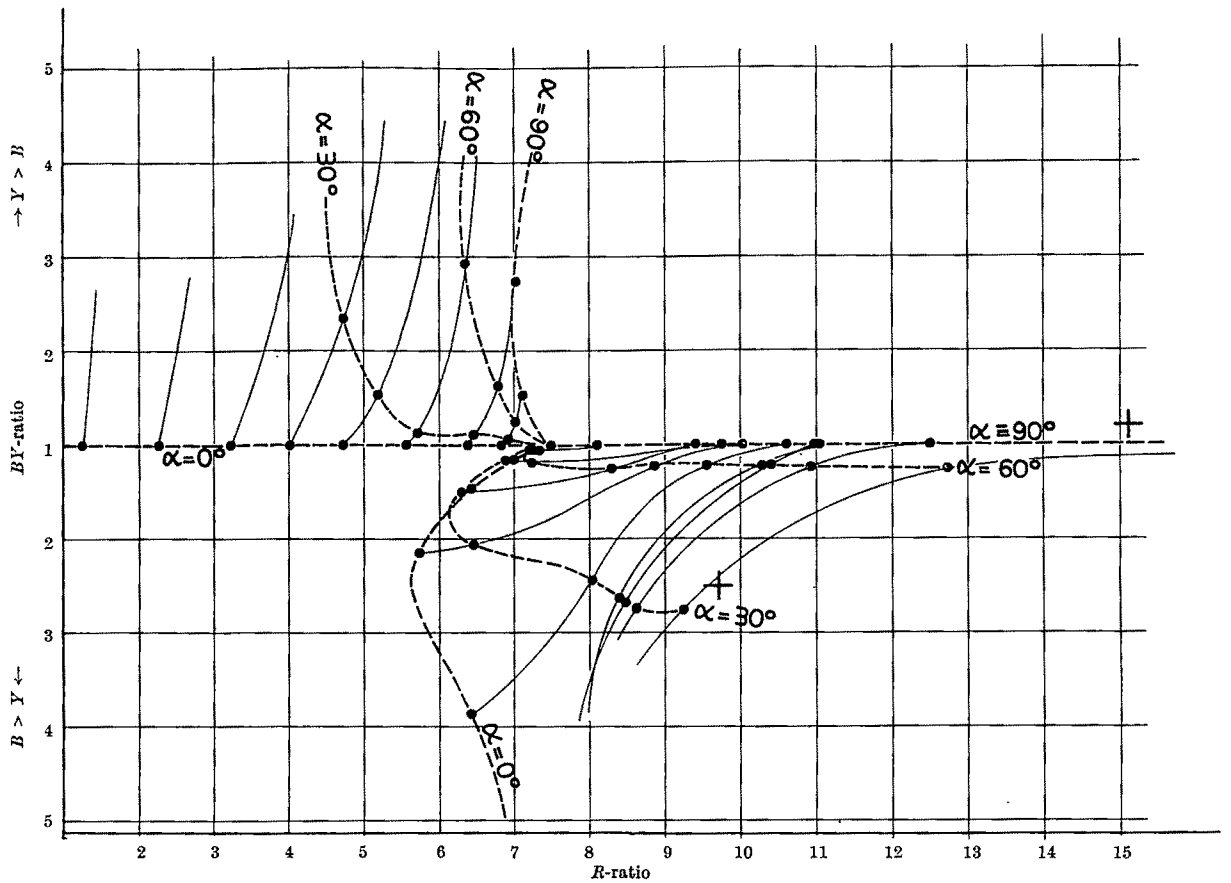


Fig. 3. *BY*–*R*-diagram of ideal girdle (lower half) and ideal pole-maximum (upper half)
α-line, thick dashed; θ-line, thin continuous. θ = 90° is random orientation and gives *R*-ratio = 7·5; θ-lines spaced at 10° intervals decreasing outwards from this point. + + = thin-sections mentioned in text

strong girdle orientation. This was an intensely rodded quartzite[6], and two sections were cut, one perpendicular, the other oblique (27°) to the rodding. These gave the following results (see Fig. 3):

perpendicular : *BY*-ratio = 1·3 (*Y* greater than B)
R-ratio = 15·1
oblique: *BY*-ratio = 2·5 (*B* greater than Y)
R-ratio = 9·7

When we remember that both the simplifications on which the curves are based are completely unlikely to occur in nature, these points fit surprisingly well:

(*a*) The line joining the two points is approximately parallel to the nearest θ-lines of the ideal and in a position not very different from that of the most 'intense' (θ → 0°) of these lines.

(*b*) The perpendicular section lies close to the 90° α-line and the oblique section lies close to the 30° α-line.

These points fit so well with the ideally expected values that we can now say with certainty that we are dealing with a girdle about the rodding axis and that this girdle must be strongly developed. The total time required for counting the sections (1,000 quartz grains in each) was 1·5 h.

In the case of simple orientation patterns (see also later cases), this method of counting two or more sections of the same hand-specimen gives a fair idea of the pattern-type and intensity of orientation ; it becomes 'semi-three-dimensional'. I do not believe, however, that it has any practical use, since for more complex patterns there are no ideal curves for comparison and as soon as more than four thin-sections have to be counted for no other reason than an investigation of the quartz fabric, then the universal stage is as quick and much more definitive.

These objections are completely reversed however if we are carrying out a systematic study of a large gneiss mass. This involves an investigation of compositional variations but up to now the fabric could not be treated systematically—the time required to measure hundreds of thin-sections using the universal stage is completely prohibitive. Now, however, with each modal analysis, a *BY*- and an *R*-ratio for the quartz is obtained without increasing the time required for each thin-section.

Let us take an imaginary example to find out what we would expect from such an investigation. We have a gneiss mass which has been worked on with universal stage methods and found to give a good girdle perpendicular to the foliation (as above) in all thin-sections measured (probably not more than 10, and 200 grains per section). During systematic modal analysis we have obtained very many pairs of *BY*- and *R*-ratios (for example, 110 in the example to be cited later, 300–1,000 grains per section) and plotted them on the same diagram as the ideal curves. Suppose also that the gneiss has only rarely a lineation; most thin-sections are cut randomly but always perpendicular to the foliation. We would expect the points to show the following features:

(*a*) α varies from thin-section to thin-section, therefore the points will be concentrated in a zone parallel to the corresponding θ-line and will be equally distributed along the length of the zone. An additional requirement is that, when a lineation is present and if the lineation and the quartz orientation are the results of the same movements, sections perpendicular to the lineation should fall near the 90° α-line, sections parallel near the 0° α-line and oblique sections intermediate.

(*b*) If θ (= intensity of development of the girdle) was completely constant throughout the gneiss mass, this zone would be very narrow and by comparison with the

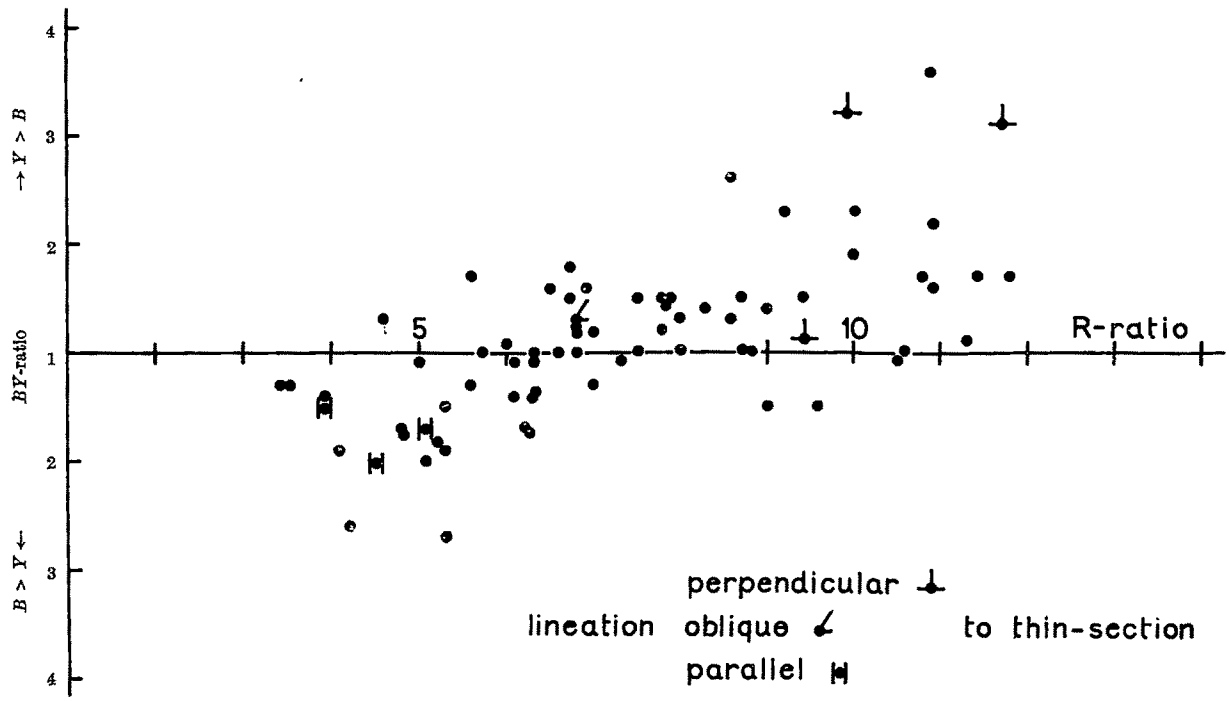


Fig. 4. *BY–R*-diagram of Antigorio gneiss

ideal curves would give a value of θ, a semi-quantitative measure of the intensity of orientation.

(*c*) If θ was not constant, the zone would tend to be broad and irregular, but still approximately parallel to the θ-lines of the ideal curves and still in the form of one elongate maximum. We then have the possibility of determining whether the less well-orientated gneisses are confined to a definite tectonic region (by plotting the points with different symbols representing different tectonic positions) or even whether the intensity of orientation depends on mineralogical or microscopic textural features (different symbols according to mineralogical or textural criteria). This will be illustrated by the example later.

(2) *Single pole-maximum in plane of foliation* (*a*-maximum).

Another common case in nature, the axis of the maximum often parallel to the direction of transport. This has been treated in exactly the same way as the girdle (upper part of Fig. 3). In this case, α = angle between axis of maximum and pole of thin-section, and θ = angular radius of a perfectly circular maximum with homogeneous pole distribution within its boundary.

The same discussion applies here also. The first obvious difference between pole-maximum and girdle is:

pole-maximum — *Y* greater than *B*
girdle — *B* greater than *Y*.

Another difference is that for a pole-maximum, *R*-ratio tends to be smaller than 7·5, while for a girdle the tendency is to be larger.

(3) *Single pole-maximum perpendicular to foliation* (*c*-maximum).

Again a common natural pattern and easily distinguishable from the above two, since however the section is cut (as long as it is perpendicular to the foliation), the maximum will have the same position in the thin-section. Hence there is no equivalent of α here and the *BY–R*-ratio diagram from a gneiss mass with such a fabric throughout must give a single non-elongate maximum, the position of which represents θ (intensity of orientation). This non-elongate maximum must lie on a line which is the same shape and in the same position as the 90° α-line of the second case, but lying on the *B* > *Y* side of the diagram.

(4) *Girdle in plane of foliation.* An unusual case in nature, the argument being similar to case 3.

Fabric analysis of the Antigorio gneiss using this method. Fig. 4 shows the results of modal analysis of 110 thin-sections of 'normal' Antigorio gneiss (that is, not including many analyses of veins, shear zones, local facies, etc.).

The points fall in a broad but definitely elongate zone which does not coincide with the ideal curves, that is, we are here dealing with a more complex pattern. That the pattern, whatever its exact shape, is constant throughout the gneiss mass, and that the *BY–R*-diagram represents one pattern and not, for example, two superimposed patterns is proved in the following way:

(*a*) Contouring the points would produce one continuous maximum.

(*b*) In most cases the gneiss is unlineated; but when a lineation was present, the orientation of the lineation with respect to the thin-section has been also noted on the *BY–R*-diagram. It is immediately obvious that thin-sections cut perpendicular to the lineation occur at one extreme end of the zone, thin-sections cut parallel at the other, and oblique sections somewhere between. By analogy, from the discussion of ideal cases above, we see that the long axis of the zone represents 'α', the orientation of the pattern with respect to the thin-section. Such a relationship can only be understood if the lineation and the quartz fabric are the results of the same deformation.

(*c*) The width of the zone must therefore represent the spread of the intensity of orientation (see above), that is, the variation of θ. The points were plotted with different symbols denoting different tectonic positions, and with different symbols according to mineralogical and textural features of the quartz in thin-section. There was never any tendency towards segregation of these symbols on the *BY–R*-diagram, further proof that one particular quartz orientation pattern is responsible and is a typical feature of this gneiss.

A few thin-sections of very strongly lineated gneisses give a second, exactly similar zone, parallel to and below the one illustrated here, outside the range of Fig. 4. Since, presumably, the strongly lineated gneisses show a

more intense quartz orientation than the normal, unlineated varieties, the displacement of the zone must represent this difference in intensity of orientation (that is, two different values of θ giving two parallel zones). The number of these sections is, however, still too small to give a good statistical basis.

From a general consideration of more complex quartz patterns, I find that the simplest pattern which could produce the distribution of Fig. 4 is an incomplete girdle (around the lineation when present), the 'gap' in the girdle being situated around *c* (the pole of the foliation). The exact shape, however, is unimportant since it is defined by the point distribution of the *BY–R*-diagram and the patterns from different gneiss masses can be compared on this basis alone. This method gives a statistical idea of the quartz fabric throughout a large rock mass, enabling one to see through the local variations towards the regional picture.

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GEOCHRONOLOGICAL INVESTIGATIONS ON ROCKS FROM NORTH WALES

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AN extensive programme of age measurements on rocks from North Wales using the potassium–argon method is being carried out at present in the laboratory of the Department of Geodesy and Geophysics, University of Cambridge. The salient features of the age-pattern emerging from this work are revealed by the preliminary results listed in Table 1. Argon volumes were estimated using the isotope dilution technique and the potassium oxide content of the samples measured by flame photometry. The geological interpretation is largely due to Fitch, while the geochronological work has been done by Miller and Meneisy. Full detailed results on a more comprehensive range of North Welsh rocks will be published later.

The proved or inferred geological ages of the rocks forming this preliminary sample range from the pre-Cambrian to the Tertiary. The pre-Cambrian (Monian) age relationships of the Coedana and Sarn granites, and of the Gabbro–Serpentine Complex of Rhoscolyn, have been discussed by Greenly[1], Matley[2], and Shackleton[3,4]. The Twt Hill granite is referred to as of younger pre-Cambrian (Arvonian) age by Greenly[5]; the Uriconian volcanics, although definitely pre-Cambrian, are not thought to correspond to the Arvonian or Monian because of their inferred relationship to the Longmyndian[6,7]. Stanner rocks, once thought to be intrusive into the Ludlovian, are now regarded by Holgate and Hallowes[8] as a slice of Uriconian basement brought up in the Church Stretton fault zone. The Ordovician volcanic rocks Carreg y Defaid have been dated on palæontological evidence as Caradocian by Matley[9]. The extensive basic sill and dyke complex of North Wales has been variously interpreted; but it is now accepted that the majority of these minor intrusions were emplaced in a late phase of the main end-Silurian Caledonian movements, as is maintained by Shackleton[7,10]. Both the Llanbedrog and Penmaenmawr plutons are examples of an extensive suite of small stocks, plutons and domes found in North Wales. They have been regarded as Ordovician in the past[11], but the majority are now known to be late-Caledonian[10,12]. The rare alkaline basic dykes of Anglesey and the mainland are thought to be Tertiary by Greenly[1,13].

Potassium–argon results obtained from this varied suite of igneous rocks can be discussed under the five age-groups into which they fall.

Early Tertiary Ages. The analcite-bearing olivine–dolerite dykes (Crinanites) at Porth Dafarch on Anglesey and near Ogwen Cottage in Snowdonia gave total rock potassium–argon ages of 73 ± 17 and 86 ± 13 million years respectively. These rocks have long been regarded as Tertiary on petrographic grounds, and this is now confirmed: the actual period of intrusion being most probably the Palæocene. An anomalous age obtained from the Rhoscolyn Gabbro–Serpentine Complex, intruded into the New Harbour Beds on Holy Island, Anglesey, which is certainly pre-Cambrian, can only be explained by postulating the proximity of the mass to Tertiary magmatism. Further field investigation is required before this anomaly can be satisfactorily understood.

Variscan Ages. Moorbath[14] has shown that Variscan mineralization is widespread in North Wales. This mineralization is associated with a fault complex which can be readily appreciated where Carboniferous rocks are present but may be confused with Caledonian faulting and mineralization in the older core of North Wales. Basic sills of tholeiitic dolerite intruded in the later phases of the Caledonian orogeny are commonly found in the folded Ordovician rocks of North Wales. These sills are related to a north-west–south-west trending dyke swarm. Two of these basic rocks, the Holyhead Dyke on Anglesey, and the Llandegai sill near Bangor, gave true Caledonian ages. Four other 'Caledonian' basic sills sampled, those at Nant, and near Llanwrst, on Tal-y-fan, near Ro Wen, at Ty-Ruttan, near Sarn, and at the Pant Quarry, Llanwrst, gave the apparently Variscan ages of 288 ± 19, 278 ± 23, 264 ± 17 and 245 ± 18 million years respectively. The view that the ages obtained are due to partial or complete argon loss consequent on the effects of nearby Variscan mineralization and movement is strengthened by the closely comparable results obtained from the demonstrably Caradocian acid volcanic rocks at Carreg y Defaid near Pwllheli. From this locality a rhyolitic vent rock and a welded eutaxitic ignimbrite gave ages of 272 ± 13 and 279 ± 13 million years respectively. All these rocks in which excessive loss of argon is suspected show some signs of hydrothermal alteration (kaolinization, silicification, chloritization, etc.) in thin section. The basic sills from the Conway Valley are geographically close to known Variscan ore bodies (for example, Trecastell Mine and Parc Mine). The Pant Quarry rock, which gives a very low result, is extremely brecciated and subject to deep

Table 1. Results of Potassium–argon Measurements

$\lambda_e = 0{\cdot}584 \times 10^{-10}.yr^{-1}$ $\lambda_\beta = 4{\cdot}72 \times 10^{-10}.yr^{-1}$

Sample	Ref.	K_2O (per cent)	Atmospheric contamination (per cent)	Vol. radiogenic A^{40} (mm^3 N.T.P.) / Weight of sample (gm)	Apparent age and error (m. yr.)
Early Tertiary Basic Dyke, Porth Dafarch, Anglesey. *M.R.* 234 801	322*c* 3/*DM*	0·64	73·5	0·00157	73 ± 17
Pre-Cambrian (Monian) Gabbro from Gabbro–Serpentine Complex Rhoscolyn, Anglesey, between *M.R.* 264 772 and 269 772	320 1/*MM*	0·28	32·4	0·000737	78 ± 17
Early Tertiary Basic Dyke, near Ogwen Cottage, Snowdonia. *M.R.* 644 605	339 1/*SM*	0·54	11·7	0·00157	86 ± 13
Caledonian Basic Sill, Pant Quarry, Llanwrst *M.R.* 783 622	334 2/*IM*	0·79	4·9	0·00682	245 ± 18
Caledonian Basic Sill, Ty-Ruttan, Sarn, Lleyn *M.R.* 231 321	186 *T*/12	0·68	24·4	0·00638	264 ± 17
Ordovician (Caradocian), Vent Rhyolite, Carreg y defaid, Lleyn *M.R.* 342 326. (Whole rock sample)	190 *V*/19	5·50	1·5	0·05322	272 ± 13
Caledonian Basic Sill, Tal-y-fan, Ro Wen *M.R.* 737 723	335 2/*AM*	0·51	25·9	0·00505	278 ± 23
Ordovician (Caradocian) welded eutaxitic ignimbrite Carreg y Defaid Lleyn *M.R.* 342 326 (whole rock sample)	189 *U*/9	6·35	2·7	0·06320	279 ± 13
Caledonian Basic Sill, Nant Llanwrst *M.R.* 785 602	333 1/*UM*	0·60	5·7	0·00617	288 ± 19
Caledonian (Post-tectonic) Granophyre, Llanbedrog, Lleyn *M.R.* 330 303 (whole rock sample)	353 2/*YM*	5·25	24·9	0·05890	312 ± 14
Pre-Cambrian (Synorogenic, Monian) Adamellite, Sarn Lleyn. *M.R.* 229 347 (whole rock sample)	184 *V*/20	3·99	6·1	0·04549	317 ± 15
Pre-Cambrian (Arvonian) Granite, Twt Hill, Caernarvon. *M.R.* 482 630 (whole rock sample)	181 *U*/12*M*	4·65	4·3	0·06008	355 ± 16
Caledonian (Post-tectonic) Microdiorite, Penmaenmawr. *M.R.* 703 762	336 2/*LM*	2·59	0·2	0·03554	375 ± 17
K-feldspar from the Pre-Cambrian (Post-tectonic, Monian) Coedana granite, Gwalchmai, Anglesey. *M.R.* 382 727	325 1/*DM* 327 3/*CM*	3·58 5·28	42·4 1·1	0·05270 0·07286	400 ± 11 377 ± 17
Caledonian Basic Sill, Llandegai, Nr. Bangor. *M.R.* 590 696	337 2/*PM*	0·39	12·1	0·00576	401 ± 29
Caledonian Basic Dyke, Holyhead Dyke, Anglesey. *M.R.* 238 808	322 1/*HM* 322 5/*Q*	0·55 0·55	3·0 0·4	0·00827 0·00845	407 ± 22 415 ± 33
Pre-Cambrian (Eastern Uriconian) Basic/Acid igneous complex, Stanner Rocks, Old Radnor. *M.R.* 262 583 (whole rock sample)	343 1/*IM*	1·76	0·1	0·02725	418 ± 23
Pre-Cambrian (Eastern Uriconian) Basalt, Leaton Quarry, Wrockwardine, Salop *M.R.* 616 113	348 1/*KM*	0·12	64·2	0·00301	638 ± 81

recent weathering. No proved Variscan mineralization is known in the Lleyn; but it would appear that much of the mineralization previously regarded as Caledonian (for example, south of Abersoch at Llanfaelrhys) must in fact be Variscan.

Intermediate Caledonian/Variscan Ages. Three of the granite masses of Western Caernarvonshire, the Llanbedrog granophyre, the Sarn Adamellite and the Twt Hill granite gave the intermediate ages of 312 ± 14, 317 ± 15 and 355 ± 16 million years respectively, from samples in which potash feldspar was the main potassium-bearing mineral. The Llanbedrog granophyre is a post-tectonic Caledonian pluton and the age obtained must reflect partial argon loss in addition to that normally lost from feldspars, due to Variscan movements and mineralization. The Sarn Ademellite and the Twt Hill granite are both pre-Cambrian in age, the Twt Hill mass being the younger of the two. Both show brecciation, and shearing of Caledonian age would be expected to give Caledonian ages on analysis by the potassium–argon method. The low value obtained from Sarn is comparable to that obtained from Llanbedrog and may be attributed to mild hydrothermal alteration of Variscan age. The smaller loss of argon suffered by the Twt Hill rock is probably related to the proximity of the Variscan Dinorwic fault rather than to the passage of hydrothermal solutions.

Caledonian Ages. All but one of the remaining rocks listed in Table 1 gave Caledonian ages only slightly modified by later events. The Penmaenmawr post-tectonic Caledonian microdiorite stock gave an age of 375 ± 17 million years from a sample in which the feldspars were not quite fresh. Two potassium-feldspars from the pre-Cambrian Coedana granite of Anglesey, which was strongly sheared and chloritized by Caledonian movements gave ages of 377 ± 17 and 401 ± 11 million years. These must be regarded as minimum ages, as slow loss of argon from feldspar occurs over geological time. Two measurements on the Holyhead Dyke and one on the Llandegai sill gave Caledonian ages of 407 ± 22, 415 ± 33 and 401 ± 29 million years respectively, and the strongly sheared Uriconian basic acid igneous complex of Stanner Rocks in the Church Stretton Fault zone gave a minimum age of 418 ± 23 million years from a whole rock sample.

Pre-Cambrian Ages. Only one rock of this group produced a pre-Cambrian age on analysis. This is the remarkably fresh Uriconian basalt from Leaton Quarry, near Wrockwardine in Shropshire, which gave an age of 638 ± 81 million years. This rock forms part of the Caledonian foreland to the south-east of the Church Stretton tectonic line and appears to have largely escaped the full force of the Caledonian deformation. The rocks in this quarry have been folded and faulted at some time, however, and the age obtained cannot be used to fix the date of the Uriconian vulcanism more closely within the pre-Cambrian.

Certain conclusions can already be drawn from this work. Potassium–argon ages obtained from rocks are shown once again to be strongly affected by later geological events which can cause partial or complete argon loss. Orogenesis or metamorphism causes complete loss of all accumulated argon, while faulting and mineralization cause argon losses of varying amounts depending on their intensity. Thus the ages obtained from North Wales are necessarily a record of later events rather than of original ages. Apart from the one rock sampled from outside the Caledonian mobile belt, no age earlier than that of the Caledonian deformation has been obtained. In North Wales the pre-Cambrian, Cambrian, Ordovician and Silurian rocks have all been subjected to strong Caledonian deformation, and this event is therefore the

oldest for which an age can be expected by this method. Measurements in other areas have suggested that the main-Caledonian movements occurred some 420 million years ago. The age results from North Wales indicate that the principal Caledonian movements and magmatism took place some 400–420 million years ago. This would be compatible with the geological evidence which suggests that the folding began during the Salopian (producing submarine slumping and facies changes) and reached its greatest intensity towards the end of the Silurian. Uplift was probably earlier and more intense in the north-west (Anglesey) than in the south-east (Shropshire). Folding movements continued in the middle Devonian and these may be represented by the Penmaenmawr age of 375 ± 17 million years as late folds can be recognized near this intrusion.

But for later Variscan and Tertiary events the older rocks of North Wales should show uniformly Caledonian ages. In the vicinity of Variscan faulting and hydrothermal mineralization and close to Tertiary intrusions partial or complete argon loss has resulted in further apparent age reductions. Ages obtained in the range 265–280 million years are clearly related to the main end-Carboniferous (Saalic) Variscan movements, post-tectonic granitic activity and mineralization, which in North Wales mainly took the form of major north-east–south-west faulting, the faults then acting as channels for localized hydrothermal mineralization. The areal pattern of potassium–argon ages will enable these faults and zones of mineralization to be closely delimited. The effects of the early Tertiary magmatism were very local in North Wales, but represent the third event recorded by the age determinations.

Intermediate ages between these three dated events represent partial losses of argon and are not significant.

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IN VITRO CYTOTOXIC EFFECT OF ISOANTIBODY MEASURED AS ISOTOPE RELEASE FROM LABELLED TARGET CELL DNA

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THE mechanisms of tissue damage in the homograft reaction are poorly understood. Although the destruction of the grafted tissue is most likely the end result of a large number of different types of reactions, it is commonly assumed that the interaction of antigen carrying (or producing) graft cells with either host antibodies or sensitized host lymphoid cells plays a predominant part in this connexion.

The mechanism of cytotoxic damage of target cells by antibodies can be studied *in vitro* in various ways. Antibodies are known to attach to the cell surface which changes its ultrastructure and probably becomes more fragile[1]. However, cell lysis is usually not observed unless complement is also present. The predominant expression of cell damage is an irreversible change in permeability of the surface of the affected cells[2]. This change results in a rapid equilibration of low-molecular substances between cell interior and outer medium. Therefore, the cytotoxic effects of antibodies can be easily studied by measuring the penetration of a dye into the damaged cells or by following the release of low-molecular substances from the cells[3]. Moreover, provided the concentration of stabilizing high-molecular substances in the medium is insufficient to counteract the imbalance of the osmotic equilibrium, the cells will also release a great deal of their cytoplasmic ribonucleic acid and protein[2]. If these components are labelled with an isotopic marker, their leakage into the medium can be easily followed[4].

The isotope method provides a convenient approach for investigations on the mechanism of immune cytotoxicity. However, under certain experimental conditions, it is difficult to avoid a cytoplasmic label leaking out to a certain extent from the control cells as well[5]. For quantitative studies of target cell damage due to immunological reactions, it would be desirable to reduce the non-specific background to a low level; this may result in some loss of sensitivity but can be useful as a complement to already existing techniques. The work recorded here represents an attempt to determine if this purpose could be achieved by labelling the DNA of the target cells with ^{14}C-thymidine. It has been known for some time that DNA is not released from the cells under the conditions described above, where the cells lose a great deal of their cytoplasmic RNA and protein[3,6,7]. However, it was expected that a release of DNA from immunologically affected target cells could be induced experimentally.

In a first series of experiments, isotope release from fresh and heat-killed ascites tumour cells was measured under various conditions. Cells of the ascites lymphoma *6C3HED* were labelled *in vivo* by injecting 0·2 μc. of thymidine-2-^{14}C (Amersham, England; spec. activity 0·1 mc./0·83 mg) intraperitoneally into *C3H* mice inoculated with the tumour 6 days earlier. The cells were harvested 48 h later. The ascitic fluid was divided into

Table 1. RELEASE OF RADIOACTIVITY FROM THE CELLS OF THE *6C3HED* LYMPHOMA LABELLED WITH ^{14}C-THYMIDINE AFTER VARIOUS PRETREATMENTS

Conditions of cells	Incubated with	Total radioactivity (c.p.m.) per sample	% release to supernatant
Fresh	—	5,082	3·2
,,	deoxyribonuclease	5,433	2·2
,,	trypsin	4,862	9·7
,,	deoxyribonuclease + trypsin	5,192	12·0
,,	sodium deoxycholate	2,616	45·8
Stored overnight in refrigerator	—	4,175	3·2
,,	deoxyribonuclease	5,324	2·3
,,	trypsin	1,013	11·9
,,	deoxyribonuclease + trypsin	8,412	2·0
Heat-killed	—	3,796	4·1
,,	deoxyribonuclease	5,680	4·6
,,	trypsin	2,177	63·0
,,	deoxyribonuclease + trypsin	2,125	69·7

Table 2. Isotope Release and Viability of Lymphoid Cells after Exposure to Isoantiserum and Complement*

Material	Exposed to	% eosin stained cells prior to addn. of complement	% eosin stained cells after incub. with complement	Cytotoxic index§	Total radioactivity in balanced salt solution treated sample	% release in supernatant	Total activity in trypsinized sample	% release in supernatant	Cytotoxic index calc. from isotope release ¶
Normal lymphoid cells (lymph nodes + spleen) of *A* mice†	Normal ACA-serum	22·1	21·2		103	22·7	82	21·4	
,,	ACA anti *A*-serum ‡	24·9	73·0	0·66	131	17·5	76	58·4	0·47
Lymphoma *L*1210-*AMR* of *DBA*/2 origin†	Normal ACA-serum	6·0	10·8		3,102	1·4	3,280	7·7	
,,	ACA anti-*A* immune serum ‡	4·8	91·0	0·90	3,087	1·6	2,395	88·5	0·88
Gross virus induced lymphoma in *C3H* × *DBA*/2 F_1 hybrid†	Normal ACA-serum	1·6	4·6		2,684	1·5	2,505	7·7	
,,	ACA anti-*A* immune serum ‡	1·8	84·8	0·84	2,824	1·7	3,454	84·4	0·84

* 2 ml. of the cell suspensions (2–8 × 10^6 cells/ml.) were incubated with 2 ml. isoantiserum at 37° C for 20 min. (Final volume of reaction mixture: 4 ml.). Afterwards, they were centrifuged and the supernatant was replaced with 2 ml. of fresh guinea pig serum. After incubation at 37° C for 45 min the test was terminated and the cells were processed further.

† The cells were labelled with 0·2 μc. thymidine-2-^{14}C, injected intraperitoneally, 48 h before collecting.

‡ The serum donors were immunized by three consecutive inoculations of pooled liver, kidney and spleen suspensions, given at 5–7-day intervals. Serum was collected 5 days after the last inoculation.

§ $\frac{\%\text{ eosin-unstained cells in control} - \%\text{ unstained cells in antiserum-treated sample}}{\%\text{ unstained cells in control.}}$

¶ $\frac{\%\text{ isotope in control trypsinized cell sediment} - \%\text{ isotope in antiserum-treated trypsinized cell sediment}}{\%\text{ isotope in control trypsinized cell sediment}}$

three main portions. One contained the fresh, untreated cells, the second cells killed by heat (30 min incubation at 56° C) and the third cells stored at +4° C overnight. Subsequently, each sample was divided into four parts each containing approximately 10^3 cells, although the distribution of cells between samples was unequal owing to cell clumping. One part was treated with trypsin (0·25 per cent trypsin, dissolved in balanced salt solution) for 30 min at room temperature under gentle stirring, one with deoxyribonuclease under the same conditions (0·01 per cent, Sigma Chemical Co., St. Louis, Mo.), one with a mixture of trypsin and deoxyribonuclease, while one was left untreated in the refrigerator. In addition, the fresh cells were exposed to sodium deoxycholate in one sample (1 per cent in balanced salt solution). Afterwards, all samples were centrifuged (2,000 r.p.m., 10 min) and the supernatants were separated from the cells. Radioactivity was measured, at infinite thinness, with a thin-windowed Geiger–Müller tube in an automatic gas flow counter, after drying and plating of aliquots as described previously[5]. The values in the tables are expressed as the total radioactivity (c.p.m.) present in cells + medium. The relative release represents the percentage of radioactivity in the medium as related to the total activity in cells plus medium.

It appears from the typical and regularly reproducible experiment illustrated in Table 1 that fresh cells showed little tendency to release isotope into the supernatant, even when treated with deoxyribonuclease, trypsin, or both. The cells stored overnight in the refrigerator behaved like the fresh cells. This was in agreement with the results of supravital staining tests with eosin[8] which did not indicate more cell damage than approximately 7 per cent compared to the fresh controls (which contained 8–12 per cent eosin-stained cells themselves). However, when the cells were lysed with the surface-active sodium deoxycholate, almost half of their isotope content was released. Heat-killed cells did not release substantial amounts of isotope unless treated with trypsin, which induced them to release about two-thirds of their total isotope content in this experiment. In some other experiments, up to 90 per cent of the nuclear isotope was liberated. Deoxyribonuclease had no such effect in any of the cases studied.

The results of these experiments indicated that trypsin treatment may be useful in inducing isotope release from dead or damaged cells which would otherwise retain their DNA. In the following series of experiments an attempt was therefore made to apply this method for the demonstration of the cytotoxic effect of anti-H-2 isoantisera. Labelled target cells, known to be sensitive to antiserum, were exposed to it, in the presence of fresh guinea pig serum as the source of complement. Afterwards, they were treated with trypsin and isotope release was measured. Control cells were treated with normal mouse serum and complement. Isotope release was also measured in an aliquot of each sample after incubation of the target cells with balanced salt solution without trypsin. As a further control, the number of cells killed by isoantiserum and complement was also estimated by the supravital eosin-staining method of Schrek[8].

Three representative experiments, performed with a suspension of normal lymphoid cells and with two different lymphomas, are shown in Table 2. As will be seen, when the antiserum-treated cells were exposed to trypsin, the cytotoxic effect of the anti-H-2 isoantibodies could be easily detected. Moreover, the cytotoxic indices obtained very closely paralleled those calculated from the results of the eosin tests.

Preliminary experiments with another tumour (the *MC1M* ascites sarcoma), under the present conditions resistant to the cytotoxic action of anti-*H*-2 antibody as judged from the eosin test[9], have so far given negative results. This was also the case when tumour cells were similarly incubated with an excess of lymphoid cells derived from the lymph nodes and spleens of appropriately sensitized mice. Such cells are known to neutralize target tumour cells so that they will not grow when implanted afterwards into neutral hosts[10]. They also seem to exhibit visible cytopathogenic effects when added to tissue cultures of the target cells in long-term (24–48 h) experiments[11]. As reported previously[12] in experiments similar to those described here but in which a cytoplasmic-labelling technique was used, a specific release of isotope from the target cells into the medium was noted after 1–2 h of incubation with lymph node cells from sensitized animals. Further experiments will have to be carried out to decide whether the present failure was due to technical inadequacies or to real differences in the cytotoxic mechanisms as revealed by the different techniques.

In conclusion, it may be said that these experiments demonstrate that trypsin treatment of cells damaged by heat or by specific antibodies leads to the release of considerable proportions of nuclear DNA. Unless a more complete dissolution of cellular membranes is brought about by sodium deoxycholate, DNA is not released without trypsin treatment. This confirms the findings of previous authors[3,6]. Antibodies plus complement

are known to produce 'functional' holes in the outer cell membrane so that large molecules and even ribosomes as large as 150 Å in diameter may escape from the interior[7]. At present we do not know whether trypsin acts by causing a more complete destruction of the outer cell membrane or by attacking the nucleus in a more direct fashion. Whatever the mechanism, the present method seems easily applicable to measuring cytotoxicity in a highly specific manner and in a variety of immunological systems.

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RADIOSENSITIVE MOLECULAR WEIGHT OF SINGLE-STRANDED VIRUS NUCLEIC ACIDS

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THE action of ionizing radiation on the macromolecules of importance in biology has been studied intensively for many years. Work with enzymes, proteins in general and nucleic acids has shown that ionization within the molecule leads to a drastic change in the biological behaviour. The idea that the statistical distribution of ionization can be used for determining the dimensions of macromolecules has been developed by Lea[1] and Pollard, Guild, Hutchinson and Setlow[2].

The development of this interesting and simple idea was followed, however, by clear indications of ways by which radiation action can be modified, notably temperature[3,4] and added protective substances[5–8]. Simple 'target' analysis thus has become a subject of some question. This fact need not, of itself, be uninformative, but it is disquieting, and forces an element of sophistication into radiation analysis which is not desirable.

Among the macromolecules which have recently received considerable study are single-stranded nucleic acids from viruses and the viruses themselves[9–14]. Such single-strand nucleic acids must be intact to function biologically, and, moreover, they possess no secondary structure that is functionally significant[10,11]. Thus an ionization, which in a covalently bonded structure must be considered as shared by the whole molecule, will cause at least one highly reactive and hence alterable bond in the structure. It is important to consider the reaction path of such an ionized molecule, since a given path can lead to a permanent alteration and hence inactivation or in certain instances, not desirable for target analysis, restitution of the molecule to its original functional form. The necessity for complete physical and chemical integrity for a particular biological function makes single-strand nucleic acids clear examples of the applicability of target analysis and a study of several of these is clearly of interest.

We have previously studied tobacco mosaic virus (TMV) and TMV-RNA[6]. To this are now added $\varphi X174$ and $\varphi X174$-DNA, which have been characterized by Sinsheimer[13] and $R17$ and $R17$-RNA. The latter virus is a small spherical bacteriophage recently isolated and characterized by Paranchych and Graham[15] and is closely related to $f2$ bacteriophage of Loeb and Zinder[16]. The investigation was divided into three parts: (*a*) an examination of the validity of target analysis for the nucleic acid alone; (*b*) an examination of whether the capsulation of the nucleic acid by protein has any effect on the analysis; (*c*) an examination of the nature and extent of protection against or potentiation of radiation action by agents in the medium in which viruses are irradiated.

For enzymes and simple viruses, the inactivation process generally obeys the expression $N/N_0 = e^{-kD}$, where N/N_0 is the fraction surviving a 'hit' after a given dose, D, measured in roentgen, r, and k is the apparent target mass. A 'hit', as the expression implies, is a quantized kill event, but just what this event precisely entails in terms of amount of energy absorbed per target molecule and how these discrete ionization events are spaced in the experimental samples has remained uncertain. By the latest approximation a gram of tissue or virus exposed to 1 r. absorbs 87 ergs (ref. 17); this energy is absorbed not homogeneously but primarily as discrete ionization events called primary ionizations or ion clusters, each of which, as experimentally determined recently in solids by Rauth and Hutchinson[18], have an average energy of about 60 eV. If we accept these values, then 1 r. will deposit $9{\cdot}0 \times 10^{11}$ ionization events per gram of target material. Inasmuch as the foregoing expression also implies that the 'hit' events are statistical in nature, so it is necessary to assume that the spacing of the ionization events must be randomly distributed. This seems not strictly true as ionization in fact occurs along tracks. But the tracks themselves occur randomly, and if the average distance between ionization clusters along a given track is large compared with the target dimensions, then effectively the clusters will be random in space and each cluster will constitute an independent kill event. It is reasonable to assume that the action probability for simple biological systems studied here is unity or close to unity inasmuch as the release of energy in the ionization cluster involves 60 eV. A model of Samuel and Magee[19] indicates that each cluster is confined on the average within a volume of 7 Å radius. The contribution of excitation energy to lethality is considered small relative to the ionization energy[2].

The irradiation sources used here are a 50 kV Picker X-ray machine equipped with a beryllium window and a cobalt-60 source for the 1 MeV gamma ray. Both sources were calibrated by Fricke chemical dosimetry[20]. All samples were irradiated over an atmosphere of air. For X-irradiation 0·05-ml. samples of virus or nucleic acid are placed on polyethylene disks 2 mm thick, put in an aluminium chamber, the top of which is covered with a thin mylar film and either dried or frozen prior to irradiation. The X-ray machine was operated at 50 kV with a dose rate usually of 23 kr./min. Control samples were placed away from the beam and covered with a lead shield 3 mm thick. For γ-irradiation, 0·3-ml. samples were placed in small glass test tubes, frozen and set in a regular cylindrical array around the cobalt source and kept frozen in crushed solid carbon dioxide during the irradiation. The dose rate was 6·7–7·4 kr./min. TMV was assayed as previously described[6]. $R17$ and $\varphi X174$ were

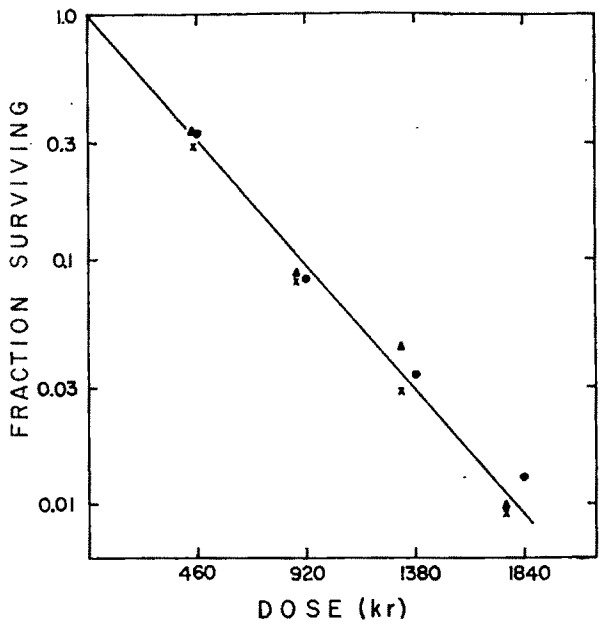


Fig. 1. X-ray inactivation curve of φX174 suspended in 1 per cent tryptone broth (Difco). Bacterial lysate in broth containing 1×10^{10} plaque-forming units/ml. of the virus was serially diluted in tryptone broth to 1×10^{8} pfu/ml. 0·05-ml. samples of the final dilution were either frozen or dried on plastic disks for the irradiation ×, Irradiated dry at 25°–30° C (average of 7 experiments); ▲, irradiated frozen at −60° C (average of 3 experiments); ●, irradiated frozen at −180° (average of 3 experiments). A 50 kV Picker beryllium window X-ray machine was operated at maximum voltage with a current of 7m.amp, dose rate at 23–24 kr/min

assayed on *Hfr* strain of *Escherichia coli* and *E. coli C* respectively, and their nucleic acids on the protoplasts of their respective hosts. The method of Gutherie and Sinsheimer[21] was followed for the assay using protoplasts, except that 30 per cent sucrose was substituted for bovine serum albumin.

Fig. 1 shows the summary of the X-ray inactivation curves of φX174 in tryptone broth from several experiments conducted over a temperature range of −180°–+30°. It can be seen that the experimental points fit a one 'hit' survival curve over at least two logarithmic decades and its slope indicates a D_{37} of 386 kr. Assuming the figures of 60 eV per ionization event and 87 ergs/g/r., we get $3{\cdot}47 \times 10^{17}$ ionization events per gram of the radiosensitive portion of the virus to effect reduction of the viral population to e^{-1}. This leads to a target molecular weight of $1{\cdot}74 \times 10^{6}$, which coincides with the physical molecular weight found for the virus DNA by Sinsheimer[13]. A range in values between 1·66 and $2{\cdot}02 \times 10^{6}$ is obtained in 13 separate experiments. It is to be noted that the target sensitivity remains invariant over the experimental temperature range. Pollard and Dimond observed earlier that over a temperature range of 20°–73°, TMV sensitivity to deuteron bombardment remains constant[23]. This is in contrast to the case of bacteriophage *T*1 and of enzyme inactivation using densely ionizing radiation[3,4,22]. This indicates a difference between these single-strand materials and other double-strand DNA or folded protein.

The dose response curves of φX174 and of its free DNA irradiated by cobalt-60 in tryptone broth at −60° are plotted in Fig. 2. A close fit of points for both the intact virus and its free DNA to a single slope is obtained. Moreover, the slope shows a D_{37} of $3{\cdot}68 \times 10^{5}$ r. for a molecular weight of $1{\cdot}82 \times 10^{6}$, which agrees closely with the result shown in Fig. 1 for X-irradiation, indicating good agreement between the two irradiation sources with regard to the dose calibration and effectiveness per r. absorbed. The result also confirms our earlier conclusion drawn from the TMV system that the inactivation of the virus by X-rays is a consequence of a single ionizing event occurring anywhere on the nucleic acid moiety of the virus[6].

Fig. 3 summarizes the results of irradiating *R*17 and *R*17-RNA, both frozen in tryptone broth at −60° by γ-rays at a dose rate of 7·4 kr./min. The D_{37} for this system is 836 kr., giving a radiosensitive molecular weight of $0{\cdot}8 \pm 0{\cdot}2 \times 10^{6}$, in good agreement with a value of $1{\cdot}0 \times 10^{6}$ we obtained by X-irradiation and with the physical molecular weight of $0{\cdot}92 \pm 0{\cdot}2 \times 10^{6}$ reported by Mitra *et al.* for the viral RNA[24]. The physical molecular weight of the intact virus calculated from sedimentation and diffusion data is $4{\cdot}9 \times 10^{6}$ (ref. 24).

We have previously noted that when TMV-RNA or catalase is irradiated while suspended in 2 per cent reduced glutathione (GSH), their sensitivity to X-ray inactivation is decreased by a factor of 2 (refs. 6 and 8). Fig. 4 shows the same effect with the φX174 and its free DNA is protected to the same extent, as is also true for the *R*17 system, indicating that the reaction is quite general, even to the extent of protection. This reaction is most likely a transfer of electrons from the reduced glutathione molecules to the ionized target, working in competition with the surrounding oxygen molecules. Some of the hit targets are thus restored to their original state. In the absence of any protective agent, oxygen reacts with the metastable target (for example, carbonium ion) with

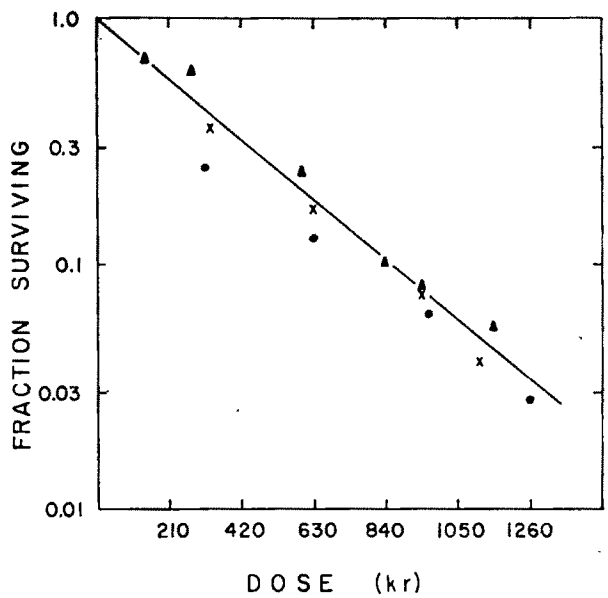


Fig. 2. Cobalt-60 inactivation curve of φX174 and φX174-DNA suspended in 1 per cent tryptone broth (Difco). 0·3-ml. samples were irradiated frozen at −60° C in glass test tubes ×, φX174; ▲, φX174 DNA, or at 30°–40° C., dried (●, φX174). Dose rate, 7·0–7·4 kr/min

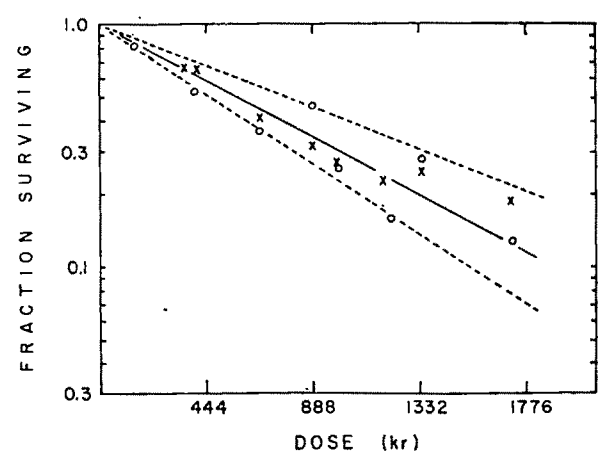


Fig. 3. Cobalt-60 inactivation of *R*17 (×) and *R*17 RNA (○) suspended and frozen at −60° C in 1 per cent tryptone broth (Difco). Upper dashed line is a slope calculated for a radiosensitive molecular weight of $0{\cdot}6 \times 10^{6}$ and the lower dashed line for $1{\cdot}0 \times 10^{6}$. Slope is exponential for at least 3 logarithmic decades in the case of X-ray inactivation

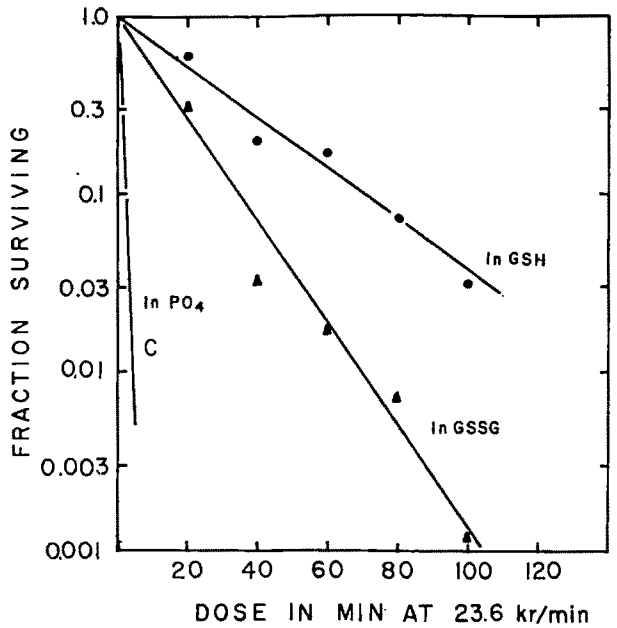


Fig. 4. Effect of 1 per cent reduced glutathione (GSH), 1 per cent oxidized glutathione (GSSG) and 0·1 M sodium phosphate buffer, *p*H 7·2 (curve *C*) on the sensitivity of φ*X*174 to X-irradiation. GSH and GSSG solutions were prepared in water and the *p*H adjusted to 6·8 with sodium hydroxide. The virus preparations in this case were irradiated dry at 25°–30° C

high efficiency forming an organic peroxide which is thought to be the principal lethal reaction[25]. In the target experiments recorded here, concentration of oxygen is not believed to be limiting because the samples are continually exposed to the atmosphere during preparation and irradiation. Moreover, the lifetime of the ionized targets while in a frozen or dried state is sufficiently long[26] to permit them to react with oxygen in the time intervening between the end of irradiation and the preparation of the samples for bioassay for the surviving population. Thus, the question of the diffusion rate of oxygen during irradiation is minimized at least under the conditions of our experiments. But, as we have shown earlier[6], glutathione must be present during irradiation to show the protective effect.

The protection is the same for the free nucleic acid as for the intact virus, indicating either that donor electrons can readily migrate through the protein shell of the virus or that the shell is not a barrier to the GSH molecules. On the other hand, the target sensitivity for either free DNA or intact virus suspended and irradiated in oxidized glutathione (GSSG) remain unchanged (Fig. 4), as might be expected, as when they are suspended in tryptone broth. This is found to be the case for TMV also.

A more serious kind of modification of target sensitivity is observed when either φ*X*174, *R*17 or their free nucleic acids are suspended in 0·1 *M* phosphate buffer, *p*H 7·2, as illustrated by Fig. 4, curve *c*, for φ*X*174. Here, as Brustad[22] has also noted, we see a striking potentiation of radiation effect, where the apparent target size is now ten-fold greater than the actual physical molecular weight. A similar effect is shown by biological materials irradiated in sodium acetate[27] and sodium chloride[6].

We have no explanation for the unusual sensitization seen here except to note that the effect: (1) is linearly dependent on the log of the concentration of phosphate buffer over the measured range of 10^{-3} to 10^{-1} molar; (2) persists after irradiation; (3) is depressed in the presence of added tryptone broth, suggesting that some reactive product is formed when this buffer is irradiated. TMV and TMV-RNA are much less sensitized than φ*X*174 and *R*17 by this buffer. This may be explained by the fact that, because of their lower plating efficiency, they must be irradiated at a concentration (> 1 mg/ml. versus 10^{-6} mg/ml. for φ*X*174 and *R*17) so high that self-protection against reactive phosphate products can be observed. This phenomenon was shown by Lea in experiments where TMV was irradiated in solution at various concentrations[1].

In Fig. 5 is shown the result of one single experiment wherein one series of TMV samples was irradiated at 2 mg/ml. with γ-rays in the presence of 0·1 M phosphate buffer, *p*H 7·2, and another in 1 per cent tryptone broth. It is obvious that there is no phosphate effect of the magnitude seen in the φ*X*174 system. It shows also that the radiation sensitivity is not modified by tryptone broth; points fit reasonably well with the curve obtained from a more extended work reported earlier[6] for both this virus and its free infectious nucleic acid. It is to be noted that by assuming the new and independently derived figures of 60 eV/pi and 87 ergs/g/r., a radiosensitive molecular weight of $2{\cdot}2 \times 10^6$ is obtained from this slope which indicates a D_{37} of $3{\cdot}0 \times 10^5$ r. The reported physical molecular weight is between $1{\cdot}9 \times 10^6$ (ref. 9) and $2{\cdot}2 \times 10^6$ (ref. 28). The target size is also consistent with the dimensions derived from the earlier radiation analyses of Lea *et al.*[1], Pollard and Dimond[23] and Buzzel, *et al.*[29].

While the phosphate and glutathione effects clearly point to the hazards involved in attempting to estimate target molecular weight of virus and enzymes in their impure state or in the cell, it must be emphasized that these demonstrations of protection and potentiation are made possible by exposing the target materials to high concentration (10^8–10^{15} molar excess over virus) of pre-

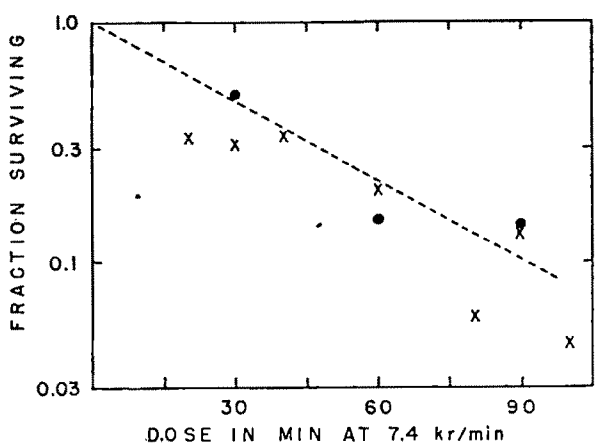


Fig. 5. Inactivation by cobalt-60 γ-ray of TMV frozen at −60° C in 0·1 M sodium phosphate buffer, *p*H 7·2 (×) and in 1 per cent tryptone broth (Difco) (●) at 2 mg virus/ml. The dashed slope (see text) shown was obtained from the earlier results of X-ray inactivation of TMV and TMV–RNA suspended and frozen in distilled water (ref. 6)

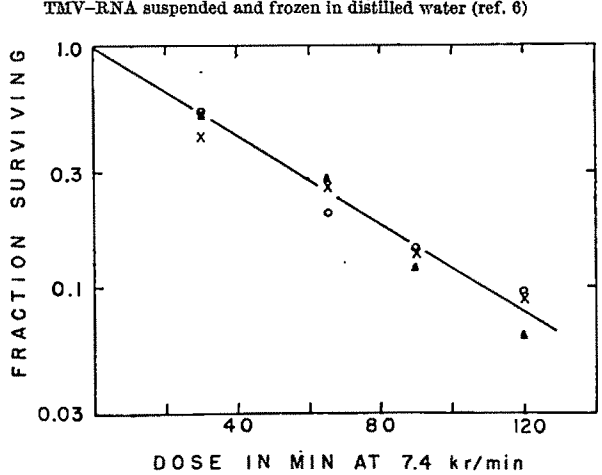


Fig. 6. Media effects on the sensitivity of φ*X*174 to cobalt-60 γ-irradiation. ○, Irradiated frozen at −60° C in 4 per cent nutrient broth (Difco); ▲, in 4 per cent tryptone broth; ×, in 30 per cent *E. coli* lysate suspended in water

selected chemical environment. Of course, the presence of radiation sensitizing micro-environment around a target inside a cell can never be ruled out; for example, Braams *et al.*[27] have shown a two-fold protection of invertase when it is irradiated in yeast cells, which they ascribed to the presence of substances rich in sulphydril groups. But Pollard *et al.*[2] demonstrated that succinic dehydrogenase and cytochrome oxidase have the same target sensitivity in or out of the *B. subtilis* cells. In Fig. 6 are plotted the present results of irradiating, at $-60°$, 1×10^9 plaque-forming units of $\varphi X174$ in bacterial lysate, which prior to irradiation is re-suspended in 30 per cent *E. coli* lysate, 4 per cent tryptone broth and 4 per cent nutrient broth. Following irradiation, the different sample series were diluted a thousand-fold into tryptone broth for assay of survivors. It is seen that the sensitivity of the virus in all media indicate closely similar target molecular weight of $1{\cdot}9–2{\cdot}0 \times 10^6$, which is well within the usual error of the assay observed in the foregoing experiments.

It can be demonstrated in all three cases of modification of direct action worked on or mentioned here that the protective or potentiating effect is dependent on the concentration of the added modifying agents[8,27]. The foregoing experiments thus suggest that, regardless of the purity of the initial virus preparation, a simple serial dilution of target samples into such innocuous media (for example, see legend, Fig. 1) as tryptone broth, nutrient broth and GSSG for irradiation would obviate any potential effect of the medium. (These media are not unique in this property since results clearly show that the heavy protein matrices of viruses do not modify the radiation sensitivity of the nucleic acid they envelop.) An empirical step of serial dilution, combined with some biological calibration of target procedure using viruses and nucleic acids of known molecular weight, should be effective in obtaining dependable target molecular weight very simply.

Taking due cognizance of radiation modifying effects, valid radiation target molecular weights can be obtained relatively simply for small viruses and their free nucleic acids with an accuracy expected from conventional physical methods. The combined experimental results obtained here from both γ- and X-irradiation bear out the assumptions that: (1) 60 eV constitute a killing event and this quantum of energy has an efficiency of 1; (2) the number of lethal events does not exceed the number of ionizing events. The results are also consistent with the assumption that an ionized target subsequently reacts in some way, probably with oxygen[25], with high efficiency and only then is the 'hit' target scored as a kill. The independence of target sensitivity with temperature between $-180°$ and $+30°$ fits the expectation for sparsely ionizing radiation that the lethal event in the nucleic acids is a direct result of ionization and not of thermal effects arising from absorption of excitation energy.

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QUANTITATIVE ESTIMATION AND IDENTIFICATION OF BARBITURATES IN BLOOD IN EMERGENCY CASES

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ANY method for the determination of barbiturate in a patient's blood must, to be of value to the clinician and, hence, to the patient, fulfil three conditions. First, it must be capable of yielding results sufficiently rapidly to allow the clinician to commence treatment before the patient's clinical condition deteriorates. By rapidly, is meant that the time required for the analysis, that is, quantitative assay of the barbiturate and its identification, should be of the order of 1 h. This would be the time reckoned from receipt of the blood by the laboratory to completion of analysis.

Secondly, the quantitative estimation must be precise to within ± 10 per cent. Greater variation than this might give misleading results if serial analyses were being performed with short time-intervals.

Thirdly, in order to be able correctly to interpret the results of the quantitative assay, it is necessary to know which particular barbiturate is present in the patient's body. In the past, it has not been possible to identify the barbiturate within 1 h, but the introduction by Street[1–4] of paper-partition chromatography at elevated temperatures has enabled the more commonly used barbiturates to be identified in a blood extract within 20 min.

Specificity is a further criterion which must be satisfied and which may be achieved in high degree by a combination of several factors. By carrying out a classical separation of a blood protein-free filtrate, a fraction can be obtained which contains only barbiturates and other weak acids. If ultra-violet spectrophotometry is used for the quantitative assay of this weak acids fraction, it is possible to arrange the conditions so that an absorption maximum occurs at a wave-length which is peculiar to a particular group of barbituric acid derivatives. For example, the

5 : 5-disubstituted barbiturates, which incidentally are the group most commonly encountered, display the characteristic phenomenon of exhibiting at *p*H 11 a peak at 240 mμ which disappears when the solution is brought to *p*H 2.

The recent work of Street and McMartin[5] has shown that a further step towards the unequivocal identification of individual barbiturates can be taken by heating at 100° C an aliquot part of the weak acids fraction with concentrated sulphuric acid for 3 min and examining the ultra-violet spectrum of the products.

Chromatography at about 90° C on tributyrin-impregnated paper with an aqueous solvent separates most of the more commonly used barbiturates in 15 min and, together with the ultra-violet spectra at *p*H 11 and *p*H 2, and the results of sulphuric acid treatment, provides a rapid procedure for the identification and assay of blood barbiturates.

The method we recommend involves preparation of a protein-free filtrate of blood. For this purpose we have modified slightly the procedure described by Folin and Wu[6] and have used Curry's[7] suggestion to heat the tungstic acid–blood mixture. The acidic and the neutral compounds are extracted from the acid filtrate by shaking with ether. Strong acids such as salicylic acid are removed by extraction of the ether with a *p*H 7·3 buffer. By shaking the ether with alkali, the neutral compounds are left in the organic phase and the weak acids extracted into the aqueous phase. Acidification of the alkaline medium and re-extraction into ether yields a fairly pure weak acids fraction.

This fraction is then divided into three parts. One portion is used for quantitative estimation by ultra-violet spectrophotometry; another portion is used for elevated-temperature chromatography; a third portion is taken for treatment with sulphuric acid to assist further in identification of the barbiturate.

De-ionized water is used throughout. The following reagents are needed:

(1) 10 per cent (w/v) sodium tungstate solution. Dissolve 100 g of $Na_2WO_4 2H_2O$ in water to 1 litre.

(2) Sulphuric acid solutions, 2 per cent, 10 per cent and 6 N. Pour 20 ml., 100 ml. and 167 ml. respectively of 'AnalaR' concentrated sulphuric acid into water and dilute to 1 litre.

(3) Ether. Shake 2½ litres of 'anæsthetic ether' (diethyl ether) with 50 ml. portions of 10 per cent sodium hydroxide solution until the alkaline extract is colourless. Wash the ether with water until the washings are free from alkali. Store the washed ether in a dark room.

(4) Phosphate buffer, *p*H 7·3 (0·5 M). Mix 80 ml. of 0·5 M $Na_2HPO_4.2H_2O$ (89 g/l.) and 20 ml. of 0·5 M KH_2PO_4 (68 g/l.).

(5) Phosphate buffer, *p*H 7·4 (M/15). Mix 80·8 ml. of M/15 $Na_2HPO_4 \cdot 2H_2O$ (11·9 g/l.) and 19·2 ml. of M/15 KH_2PO_4 (9 g/l.).

(6) Sodium hydroxide solution (2·5 per cent). Dissolve 25 g sodium hydroxide pellets in water and dilute to 1 litre.

(7) Sodium sulphate (anhydrous).

(8) Ammonia solutions (0·5 N and 4 N). Dilute 7·1 ml. and 57 ml. respectively of concentrated ammonia solution (sp. gr. 0·88) to 250 ml. with water.

(9) Tributyrin solution. 10 per cent (v/v). Dilute 25 ml. of tributyrin (glyceryl tributyrate) to 250 ml. with acetone.

(10) Sulphuric acid, 95 per cent. Carefully add 5 ml. of water to 95 ml. of 'AnalaR' concentrated sulphuric acid. Mix thoroughly and store in a well-stoppered glass bottle.

(11) Potassium permanganate solution (0·1 per cent (w/v)). Dissolve approximately 100 mg of potassium permanganate crystals in 100 ml. of water. Prepare freshly as required.

To 65 ml. of water contained in a 40 mm × 240 mm test-tube add 5 ml. of blood. Mix and allow to stand for at least 2 min for the cells to lake. Add 15 ml. of 10 per cent sodium tungstate solution, mix and then run in 15 ml. of 2 per cent sulphuric acid solution. Stopper the tube and shake the mixture until its colour changes from red to reddish-brown or brown. This takes about 1 min. Remove the stopper and immerse the tube in a bath containing vigorously boiling water for 3 min. The contents of the tube must be beneath the water surface. Remove the tube and filter the hot mixture through a Whatman No. 1 paper into a 100-ml. measuring cylinder. Cool the filtrate and measure its volume (usually about 90 ml.).

Extract the cooled filtrate by shaking with 50 ml. of washed ether. Allow the liquids to separate and run off the aqueous layer. Shake the ether phase with 10 ml. of 0·5 M phosphate buffer, *p*H 7·3 and discard the aqueous layer. Extract the organic phase with two separate 3-ml. portions of 2·5 per cent sodium hydroxide solution. Run the alkaline extracts immediately into 5 ml. of 10 per cent sulphuric acid solution contained in a clean separating funnel. Shake this acidified solution with 25 ml. of washed ether. Discard the aqueous phase and dry the ether layer by shaking with about 2 g of anhydrous sodium sulphate. Pour the ether extract into a 25-ml. measuring cylinder and divide into three separate portions in 50-ml. flasks *A*, *B* and *C* as follows: Into each of flasks *A* and *C* place two-tenths of the ether extract (*N.B.*, flask *C* should be round-bottomed). Into flask *B* place six-tenths of the ether extract. Evaporate the contents of each flask to dryness on a boiling-water bath. Remove the flasks from the bath immediately the ether has evaporated and place them on a cold slab of metal to cool.

Flask A. Dissolve the residue in 3 ml. of 0·5 N ammonia solution. Measure 2·5 ml. of the solution into a silica or quartz cuvette (1 cm light path) and plot its absorption curve against 2·5 ml. of 0·5 N ammonia solution from 200 to 350 mμ. Add 0·5 ml. of 6 N sulphuric acid to both 'test' and 'blank' cuvettes. Mix the solutions and replot the ultra-violet spectrum. Let R_{OH} be the absorbance of the ammoniacal 'test' solution at 240 mμ and R_H the absorbance of the acid 'test' solution at 240 mμ, then:

$$\text{blood barbiturate} = \frac{(R_{OH} - R_H)}{S} \times \frac{3}{50} \times \frac{10}{2} \times \frac{100}{F} \times \frac{100}{5} \text{ mg/100 ml.}$$

$$= \frac{(R_{OH} - R_H)}{S \times F} \times 600 \text{ mg/100 ml.}$$

where $S = (R_{OH} - R_H)$ for standard barbiturate solution (see below) and F = ml. of tungstic acid filtrate used.

If the ultra-violet spectrum shows no maximum at 240 mμ, it may be assumed that barbiturate is either not present in the blood sample being analysed or is present in insignificant amount.

Flask B. Dissolve the residue in about 6 drops of chloroform and transfer the solution with a glass capillary tube to the starting line of a previously prepared sheet of tributyrin-impregnated Whatman No. 3 paper. (Dip the papers in a 10 per cent (v/v) solution of tributyrin in acetone, press between sheets of filter paper and allow to dry in air. Papers should be impregnated not more than 2 h before being used) (Fig. 1*a*). As reference standards, put about 2 μl. of 2 per cent ethanolic barbiturate solutions also on the starting line. The size of spot should, preferably, be not greater than 3 mm diameter. A mixture of barbitone, phenobarbitone, butobarbitone and pentobarbitone may be placed on one side of the unknown spot and a mixture of amylobarbitone and quinalbarbitone on the other side. Fold the paper into a cylinder, fasten with a paper-clip as shown in Fig. 1*b* and carry out chromatography for 17 min.

This is done by placing the paper cylinder in a 2-litre beaker containing M/15 phosphate buffer *p*H 7·4. The beaker and buffer have, about 0·5 h previously, been put in an oven maintained at a temperature of about 95° C.

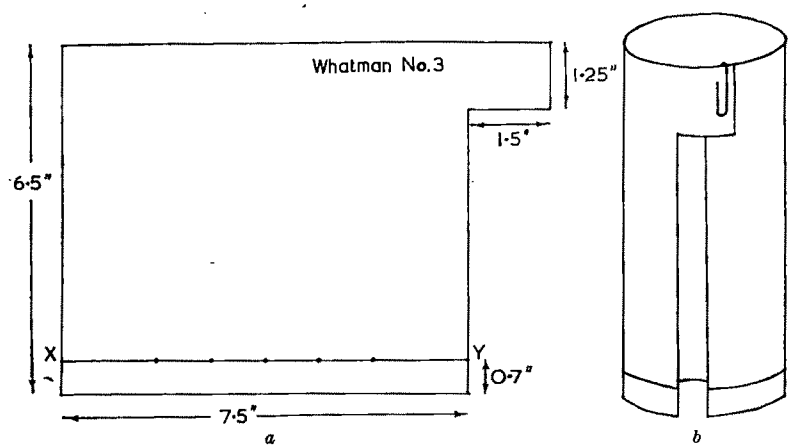


Fig. 1. *a*, Size and shape of Whatman No. 3 paper used for reversed phase paper chromatography at 95° C. After the paper has been impregnated with tributyrin, the compounds to be separated are applied at points on the line *XY*; *b*, paper folded into cylinder and fastened with a paper-clip prior to carrying out reversed phase chromatography at 95° C

A disk of plate glass smeared with silicone grease is used to seal the beaker. The amount of buffer in the beaker is such as to give a depth of about 3/8 in. of liquid. The paper cylinder is placed in the beaker without removing the beaker from the oven.

After 17 min, the solvent will have run about 5 in. up the paper from the starting line. Remove the paper from the beaker and examine in 254 mμ radiation (a Hanovia 'Chromatolite' lamp is suitable for this purpose). Expose the paper to ammonia gas and re-examine under the lamp. Note the position of any absorbing areas (Fig. 2).

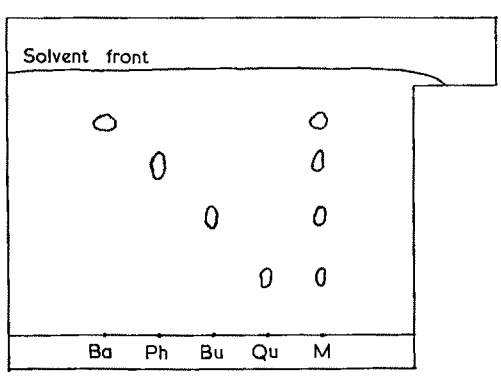


Fig. 2. Separation of four barbiturates by reversed phase chromatography at 95° C on Whatman No. 3 paper impregnated with tributyrin. Solvent, M/15 PO_4, *p*H 7·4; time of run, 17 min

Ba, barbitone; *Ph*, phenobarbitone; *Bu*, butobarbitone; *Qu*, quinalbarbitone; *M*, mixture of these four barbiturates

The paper may now be dried with a hair-dryer and then dipped in 0·1 per cent solution of potassium permanganate. Those barbiturates containing an unsaturated group in their side chain(s) will show up as yellow spots on a pink background.

Flask C. Add 0·2 ml. of 95 per cent sulphuric acid to flask *C*, stopper the flask and make sure that the acid is run over the residue. Heat the flask in boiling water for 3 min, cool and add 4 ml. of water. Pour the solution into a separating funnel, rinse the flask with 1 ml. of water and add this rinsing to the separating funnel. Shake with 25 ml. of ether and allow the layers to separate.

Aqueous phase. Run the aqueous phase into a graduated test-tube and pass a stream of nitrogen through the solution for about 2 min to remove the ether. Then add an equal volume of 4 N ammonia solution, mix and plot the absorption curve from 200 mμ to 350 mμ. Use silica or quartz cuvettes of 4 cm light path. Add 2·0 ml. of 6 N sulphuric acid solution and replot the absorption curve (Fig. 3). The 'blank' in this case is a mixture of 0·2 ml. of 95 per cent sulphuric acid, 5 ml. of water and 5·2 ml. of 4 N ammonia solution.

Ether phase. Dry the ether phase by shaking with about 1 g of anhydrous sodium sulphate, decant, and evaporate to dryness on a boiling-water bath. Remove the flask immediately the last drop of ether has evaporated. Cool the flask and dissolve the residue in 3 ml. of 0·5 N ammonia solution. Plot the absorption curve from 200 mμ to 350 mμ against 0·5 N ammonia solution. To 2·5 ml. of solution, add 0·5 ml. of 6 N sulphuric acid solution, mix and replot the absorption curve (Fig. 3).

A fuller description of the heating of barbiturates with sulphuric acid is given by Street and McMartin[5].

Standard curves should be constructed only from freshly prepared solutions.

Dissolve 20 mg of pure barbituric acid derivative in 200 ml. of 0·5 N ammonia solution. Add 1 ml. of this solution to 4 ml. of 0·5 N ammonia solution and plot the absorption curve from 200 mμ to 350 mμ. Use silica or quartz cuvettes of 1 cm light path. To 2·5 ml. of this solution add 0·5 ml. of 6 N sulphuric acid solution and replot the absorption curve.

The difference, *S*, between the absorbance in ammonia (R_{OH}) at 240 mμ and that in acid (R_H) at 240 mμ is used for calculation of the amount of barbiturate in blood (see foregoing), that is:

$$S = (R_{OH} - R_H)$$

For the 5 : 5-disubstituted derivatives, $S = 0{\cdot}9 \pm 0{\cdot}1$ (see also ref. 7).

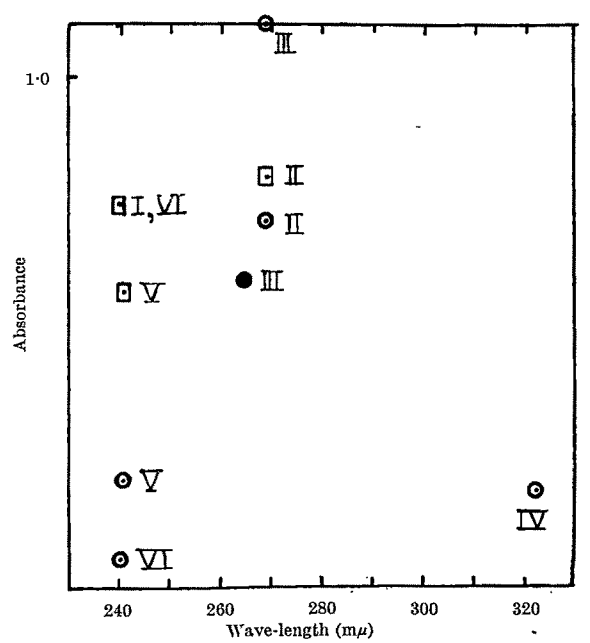


Fig. 3. Relative absorbances of products obtained from 50 μg of barbiturate subjected to treatment with sulphuric acid (see text)

⊡, absorption maximum of ether-soluble products in alkaline solution;

⊙, absorption maximum of water-soluble products in alkaline solution;

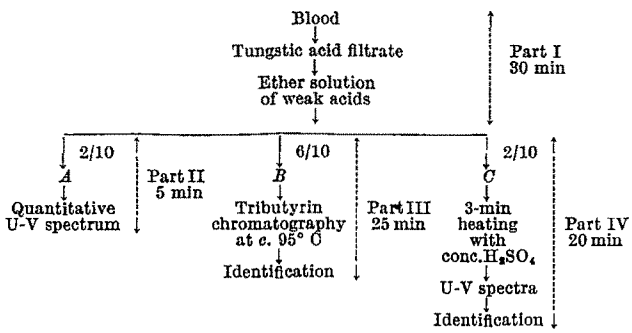
●, absorption maximum of water-soluble products in acidic solution

I	II	III	IV	V	VI
Products of: Barbitone, Butobarbitone, Amylobarbitone.	Products of: Pentobarbitone, Cyclobarbitone	Products of: Allyl isopropyl barbituric acid. Quinalbarbitone.	Products of: Dial.	Products of: Allyl isobutyl barbituric acid.	Products of: Phenobarbitone Rutonal

Discussion

A diagrammatic plan of the various steps in the analysis is shown in Table 1. Part I of the analysis, which is to prepare a protein-free filtrate of the blood and produce a partially purified ether extract of this filtrate requires 30 min. Parts II, III and IV need 5, 25 and 20 min, respectively. It is suggested that one worker carry out parts I and IV and another worker do parts II and III. In this way, a complete analysis can be carried out in 55 min.

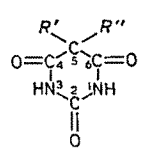
Table 1



There are several steps in the procedure which require amplification and emphasis. Thorough washing of the ether is essential for production of 'low blank readings'; loss of barbiturate can occur if, during evaporation of an ether extract, the flask is not removed from the bath immediately the last drop of ether has disappeared; it is imperative that the sulphuric acid added to flask *C* should come into contact with the whole of the residue; the filters used in the 'Chromatolite' 254 mμ lamp should be tested regularly with a spot of a known barbiturate on tributyrin-impregnated paper in the presence of ammonia gas. The 'life' of some of these filters may be as short as 800 h.

The limitations of the procedure are such that a level of 0·5 mg of barbiturate per 100 ml. of blood can just be measured quantitatively when starting with 5 ml. of blood but this concentration is insufficient for detection by chromatography of the residue in flask *B*. Above this level, no difficulty should be experienced with either the quantitative or qualitative estimation.

In the quantitative assay, it has been assumed that the barbiturates being measured are dialkyl derivatives substituted only on carbon atom 5.

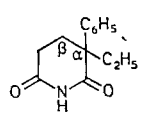


This assumption has been made because the 5 : 5-di-substituted derivatives are in commonest use. However, 1 : 5 : 5-tri-substituted derivatives may occasionally be encountered. The *N*-methyl derivatives and also the thiobarbiturates (which are used exclusively as anæsthetics) may be distinguished by the wave-length of their absorption maxima at different *p*H values (Table 2). Chromatography provides confirmation.

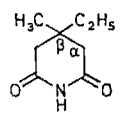
Table 2

Derivative	Wave-length maxima (mμ) at *p*H 2	at *p*H 11	at *p*H 14
1 : 5 : 5-	nil	245	245
5 : 5-	nil	240	255
Thio-	239,288	255,303	303

In the recommended method, no serious interference from substances which are not barbiturates will be encountered, and, in this connexion, only salicylates and glutarimides will, therefore, be considered. Salicylate will be removed by washing with the *p*H 7·3 buffer. If present in large amount, some will escape into the weak acids fraction and will be seen on the chromatogram in 254 mμ light as a light-blue fluorescent spot (R_F about 0·9) and will also distort the ultra-violet absorption curve. It is, therefore, wise to test a portion of the original blood sample with Trinder's[8] reagent. This takes only a few minutes and can be done while the tungstic acid/blood mixture is filtering. If the test is positive, then the excess salicylate can be removed by three washes with the *p*H 7·3 buffer. If Trinder's test is negative, the single wash with *p*H 7·3 buffer should not be omitted as this serves to remove some of the acidic fatty material encountered in all blood/tungstic acid filtrates. Of the glutarimides, only glutethimide and bemegride need be considered.



Glutethimide



Bemegride

In 0·5 N-ammonia solution, glutethimide and bemegride have absorption maxima at 235 mμ and 230 mμ respectively. The glutarimide ring is unstable in alkaline solution and both these compounds may be detected, and also estimated, by observing the decrease in absorbance (at the wave-length maximum) with time.

Part of this work has been supported by a grant from the Scottish Hospital Endowments Research Trust.

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HÆMOSTATIC CHANGES ASSOCIATED WITH EXERCISE

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PHYSICAL exercise is known to affect the hæmostatic balance. Even if some hæmostatic indices have been found to behave inconsistently in response to exercise, possibly owing to technical differences, observations on the time of coagulation[1,2], like thromboelastographic results[2,3], have generally yielded evidence suggesting that exercise gives rise to hypercoagulability. Of the coagulation factors of the plasma, the levels of Factor V[2], Factor VIII (AHG)[4] and Factor XII (Hageman factor)[5] have been reported to be raised concurrently. The platelet count

has also been stated to increase[3,6], but the count appears to be rapidly restored in the post-exercise period[3]. On the other hand, physical exercise has been shown to increase fibrinolytic activity[5,7].

The work described here was undertaken to explore exercise-induced changes in all the main hæmostatic parameters, with reference also to their behaviour in the period following exercise.

The tests were made on 10 apparently healthy medical students. They were, after over-night fasting, subjected to 6 min heavy muscular exertion (1,250 kg. m./min) by use of a bicycle ergometer. Blood samples were taken before and immediately after the exertion, as well as 10, 30 and 60 min following completion of the work. The methods for the preparation of plasma and serum and for the clotting tests were, if not otherwise stated, those described previously[8]. The following tests were carried out: thromboelastography (Hellige), recalcification time, prothrombin consumption index, Quick thromboplastin time, platelet count[9], thromboplastin generation test, fibrinogen, prothrombin, Factor V, Factor VII-complex, $P+P$, Factor VIII[10], Factor IX[10], Factor XI+XII ('contact factor' by Waaler's method)[11], and fibrin plates[12]. The plates used were as follows: (1) activator (+plasmin): euglobulin on unheated plates; (2) plasmin: euglobulin on heated plates; (3) plasminogen: euglobulin+streptokinase (8,000 units per ml. of euglobulin) on heated plates.

An outline of the behaviour of the coagulation parameters before and following the performed work is given here. The changes observed are shown in Fig. 1.

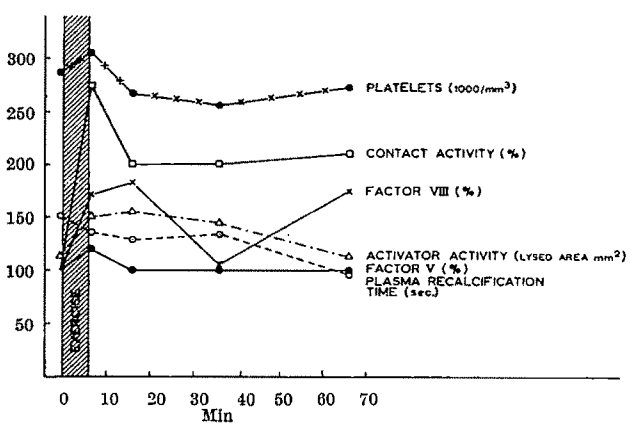


Fig. 1. Behaviour of the coagulation parameters exhibiting most evident changes in response to exercise. The values represent median ones

In all the subjects examined there was a clear-cut trend to hypercoagulability in the blood samples taken in the post-exercise period. This trend was most obvious in the samples taken immediately after completion of the work when there was, as compared with the pre-exercise values, a statistically significant shortening in the recalcification time (from 106–197 sec to 101–186 sec) with an associated, statistically significant increase in the plasma-levels of Factor V (to 100–200 per cent), Factor VIII (to 120–540 per cent) and 'contact factor' (to 240–1,000 per cent). In the later post-exercise period these hæmostatic indices acted somewhat inconsistently; but signs of hypercoagulability were still present. The recalcification time continued to shorten. The difference between the recalcification time obtained prior to exercise (106–197 sec) and that observed 60 min following completion of the work (84–152 sec) was statistically significant, and the same held true in the case of 'contact activity'. Meanwhile, the levels of Factor V became normalized whereas those of Factor VIII could be either normalized or raised.

The thromboelastographic r-time appeared to shorten in conformity to the decrease in recalcification time, even if no statistical significance could be revealed. In the thromboplastin generation test increased activity was found both in the combination of test subject plasma—normal serum and in that of test subject serum—normal plasma. However, the changes were not equally marked as were those in the levels of Factor VIII and 'contact factor'.

The parameters remaining unaltered were fibrinogen, prothrombin consumption index, Quick thromboplastin time, $P+P$, Factor VII-complex and Factor IX.

The platelet count appeared to be increased in the samples taken immediately after exercise; but in the subsequent post-exercise period a restoration of the count seemed to take place. The changes were not statistically significant, however.

As for the fibrinolytic activities, there was a statistically significant increase in activator activity in the samples obtained immediately after completion of the work. In the period following, the activator activity showed considerable individual variation. Yet in most instances the normal activity-levels were attained 60 min following the completed work. In two individuals, who exhibited the most pronounced elevation in the activator activity, some increase in the plasmin activity occurred. The plasminogen activity remained unchanged.

In evaluating the results obtained one may proceed from the possible sources or error. In the first place, venous stasis, as used in our tests, has been found to cause hypercoagulability (own observations) and increased fibrinolytic activity[13]. Hence it seems possible that a circulatory activation, following the exercise[3,6], accentuated the stasis-dependent changes especially in the first post-exercise samples and thus contributed to the rather marked hæmostatic alterations encountered at this stage. Secondly, it should be noted that in assaying the coagulation factors, the activity of which is reckoned from dilution curves obtained by use of normal plasma, the accuracy of determinations decreases as the measurable activities increase. Because even at the optimum levels of measurability the accuracy of Factor VIII determinations, for example, lies between 60 and 140 per cent of the figures obtained, it is clear that the accuracy of the determinations is relatively unreliable. Thirdly, as regards the changes in coagulation observed, the response of the individuals to a given work load may vary. Moreover, it is known that anxiety, the degree of which in the present tests, for example, may have varied from case to case and even from one experimental stage to another, readily evokes hypercoagulability with a concurrent increase in the fibrinolytic activity[14]. As regards the work recorded here, however, we are justified in concluding that the changes were great and uniform enough to exclude the possibility that they had resulted merely from an interplay of the factors just discussed and that, accordingly, the changes were occasioned mainly by the work performed.

Our observations on the shortening of the recalcification time and the thromboelastographic r-time with the concomitant rise in the levels of Factor V, Factor VIII and 'contact factor' are in keeping with those already published[1–7]. Whether there was an actual rise in all these activities or whether the results could be traced solely to the increased 'contact activity' or to a development of some new, unknown principle of activity, remains unsettled.

The platelet count was slightly and not wholly consistently increased in the samples of blood taken immediately after exercise. According to Wachholder *et al.*[3], the platelet count is restored within a few minutes following muscular work. Since, in our investigations, the time elapsed from the completion of the work to the time of taking the blood sample for platelet counting was some 1–2 min, it is possible that this delay was sufficient to reduce or abolish thrombocytosis. Within about the same time the exercise-induced circulatory activation, by which mechanism the exercise-thrombocytosis has been explained[6], may disappear[3,15].

In the fibrinolytic activities examined the activator activity was found to exhibit a clear-cut increase. Iatridis *et al.*[5] expressed the belief that in exercise it is mainly the activity of lysokinase, that is, of a factor playing an integral part in the generation of the activator activity, that is increased.

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FUNCTIONAL VASCULAR FIELDS IN THE PITUITARY STALK OF THE MOUSE

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THE hypophyseal portal system of blood vessels has been amply demonstrated to be the morphological basis for the functional link between the hypothalamus and the anterior pituitary. The basic essentials of this system are as follows: (1) a set of primary capillaries (primary capillary 'plexus') in the neurohypophysis, particularly the median eminence and neural portion of the stalk; (2) those nerve fibres of the hypothalamico-hypophyseal tracts which have terminations near the primary capillaries; (3) a series of portal trunks connecting the primary 'plexus' with (4) the 'sinusoids' or secondary capillary 'plexus' in the anterior pituitary. There is an abundance of convincing evidence that neurohumoral substances from hypothalamic nerve endings pass by way of this system into the humoral environment of the glandular cells of the anterior lobe. (For recent reviews of the literature on neuroendocrine control of the anterior pituitary see Harris[1] and the Neuroendocrine Symposium, Miami, 1961 [2], and on the vascular anatomy and microcirculation, Worthington[3].)

Although much has been written concerning the morphology and anatomical distribution of these vessels, comparatively little is known of their physiology[4–7], and nothing is known concerning the possibility of functional specialization of separate vessels or groups of vessels within the system. If one considers particularly the primary capillaries, no evidence has been presented which goes beyond the concept of a functionally uniform network acting as a drainage unit for substances released from nerve endings. Implicit in most discussions of the role of the primary capillaries in the system is the conclusion that this capillary bed is functionally uniform throughout and the vessels freely anastomotic. There is little evidence indicating that there might be functional localization within this capillary bed.

It seems beyond question that the hypophyseal portal system taken as a whole is functionally unique and highly specialized. It also seems possible that specialization occurs within its major sub-divisions. The anatomical evidence concerning the primary capillary system is quite suggestive. The mouse shows an arrangement of groups of capillaries difficult to discern in injected or sectioned material but clear enough if the living stalk and median eminence are examined carefully, which suggests some degree of specialization. In man the vascular tufts and 'glomerular' formations which have been found in injected specimens suggest areas of specialized function[8–11]. Since there is now evidence that more than one substance is released into the primary capillary system for delivery to the sinusoids of the anterior lobe[12–14], and since there is no evidence to indicate whether or not parts of the median eminence and stalk are carrying out special functions, it seemed desirable to determine whether or not there are in the primary capillary area delimited vascular fields which might be functionally separable.

An investigation of this question was carried out by applying a phenomenon which was observed many years ago but has received little attention in recent times. In dead animals the neurohypophysis is intensely stained when injected with methylene blue in dilute solution. This was first described by Dandy[15] in 1913 and was discussed briefly by Bucy[16] in 1930, but seems to have been neglected since. The hypophyseal stalk can also be stained intensely in the living mouse.

In excess of 40 mice were used in this series of studies. The ventral surface of the pituitary stalk and median eminence and portions of the hypothalamus and anterior lobe were prepared under intraperitoneal sodium pentobarbital anæsthesia for direct microscopic observation by a procedure previously reported[4,5]. In brief, this consists of the careful exposure of the structures already described using a ventral approach through the neck. The pharynx, trachea and œsophagus are moved aside and a dental drill is used to make a window in the basisphenoid bone.

The pituitary stalks which have been studied in this series were examined either without further procedures or after the insertion of a small cannula (fashioned from a 30-gauge needle and a piece of *P.E.* 10 polyethylene tubing) into the femoral vein for the administration of a 1 per cent solution of methylene blue.

Observations were made with a Zeiss dissecting microscope giving magnifications of up to 40 times or a Bausch and Lomb binocular compound microscope equipped with 10× or 15× eyepieces and a 10× objective. Effective magnifications of 150 times are attainable with the latter, and resolution is much better than with the dissecting microscope. The Zeiss microscope has a built-in illuminator. A special lamp fitted with two infra-red absorbing filters was used for incident illumination with the compound microscope. In some of the early observations a Leitz Greenough binocular dissecting microscope was used rather than the Zeiss.

Of the more than 40 animals used for this work, 20 were subjected to the interruption of one or more arterial vessels to the median eminence or stalk. This procedure was carried out in the following manner: using number 000 insect pins as dissecting instruments, a very small hole was placed in the transparent dura mater in the vicinity of the blood vessel which had been chosen for

interruption. A second insect pin with a small hook at the tip (made by tapping the pin's point gently on a hard surface) was used to cut the vessel by placing the hook around it and lifting. On occasion sufficient bleeding was caused by this procedure to require that the animal be discarded, but this was unusual. Usually there was sufficient contraction and retraction of the cut tip that bleeding was either absent or minimal. The animals were prepared as outlined either with or without the interruption of small blood vessels and were then injected with from 0·01 to 0·02 c.c./g of body-weight of 1 per cent solution of polychrome methylene blue. Occasionally toluidine blue was used, but it seemed to offer no particular advantage. Observations were made before, during and following the administration of the dye.

General observations and the initial appearance of the dye in the neurohypophyseal tissues in intact animals. Very soon after the beginning of the injection, the dye appears as a very thin, barely detectable, blue line immediately adjacent to some of the capillaries in the median eminence and stalk. Since the dye does not appear in all areas of the visible portions of the neurohypophyseal tissue at the same time, or in the same initial concentration, staining appears at first to be patchy and irregular. As the injection proceeds, the initial thin blue lines take on a deeper colour, gradually take on a granular appearance, and finally fill all the tissue between the capillaries. Finally, after the injection of a total dose of 0·01–0·02 c.c./g of body-weight, the stalk and median eminence are usually uniformly stained. If an area appears to be unstained or considerably more lightly stained than adjacent areas in an animal in which no deliberate cutting of the arterial vessels has taken place such an area has always been observed to have a reduced rate of blood flow in the capillaries. Animals in which several areas of markedly reduced flow were observed before the injection of methylene blue were seldom used for interruption of blood vessels although they can be mapped and their relation to the areas which are affected by manipulation can be determined (Fig. 2).

With the concentration of dye used, the neurohypophysis is the only part of the area under observation which is fairly intensely stained. The surrounding hypothalamic tissue is unstained to all practical purposes, and a small amount of the dye may be found along the anterior edge of the anterior lobe. The continued injection of dye in excess of the dose described above gives a gradually deepening colour to the median eminence and stalk; the neurohypophyseal area becomes blue-black, and the anterior margins of the anterior lobe become considerably more heavily stained. The effect of excess amounts of dye solution given intravenously is finally to interfere with the circulation in the hypophyseal portal system. The capillaries become clogged, and the portal blood flow slows over a period of time and finally stops with the only remaining detectable blood flow occurring in the larger arterial vessels around the stalk. An excessive dose of methylene blue was not routinely given.

If the injection of the dye is discontinued and the stained area observed for a period of time thereafter there is gradual fading of the blue colour. Whether this is due to a diffusion of the dye out of the stalk or the breakdown of the dye to a colourless form is not known. Evidently some diffusion of the dye takes place, but this does not occur so rapidly that small well-delineated vascular areas in the stalk cannot be observed by this technique.

Observation on excised specimens of hypothalamus and neurohypophysis following dye injections. In order to determine something of the more precise localization of the dye in the neurohypophyseal tissue, some specimens were excised following injection of varying amounts of the dye. These were observed *in vitro* with a water immersion lens without the application of a cover slip in order to maintain the dye in the oxidized (coloured) state. The information which has been derived from such observations is primarily negative. Very little can be determined about the nature of the dye and its distribution since, after the tissue has been excised, there is apparently acceleration of the diffusion process. In the living animal the dye appears granular at the levels of magnification obtainable (100–150 times). It also appears to lie rather superficially in the median eminence and stalk between the capillaries. So far it has been impossible to devise a method for obtaining a sufficiently well-preserved cross-section of the unfixed stalk to be able to describe accurately the localization of the dye in the deeper regions. The significant negative observation is that in no specimen that we have examined (either those described in this article or others) have we seen any staining of the small unmyelinated nerve fibres of the hypothalamico-hypophyseal tracts by methylene blue.

This negative evidence is strengthened considerably by the fact that in many of our preparations we have seen numbers of irregularly distributed nerve fibres in the hypothalamus as well as in and just behind the optic chiasma which are very dramatically stained by methylene blue. Occasionally I have seen fibres which are presumed to be vasomotor fibres passing along the courses of the hypophyseal and hypothalamic arteries and on numbers of occasions nerve fibres lying superficially in the hypothalamus (which must be either within the pia or immediately sub-pial) were seen passing into the median eminence and stalk. This was quite dramatically demonstrated when small blood vessels to the primary capillary plexus were interrupted leaving an unstained area in the middle of the stalk. One or two nerve fibres could be seen standing out against a light background. It should be emphaized that these fibres are not the well-organized fibres of the hypothalamico-hypophyseal tracts which stain with silver stains.

Interruption of arterial vessels of the median eminence and stalk. The arterial vessels around the median eminence and stalk and portions of the hypothalamus as they appear in the living animal have been described previously[4,5]. An excellent recent description of injected material has been given by Enemar[17]. The courses of the vessels will not be described in further detail here except where such a description relates directly to the delineation of vascular fields by the methylene blue technique. The result of cutting a small arteriole to the median eminence is shown in Fig. 1. Little or no dye appears in a small fairly well-delineated field. It must be emphasized at the outset that there is no vascular stasis in this small field. Blood continues to flow through the capillaries but at a markedly reduced rate when compared with other capillaries outside of the field. The line of demarcation between stained and unstained tissue is usually quite sharp and falls between capillaries of separate groups. Since no other direct arteriolar supply to this small unstained area can be observed, it is deduced that a reduced capillary blood flow in the area is maintained by anastomoses with surrounding capillaries[4]. It is not impossible that even smaller areas than that shown in Fig. 1 might be affected by this procedure if there were not a limit on the size and location of arteriole which may be interrupted by freehand methods. A larger area of involvement is demonstrated in Fig. 2 after the interruption of a more proximal arterial vessel. Animals in which vessels were removed from one side of the stalk and median eminence are illustrated in Fig. 3. The demarcation between the stained and unstained areas roughly bisect the stalk.

Our experiments using this method suggest very strongly that the blood supply of the upper part of the stalk and median eminence are relatively independent of that of the lower stalk. The lower stalk must have its arterial supply intact to receive dye from the general circulation in sufficient concentration for it to appear in the tissue. The result of interrupting vessels to the upper and lower stalk

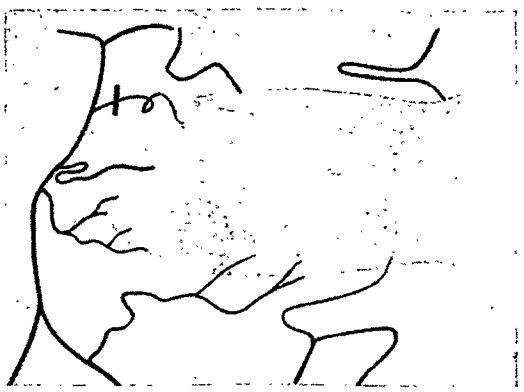


Fig. 1. Arteries and arterioles of the median eminence and stalk of the living mouse. The shaded area outlines the area of distribution of methylene blue in this region. The bar indicates the point at which an arteriole has been cut and the light area, the field in which no dye appeared although blood continued to flow through its capillary bed at a reduced rate. Artist's diagram from author's sketch of the living preparation



Fig. 2. Similar preparation to Fig. 1. More proximal branch of an artery cut as indicated by the bar. There is a much larger area of involvement. The blood flowed at a reduced rate through much of the lighter stained area. The oval area in the upper left part of the diagram represents a less well-stained area in which there was reduced blood flow before cutting stalk vessels

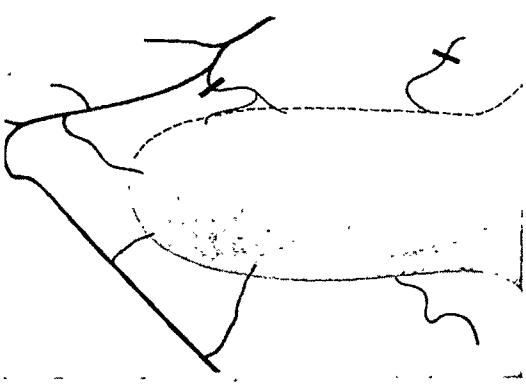


Fig. 3. Interruption of arteries of the left side of the median eminence and stalk

is illustrated in Figs. 4 and 5. The lower lateral margins of the stalk show no dye concentration after cutting the vessel on either side supplying this area (Fig. 4). If the reverse procedure is carried out and these vessels are left intact and others in the upper part of the stalk cut, something of the reverse of this picture is observed (Fig. 5). When only the vessels in the lower part of the stalk (sometimes only a single vessel) are left intact and all the other vessels of the upper stalk and median eminence are cut, one observes considerably more interference with the circulation in the primary capillary system and portal trunks from the upper stalk and median eminence than when some of the vessels in the upper part of the stalk are left intact. Under the former conditions one may see stasis in primary capillaries and in one or more portal vessels. Fig. 6 illustrates the stalk of an animal in which all the hypophyseal arterial vessels in the region had been cut except one which was partially closed as a result of mechanical manipulation. Those areas which are stained still showed fairly rapid flow in the capillaries.

The dye, in order to reach the tissues in sufficient concentration to become visible, must arrive there by way of the arterial vessels and not by capillary anastomoses from the upper part of the stalk and median eminence or by diffusion through the walls of portal trunks. In prepara-



Fig. 4. Interruption of arteries to lower lateral areas of the stalk. Vessels to the upper stalk and median eminence intact

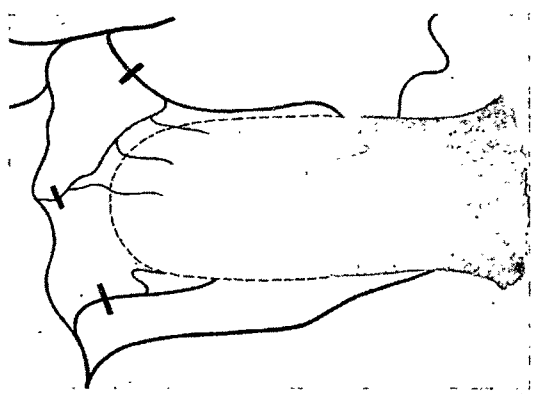


Fig. 5. Interruption of arteries and arterioles to the upper stalk and median eminence. Vessels to lower stalk intact

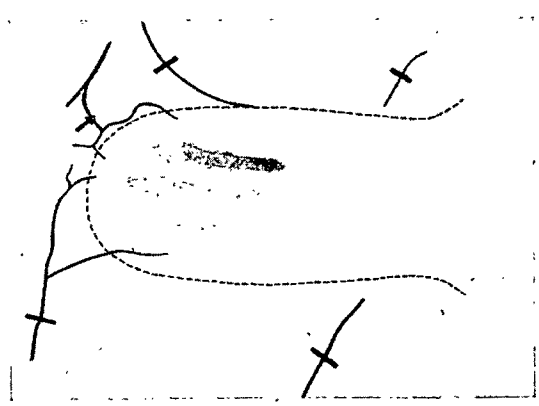


Fig. 6. All vessels to stalk and median eminence cut except that indicated by the arrow. The latter had been manipulated and was constricted, but rapid blood flow was still present. Fairly rapid capillary blood flow remained in the stained areas

tions in which a portal trunk traversed a lightly stained or unstained area the arterial supply of which was cut, no dye appeared in the area although both capillary anastomoses and the portal trunk passed from stained to unstained areas. Occasionally dye appears in an area from which we feel that we have removed the arterial supply or conversely a light area appears but no direct interference with the arterial supply has occurred.

These cases are infrequent. When it is a lighter area which was not anticipated (Fig. 2) there is always a reduced blood flow in this area. Whether this reduced flow is due to arteriolar constriction and reduction in rate of blood flow by physiologic mechanisms or through some incidental and unnoticed trauma during the procedure is not known. In the case of the appearance of dye in an area in which we thought we had removed the arteriolar supply, I afterwards found an arteriole coming from another area, or an intact arteriole closely following the course of the one which had been cut (Fig. 7). Rarely, dye was found in an area in which no arteriolar supply could be demonstrated. These are assumed to receive their supply from the lower stalk blood supply through the under surface. For reasons which should be obvious any animals showing even the slightest sign of trauma directly to the primary capillary system, portal vessels or their arterial supply were discarded and no dye injection investigations were made.

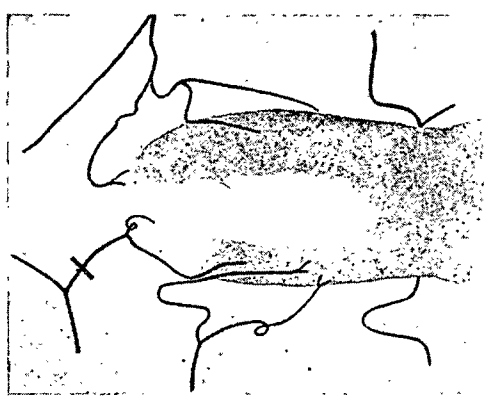


Fig. 7. Dye appears in an area which is served by an arteriole which has been cut. An intact arteriole passes very close to the cut vessel along part of its course

Preliminary observations on differences in dye concentration in animals in different physiological states. There is a distinct difference between pregnant animals and non-pregnant female or male animals in the amount of dye taken up by the pituitary stalk. In the pregnant female comparatively small amounts of the dye injected into the femoral vein impart an intense blue-black colour to the neurohypophysis much more rapidly than one finds in the non-pregnant female and the male animal. This is an incidental observation and thus far no intensive investigation has been made of it.

Discussion

The intense blue colour imparted by methylene blue to the neurohypophysis was observed by Dandy[15] in 1913 and may have been known earlier although he makes no reference to previous observations. Dandy's primary purpose was an investigation of the vasomotor nerve fibres accompanying the blood vessels and he quite correctly stated that he saw no staining of nerve fibres or tracts within the neurohypophysis other than those fibres accompanying blood vessels on their way to the anterior pituitary. The statement by Bucy[16] that it had become apparent since Dandy's work that the intense blue colour of the neurohypophysis is due to the presence of the many unmyelinated nerve fibres which are present is not supported by the observations reported in this article. In none of these were we able to find staining of the hypothalamico–hypophyseal tracts, even in the presence of staining of other hypothalamic nerve fibres. This leaves the nature of the methylene blue staining of the neurohypophysis unresolved. A number of conjectures may be made concerning this matter. The first of these is that the blue colour in the stalk is due entirely to the presence of precipitated methylene blue and that its presence here in higher concentrations than in surrounding brain tissue or in the anterior lobe is due to some fundamental difference in the permeability of the capillary or sinusoidal walls to methylene blue.

The second possibility is that there is some substance which either maintains the methylene blue in the oxidized state more readily in the neurohypophysis, or, conversely, some material which keeps the methylene blue in a reduced state in the surrounding brain tissue and the adenohypophysis. That there is present in the neurohypophysis a substance which maintains the dye in the oxidized state is doubtful. When these pituitary stalks are placed under cover-slips the oxygen is very rapidly used up and fading very rapidly occurs. The colour can be restored to the tissues when the cover-slip is removed and the tissue is allowed to come in contact with the air. In this connexion it may be conjectured that the interruption of the small blood vessels constituting the arterial supply to localized areas of the stalk is not actually reducing the concentration of the dye arriving at these limited areas of the stalk but merely reducing the oxygen tension to the point where the dye is not being maintained in the oxidized state. This latter interpretation is unlikely since the tissues in this region of the mouse are extremely thin and they should receive a supply of oxygen from the air, which is adequate for maintaining the blue colour. The previously mentioned fact that the dye is very rapidly reduced when one of the specimens is placed under a cover-slip would also make the latter explanation unlikely. In either case, however, this method is effective in establishing the presence of fairly well-delineated areas of vascular supply in the neurohypophysis.

Still another possibility concerning the nature of the staining reaction is that there is a specific affinity for some material in the neurohypophysis for the methylene blue. The first material which should be considered is neurosecretory material in the classic Scharrer sense[18]. This seems unlikely since there is a marked discrepancy between the amount of neurosecretory material which occurs in the mouse neurohypophysis stained with the chrome–alum hæmatoxylin technique, and the amount of methylene blue stained material which appears there in the living animal. The former is very sparse[19,20], the latter very heavy. These considerations still do not eliminate the possibility that this stained substance might be neurosecretory material since methylene blue may stain some part of the molecule of this material which does not stain by the chrome–alum hæmatoxylin and might in fact be a better stain for neurosecretory material in the mouse than chrome–alum hæmatoxylin.

These studies with methylene blue have a direct bearing on several aspects of pituitary stalk, neurohypophyseal, and hypophyseal portal function. The unmodified view that the primary capillary plexus is specialized in function only by virtue of location and vascular connexions pervades the literature on pituitary–hypothalamic relationships. Anatomical evidence for distinct groupings of capillaries in man and mouse was mentioned in the introductory paragraphs of this report.

The experiments reported here show that the pituitary stalk and median eminence can be sub-divided, on a vascular basis, into various areas which are functionally separate although there are capillary anastomoses between them. A sufficient rate of perfusion of blood through the capillaries of these areas by way of arterioles must be maintained for a uniform concentration of methylene blue in the blood to diffuse into the neurohypophyseal tissue. It is not an unreasonable hypothesis that this

might be true of other materials as well. These experiments show further that the upper and lower parts of the stalk have a relatively independent vascular supply.

The demonstration of more than one adenohypophyseal trophic factor from the hypothalamus[11–13] leads one to the question whether these materials are being released uniformly throughout the median eminence and stalk, or whether, alternatively, certain functions are located in separate areas of the stalk. One may ask further, whether the rate of release of these materials is governed completely by some functional attribute of the neuron or neuron terminals from which they are released or whether variations in the rate of blood flow through independent vascular fields contributes something to the rate of delivery of these materials into the portal trunks and thence into the adenohypophysis.

Although any conclusion on these matters at the present time would be premature, the experiments reported here demonstrate that such functional areas could exist and could be controlled in some measure by vasomotor control of blood flow into a given area. Experiments demonstrating chronic insufficiency of specific stalk-mediated functions by interrupting vessels to specific areas and maintaining the animals for long periods of time have been attempted. They have all so far failed because animals have not survived the experiment. The factors involved are not entirely clear although it does not involve the interruption of hypothalamic blood vessels or damage to the hypothalamus or to the pituitary stalk. Sham-operated animals with no manipulation of stalk vessels or hypothalamic damage also failed to survive.

The second major point which these experiments demostrate is that 'titre' or concentration of material in the circulating blood is not the only factor controlling the rate at which materials can arrive in the median eminence and stalk or in the anterior lobe.

It is obvious from these experiments that rate of capillary blood flow markedly influences the ability of the neurohypophysis to take up and concentrate the dye methylene blue. In every case where there were lighter areas of the stalk after injection of the dye there was a reduced blood flow rate. It can be concluded that there was a sufficient concentration of the dye in the blood of the stalk capillaries flowing at a rate insufficient to bring about a visible accumulation in the tissues.

In summary, these experiments with a dye which diffuses readily from primary capillaries into the neurohypophyseal tissue lends support to the following hypotheses: (1) That small regions of the pituitary stalk and median eminence are functionally separable on the basis of the arterial supply to different parts of the primary capillary system. (2) That capillary perfusion rate is an important factor in determining the amounts of materials which pass from primary capillaries to neurohypophyseal tissue. (3) That the arterial blood supply to the upper stalk and median eminence is functionally relatively independent of the lower stalk. (4) That the permeability of the primary capillaries varies with physiological state. It is possible that all these factors are involved in the fine control of pituitary function by hypothalamic neurohumours and/or materials from the general circulation.

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A WATER-SOLUBLE POLYPEPTIDE PREPARED FROM ZYMOSAN

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ZYMOSAN, an insoluble lipoprotein-polysaccharide derived from the cell wall of yeast cells, inactivates the third component of complement[1,2], produces hyperplasia and hyperfunction of the reticuloendothelial system[3–7]. Furthermore, it increases the resistance of mice towards bacterial infections[8] and ionizing radiation[9]. It enhances the formation of antibody[5] and also prevents the development of tumours[10] in animals and is pyrogenic and antigenic in rabbit[11].

This article describes a method for producing from zymosan a water-soluble polypeptide. This material protects, after intraperitoneal injection, mice against *E. coli* infection. Furthermore, it diminishes the lesion produced by vaccinia virus in rabbit skin. It also provides some protection of chick embryo against vaccinia virus. Finally, in tissue culture it inhibits the mitotic activity of HeLa cells.

2 g of commercial zymosan (from fresh yeast, type *A*, L. Light and Co., Colnbrook, England) prepared from Fleischmann yeast was extracted at 37° C with 150 ml. of 90 per cent aqueous phenol for 2 days. Undissolved material was removed by centrifuging and the clear

supernatant was shaken with 750 ml. of ether and 500 ml. of water. The aqueous phase containing a suspension of precipitated material was re-extracted with ether to remove phenol and the precipitate subsequently brought into solution by adjusting the *p*H to 9. The solution was concentrated in vacuum to a small volume and passed through a 'Sephadex *G* 50' (40 cm high × 4 cm diam.) column previously equilibrated with 0·01 N sodium hydroxide. The high molecular fraction was collected, neutralized with 'Amberlite *IR* 120 (H)' to *p*H 7·0 and freeze-dried. 0·07 g of a white solid was obtained. To purify it further, the white solid was dissolved in water (50 ml.) and centrifuged at 20,000*g*. To the clear supernatant was added 2 ml. of 20 per cent of sulphosalicylic acid, the precipitate redissolved in water by adjusting *p*H to 7 with sodium hydroxide, dialysed at +4° C against distilled water for 24 h and freeze-dried.

It is seen from Table 1 that the material is largely a polypeptide and contains only small amounts of carbohydrate. 'Sephadex *G* 50' excludes material with molecular weight equal or greater than 8,000–10,000. These figures could therefore represent a lower limit for the molecular weight of the polypeptide. It is precipitated on acidification and by sulphosalicylic acid, phosphotungstic acid and ammonium sulphate (completely precipitated at 50 per cent saturation).

Table 1. Analytical Results of the Material Prepared from Zymosan

	Per cent
Nitrogen*	12·69
Biuret†	92·0
Hexose content‡	1·9
Hexosamin content §	0

* Material dried at 105° C over P_2O_5 and analysed by the Dumas method. Bovine serum albumin by the same method gave 14·8 per cent (carried out by Dr. A. Schoeller, Kronach).
† The Biuret method of Gornall *et al.* (ref. 12) was used. The figure given is in percentage of the colour produced by an equal amount of serum albumin.
‡ The anthrone method of Goa (ref. 13) was used.
§ The modified Morgan–Elson method of Svennerholm (ref. 14) was used.

On electrophoresis in veronal buffer at *p*H 8·6 (ionic strength 0·1) on Whatman No. 1 followed by staining with Amido-Schwarz, the main part of the stainable material is negatively charged and moves as a homogeneous band. The distance travelled is 35 per cent of that travelled by bovine serum albumin.

Hydrolysis in 6 N hydrochloric acid at 110° for 20 h, followed by two-dimensional chromatography in *n*-butanol–acetic acid–water (200 : 48 : 200) and *n*-butanol–methylethylketone–water (200 : 200 : 1) in *cyc*lohexylamine, revealed the presence of glutamic acid, aspartic acid, arginine, lysine, histidine, glycine, serine, proline, alanine, valine, tyrosine, threonine, phenylalanine, leucine and isoleucine.

The ultra-violet absorption spectrum at *p*H 7·0 in *tris*-hydrochloric acid is seen in Fig. 1.

An aqueous solution of the material exhibits in the ultra-violet light a bluish fluorescence.

Pyrogenicity. 1 mg of the material was dissolved in 1 ml. of saline and injected intravenously into each of four rabbits and the temperature recorded as described previously[15] over a 3-h period. The maximum increase in temperature in each of the four rabbits respectively was 0·4°, 0·4°, 0·5°, 0·6° C, and shows that the material was not pyrogenic at this dose level.

Bacterial challenge. The effect of the intraperitoneal injection of 50 μg of material prior to the intraperitoneal

Table 2. Effect of the Material Isolated from Zymosan on *E. coli* Challenge in Mice

Hours between injection of material and bacterial challenge	Survivors/total mice in experiment
3	11/18
6	11/18
24	8/18
Controls	2/18

0·1 ml. pyrogen-free saline containing 50 μg of material was injected intraperitoneally into Swiss albino mice weighing about 20 g. The mice, including the controls, were challenged intraperitoneally with 0·25 ml. of a suspension of 1·25 × 10^8 *E. coli* grown and prepared as described previously (ref. 15).

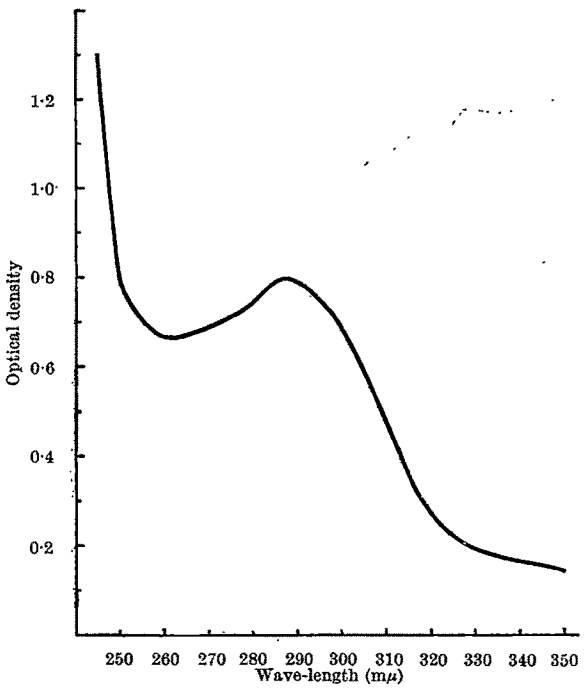


Fig. 1. The ultra-violet absorption spectrum of material prepared from zymosan in *tris*-hydrochloric acid buffer 0·1 M, *p*H 7·0 (0·33 mg/ml.)

injection of *E. coli* in mice is seen in Table 2. It is apparent that the material offers some protection against the bacterial challenge.

Vaccinia virus in rabbit skin. The effect of different quantities of material injected intracutaneously into rabbit skin prior to infection (on the same site) with vaccinia virus is seen in Table 3. It is apparent that the material reduces the development of lesions.

Vaccinia virus in chick embryo. The result of administering the material to chick embryo 24 h prior to infection with vaccinia virus is shown in Table 4. It is seen that the material provides some protection of chick embryo against vaccinia virus.

Mitotic activity in HeLa cells. The effect of the material on the mitotic activity of HeLa cells grown in the *E2a* medium of Puck, Cieciura and Fisher[16] is seen in Fig. 2. It is apparent that 0·33 mg/ml. reduces the mitotic index

Table 3. Effect of the Material Prepared from Zymosan on the Development of Vaccinia Virus in Rabbit Skin

Amount of material injected (μg)	Area free of lesions	
	Rabbit 1	Rabbit 2
400	1 cm × 0·5 cm	0·5 cm × 0·5 cm
200	0·5 cm × 0·3 cm	1 cm × 0·5 cm
100	No area free of lesions	No area free of lesions
Controls	No area free of lesions	No area free of lesions

White land rabbits weighing 3–4 kg were used. The animals were epilated with barium sulphide on the flanks. 0·2 ml. of a sterile saline solution of the material was injected intracutaneously (0·2 ml. saline as control), the area at and around the injection site scarified (ref. 15) and a solution of vaccinia virus applied to the area. The vaccinia virus used was a vaccine prepared from chick embryo infected with vaccinia virus and was free of bacteria. The results were recorded after 10 days.

Table 4. Effect of the Material Prepared from Zymosan on Chick Embryo Infected with Vaccinia Virus

Exp. No.	Amounts of material administered to each embryo (μg)	Survivors/total embryos	
1	2	23/37	
	controls	3/35	
2	10	5/5	Total 20/20
	5	5/5	
	2·5	5/5	
	1·0	5/5	
	controls	4/10	

Eggs from White Leghorn were used. After 10 days at 37° a 0·5 cm × 0·5 cm window was drilled over the chorio-allantoic membrane and the membrane dropped by suction over the air sack. 0·05 ml. of a sterile solution in saline of the material to be tested was applied to the membrane. 0·2 ml. of a vaccine (free of bacteria) prepared from a cell culture of calf embryo epithelial cells infected with vaccinia virus (ref. 15) was applied on the same spot after 24 h at 37°. The window was sealed with cellophane tape, eggs incubated for 4 days and deaths determined.

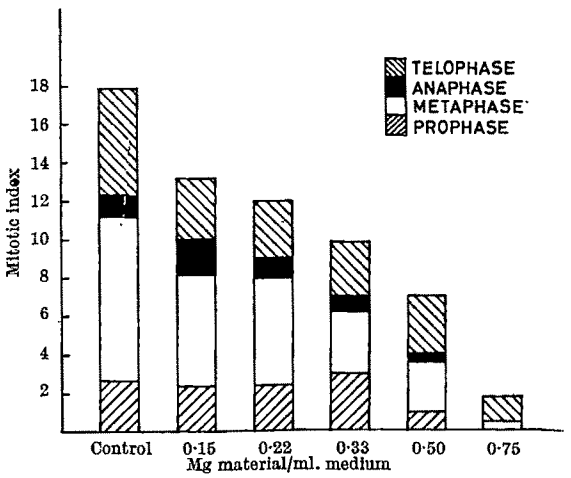


Fig. 2. The effect of the material prepared from zymosan on the mitotic index of HeLa cells
HeLa cells strain *S3* were grown in tubes as a monolayer on glass plates in *E2a* medium at 37°. The control is the average of the results from 10 tubes and the experiments the average of results from five tubes. The mitotic index was determined after 24 h exposure to the material. For further details of the experimental technique see Oftebro, Laland, Dedichen, Laland and Thorsdalen (ref. 17)

the HeLa cells with 50 per cent. Furthermore, the results suggest that the material interferes with some processes in the interphase. No chromosomal abnormalities were seen.

The experiments described here demonstrate that a high molecular polypeptide can be extracted from zymosan. This is rather surprising since the preparation of zymosan involves exhaustive digestion with trypsin[1].

The polypeptide material prepared from zymosan resembles in its biological properties material isolated from liver and blood and described previously[15]. These latter materials are, as the zymosan material, essentially polypeptides[16]. However, they differ from the zymosan material in being more soluble at acid *p*H. They are similar so far as they all exhibit in aqueous solution a bluish fluorescence in ultra-violet light. It is tempting to speculate that the fluorescent substance is required for biological activity and that this substance is the same or a closely related substance to those present in the materials prepared from liver and blood.

The reported biological properties of the polypeptide material prepared from zymosan indicate that it increases the resistance of the animal both against bacterial and viral challenge. It is thought that this might be caused by a general cellular effect. The effect on the mitotic activity of HeLa cells provides an example of the cellular effect of the material on one particular kind of cells.

The substance in zymosan responsible for its biological properties is still a question of disagreement. Heller[19] reported to have isolated from zymosan a non-toxic lipid which stimulated the reticuloendothelial system whereas Riggi and di Luzio[20] claim that the glucan part of zymosan is responsible for the stimulation of the reticuloendothelial system. Whether or not the water-soluble polypeptide prepared from zymosan and described in this paper is responsible for all the biological properties of zymosan cannot be concluded from the present experiment.

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ELECTRON-DONOR OR ELECTRON-ACCEPTOR PROPERTIES AND CARCINOGENIC ACTIVITY OF ORGANIC MOLECULES

THE possible correlation between the electron-donor or -acceptor capacities of aromatic molecules (as exemplified by their ionization or reduction potentials) and their carcinogenic activity was examined for the first time in 1955 [1]. We concluded that no significant correlation existed between these two types of property although it was pointed out that some restricted correlations between the electron-donor capacity and the ability to produce tumours could be observed in some limited series of active molecules. Recently the problem of the possible involvement of the electron-donor or -acceptor properties of molecules in promoting carcinogenic activity has been raised again by some authors[2–5], and we have already discussed some of these contributions[6,7]. This type of approach seems to have culminated in the very recent paper by Allison and Nash[8], who postulate that "carcinogenicity arises as a result of a suitable combination of *both* electron-donating and electron-accepting properties in a compound". The authors explicitly recognize that neither of these properties alone is sufficient by itself for carcinogenesis, because as they say "many drugs which are apparently not carcinogenic are either donors or acceptors of electrons". They consider that the combination of both electron-donating and electron-accepting properties in the same molecules is, however, rare, and that it is a particular characteristic of carcinogenic compounds.

The erroneous nature of this proposition may be demonstrated in a number of ways. Thus let us consider the aromatic polybenzenoid hydrocarbons, which not only represent one of the most important and largest group of compounds investigated for carcinogenic activity but which are also the most advantageous example, from the point of view of Allison and Nash, because, as is well known from general quantum chemistry, the electron-donor and electron-acceptor properties of these molecules run parallel, so that in this series the same molecules combine these two properties. This situation already destroys one of the arguments of Allison and Nash. In these molecules, at least, the properties of being a good donor and good acceptor always go together and are in no way an exceptional situation. Moreover, it results from this fact that if there is no correlation in this series of molecules between their carcinogenic activity and their electron-donor or their electron-acceptor capacity (and

the authors themselves admit that there is none), there cannot be either any correlation between carcinogenicity and the combination of these two properties.

That this is really the situation is obvious already from an objective examination of the data of Allison and Nash themselves. These authors base their proposition on the results of charge-transfer complex formation of carcinogenic molecules with chloranil and 1,3,5-trinitrobenzene and on the polarographic determination of the electron donating and accepting properties of these molecules. A typical situation for the fundamental unsubstituted hydrocarbons is illustrated in Table 1, extracted from these authors' data, where the energy of the charge-transfer band with chloranil, E_{CT}, is listed against the carcinogenic activity. The electron-donor properties of the hydrocarbons are considered to be the greater, the smaller E_{CT}.

Table 1. CHARGE-TRANSFER FORMATION OF AROMATIC HYDROCARBONS WITH CHLORANIL AND CARCINOGENICITY (Results of Allison and Nash)

Compound	E_{CT} (eV) chloranil	Carcinogenicity
Phenanthrene	2·72	−
Naphthalene	2·64	−
Chrysene	2·52	−
Triphenylene	2·44	−
1,2-Benzpyrene	2·18	−
1,2,7,8-Dibenzanthracene	2·18	+
1,2,5,6-Dibenzanthracene	2·16	+ +
Pyrene	2·13	−
Coronene	2·08	−
1,2-Benzanthracene	2·05	±
Anthracene	1·98	−
1,2,3,4-Dibenzpyrene	1·91	+ +
3,4,9,10-Dibenzpyrene	1·91	+ + +
3,4-Benzpyrene	1·79	+ + +
1,2-Benzperylene	1·85	± (?)
Perylene	1·83	−
3,4,8,9-Dipenzpyrene	1·82	+ + +
Naphthacene	1·67	−

The conclusion which these authors draw from such a table, namely, that as a rule the carcinogenic hydrocarbons are stronger donors than other hydrocarbons, is not justified. Thus, if the value of E_{CT} in 1,2,7,8-dibenzanthracene, a weak carcinogen, is taken as the threshold, six inactive compounds out of ten studied should have been carcinogenic. In fact, such completely inactive compounds like perylene or naphthacene are among the best donors. This situation is so striking that these authors themselves suggest that there must be other factors playing a part and responsible for these 'exceptions'. We shall discuss these other factors later. What must immediately be stressed here is that the real situation is much worse than appearing from the limited data of Table 1. Thus Allison and Nash have examined the majority of the active aromatic hydrocarbons, but only 12 out of the 32 polybenzenoid hydrocarbons known to be non-carcinogenic. Moreover, most of these 12 inactive molecules are of relatively small dimensions (composed of 4 or less fused benzene rings) and have thus *a priori* relatively low electron-donor or -acceptor properties, while the important carcinogens are of a bigger size (composed of 5 or 6 fused benzene rings).

The type of results which Allison and Nash would have obtained had they included in their investigation a larger number of non-carcinogenic molecules of dimensions comparable with those of the strongly carcinogenic ones may already be guessed from the case of naphthacene and perylene in Table 1 and is shown in more detail in Table 2. This last table represents the absolute value $|k|$ of the energy coefficients of the highest filled and the lowest empty molecular orbitals in all the polybenzenoid aromatic hydrocarbons which have been studied for carcinogenic activity. As is well known[9], these coefficients obtained by molecular orbital calculations measure, respectively, the electron-donor and the electron-acceptor properties of molecules and in the particular case of the aromatic hydrocarbons the smaller the coefficient of Table 2, the greater both the electron-donor and the electron-acceptor properties of these molecules. The reliability of these coefficients for the measurement of these properties of aromatic hydrocarbons is substantiated by the available correlations between these coefficients and ionization potentials[9–11], polarographic oxidation potentials[12], electron affinities[9,10], polarographic reduction potentials[13,14] and the wave-length of charge-transfer bands, in particular in complexes with a given acceptor (for example, refs. 15–17).

A glance at Table 2 immediately shows that if such a wider study is made, the correlation postulated by Allison and Nash vanishes completely, the carcinogenic molecules and the related non-carcinogenic ones being indistinguishable from the point of view of their electron-donor or acceptor properties.

Still restricting ourselves to the aromatic hydrocarbons, which means giving the theory of Allison and Nash its best chances, we may also present a more general criticism of their proposition. Thus, proposing that carcinogenesis is dependent on great electron-donor and -acceptor properties of molecules, leads naturally to some general predictions. One of such predictions would be that acenes, which are by far both better electron-donors and better electron-acceptors than the isomeric phenes, should also be better carcinogens. Such a prediction is in complete contradiction with the fundamental facts of carcinogenesis by aromatic molecules, where only phenes are carcinogenic and where even the presence of a small acenic fragment in a molecule is incompatible with activity[18]. Another prediction would be that large hydrocarbons should, in general, be better carcinogens than small ones, the electron-donor and -acceptor properties increasing, as a mean, with molecular size in these conjugated systems. This again is in contradiction with experiment, which shows that the activity is maximum for compounds composed of 5 or 6 fused benzene rings. If steric effects may surely be invoked to explain the inactivity of bigger molecules or of substituted derivatives containing long substituent chains[19], they cannot account for the difference in activity among the hydrocarbons composed of 5 and 6 fused benzene rings, where the active and inactive molecules are frequently of a very comparable size and shape. Finally, a third prediction which may naturally be made on the basis of Allison and Nash's general postulate is that, say, the different methylated derivatives of the same hydrocarbon (for example, the different methylbenzanthracenes) should all behave similarly from the point of view of their carcinogenic

Table 2. ELECTRON-DONOR (OR ACCEPTOR) PROPERTIES OF HYDROCARBONS AS MEASURED BY THE ABSOLUTE VALUE OF THE COEFFICIENT $|k|$ OF THEIR HIGHEST FILLED (OR LOWEST EMPTY) MOLECULAR ORBITAL

Compound	$\|k\|$	Carcinogenicity	Compound	$\|k\|$	Carcinogenicity	Compound	$\|k\|$	Carcinogenicity
Benzene	1	−	1,2,5,6-Dibenzanthracene	0·473	+ +	1,2,3,4-Dibenznaphthacene	0·356	−
Triphenylene	0·684	−	1,2-Benzanthracene	0·452	±	2,3,8,9-Dibenzperylene	0·356	−
Napthalene	0·618	−	Pyrene	0·445	−	2′,1′-Anthra-1,2-anthracene	0·348	−
Phenanthrene	0·605	−	1,2-Benzperylene	0·439	−	Perylene	0·347	−
3,4-Benzphenanthrene	0·566	+	Pentaphene	0·437	−	3,4,9,10-Dibenzpyrene	0·342	+ + +
1,2,6,7-Dibenzpyrene	0·555	−	2,3,5,6-Dibenzphenanthrene	0·419	−	1,2-Benznaphthacene	0·327	−
1,2,5,6-Dibenzphenanthrene	0·550	+	Anthracene	0·414	−	3,4,8,9-Dibenzpyrene	0·303	+ + +
Coronene	0·539	−	2,3,7,8-Dibenzphenanthrene	0·405	−	2′,3′-Naphtho-3,4-pyrene	0·303	−
3,4,5,6-Dibenzphenanthrene	0·535	−	1,2,3,4-Dibenzpyrene	0·398	+ +	Naphthacene	0·295	−
1,2,3,4-Dibenzphenanthrene	0·532	+	4,5,10,11-Di(1′,2′-naphtho)-chrysene	0·383	−	Anthanthrene	0·291	−
Chrysene	0·520	−	3,4-Benzpyrene	0·371	+ + +	3,4,9,10-Di(2′,3′-naphtho)-pyrene	0·273	−
Picene	0·501	−	1,2,9,10-Dibenznaphthacene	0·361	−	Pentacene	0·220	−
1,2,3,4-Dibenzanthracene	0·499	−	1′,2′-Anthra-1,2-anthracene	0·360	−	Naphthodianthrene	0·177	−
1,2-Benzpyrene	0·497	−	1,2,7,8-Dibenznaphthacene	0·358	−			
1,2,7,8-Dibenzanthracene	0·492	+						

potency, because they all have rather similar electron-donor or -acceptor properties (a fact verified by the proper results of Allison and Nash). Such a prediction would again be in disagreement with experiment, the monomethylbenzanthracenes ranging from inactive to quite powerful carcinogens.

This last remark brings us to discuss the other groups of molecules quoted by Allison and Nash in support of their theory.

In these cases again there seems to be no contact between the very data presented by these authors and their interpretation of them. Thus Allison and Nash state that their results indicate that the carcinogenic aromatic amines are better electron donors than the non-carcinogenic ones. In fact, nothing of this kind is apparent from their data. They have studied 14 carcinogenic aromatic amines and only 5 non-carcinogenic ones. Even so, not the slightest distinction can be made between the electron-donor properties of these two groups of molecules. The carcinogenic and non-carcinogenic aromatic amines are intertwined in an indistinguishable way. In fact, some of the non-carcinogenic ones are better electron-donors than the carcinogenic ones. The same situation obviously prevails also, as can be seen in the very numerical data given by these authors (and in contrast to their interpretation of them), in the group of heterocyclic-*N*-compounds. Finally, in the groups of steroids the authors observe and consider it significant that only œstrogens, which are the most carcinogenic among the steroids, are relatively good electron-donors. To this it may, however, be objected that they seem to be particularly bad electron acceptors[20]. Now it is the combination of both properties that Allison and Nash consider as important for carcinogenesis. If one of these properties alone is sufficient, this could be equally well electron acceptance, a property particularly outstanding, among the steroids, in the adrenocortical hormones[20]. Moreover, although apparently devoid of electron-donor properties[8] and possessing only moderate electron-acceptor properties[20], the androgens are not without carcinogenic potentialities[21].

It seems therefore obvious that no correlations can be claimed to exist between the carcinogenic activity of organic molecules and their electron-donor or electron-acceptor properties or the combination of both. The results of Allison and Nash, when looked at objectively, preclude the establishment of any such correlations. In the rare cases where some doubts could exist about this situation a study of some supplementary compounds can settle the problem quickly. This state of affairs does not preclude, of course, that some forms of electron-transfer reactions may play a part in the interaction between the carcinogens and their tissue receptors. There is even some evidence that such may be the case[6,22]. The involvement of such a transfer in carcinogenesis is, however, a secondary feature subject to the fulfilment of other primary conditions which, for example, in the aromatic hydrocarbons, are based on the characteristics and probably the reactivities of the *K* and *L* regions[1,18]. Statements, such as the following, found in a recent publication[23]: "Because polynuclear aromatic hydrocarbons are devoid of polar functions, such as —OH or —NH_2 groups, the only possible bonding of such compounds to biological systems is that of charge-transfer complex formation", are entirely unwarranted and ignore the numerous chemical potentialities of such molecules. These molecules undergo many chemical reactions on their *K* and *L* regions or at their reactive carbons and there is no apparent reason why the same active centres should not be involved in their biochemical reactions. The work of Heidelberger *et al.*[24] shows that they are so involved.

One of the temptations which probably induce some authors to develop theories of the type of that proposed by Allison and Nash is that such theories have the apparent attraction of being susceptible to offer a unique concept for the mode of action of the different types of known chemical carcinogens, a pretention which the *K–L* regions theory has not. In this respect it may, however, be remarked that while most probably the final intimate unknown event representing carcinogenesis is common to all carcinogens, it is, on the contrary, quite improbable that a unique type of "a reaction" and thus a unique characteristic could be responsible for the activity of the different types of carcinogens. This conclusion is substantiated by the multitude of different processes by which these different types of molecule may interact with probable cellular receptors such as, for example, the nucleic acids[9,25,26] and also by the results of the recent studies on the mechanism of action of the related mutagens, which show that the molecular event representing a mutation, and which most probably consist of the perturbation of the normal sequence of the complementary base-pairs of the nucleic acids, may be brought about by a variety of different types of reaction occurring between the mutagens and their target, at different sites of the target.

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In our papers we were careful not to criticize the Pullmans' work since we believe that it is not only permissible, but desirable, that alternative approaches be tried to the elucidation of carcinogenic mechanisms, about which so little is known. In particular, theoretical and experimental approaches to the physico-chemical problems involved are complementary, and each has something to offer the other. However, the attack which the Pullmans have delivered on our paper compels us to defend our hypothesis and compare it with theirs.

In assessing the Pullmans' arguments it must be borne in mind that the quantum-mechanical calculations they make involve a series of approximations. As Szent-

Gyorgyi has put it[1]: "the k values obtained depend on the parameters used, which may be subject to change. As mentioned before, experience and intuition are needed in this line, which also means that there is no numerical rigidity. Another, even more serious, shortcoming is that the k values of substances belonging to different chemical series cannot be strictly compared, since both α and β [the 'Coulomb integral' and 'exchange integral' of the method] may vary in different chemical series by as much as 25%. If the numerical value of α is around 6 eV, 25% means 1·5 eV, more than the total energy of biological oxidation". It may be added that the differences with which we are concerned seldom exceed 1·5 eV. Thus, what the Pullmans claim to be "measurements" of k values are merely subjective estimates which have some measure of reliability in the case of unsubstituted hydrocarbons (though absolute values for electron-donating or -accepting energies still have to come from experiments), but become progressively more uncertain in substituted and heterocyclic compounds. Fortunately, it is now possible to supplement such estimates with measurements of electron-donating and electron-accepting capacities of aromatic hydrocarbons and other compounds by several methods, which give very similar results. Sometimes these results have been found to differ markedly from those calculated theoretically, for example, in the case of electron acceptance by tropylium and pyridinium ions[2,3] and electron donation by chlorpromazine[4,5]. Hence theoretical calculations are no substitute for experimental results of the kind which we presented.

Our main point is that a wide range of chemical carcinogens, other than alkylating agents, are powerful electron donors and are also electron acceptors. The Pullmans have not attempted to deny this interesting fact. Nor can it be supposed that these properties recur in many other classes of organic compounds. We have been looking for compounds having the right electronic properties, lipid solubility, and stability under physiological conditions, and have found very few; suggestions from readers as to suitable compounds would, in fact, be welcome. However, it is also true, as we were careful to point out, that some compounds related to carcinogens have similar electronic properties but are inactive in biological tests. We offered three explanations why this might be so: first, that steric factors could prevent charge transfer with the relevant biological constituents; secondly, that the electron distribution could be unfavourable for such charge transfer; and thirdly, that the carcinogenic compounds could be more stable and less easily metabolized to inactive products than the non-carcinogenic compounds.

Most of the points raised by the Pullmans were explicitly and fully discussed in our papers, so there is no need to recapitulate the arguments. We find ourselves unable to agree with any of the new points made by the Pullmans:

(1) While it is unquestionably true that examination of unsubstituted polybenzenoid hydrocarbons gives the K–L region hypothesis its greatest chance of success (the calculations being less uncertain than for other compounds), this is by no means true of our hypothesis. Indeed, the whole point of our hypothesis is its applicability to a wide range of compounds of different kinds.

(2) The Pullmans state that electron-donor and electron-acceptor properties increase with increasing size in polycyclic aromatic hydrocarbons. This is by no means true, as our results (and even the k values in their Table 2) show. To take a few examples among many, chrysene (4 rings), pyrene (4 rings), 1,2-benzyprene (5 rings) and coronene (7 rings) are weaker donors than anthracene (3 rings).

(3) According to the Pullmans, in aromatic polybenzenoid hydrocarbons electron-donor and electron-acceptor properties always run in parallel. However, the experimental results presented in Table 1 of our second paper show that in all such compounds tested electron-donating capacity is much greater than electron-accepting capacity. While in the unsubstituted compounds parallelism exists in the sense that stronger donors are also stronger acceptors, this relationship is broken down in substituted compounds, as Streitweiser and Schwager have shown[6]. Our own results demonstrate, for example, that 9,10-dimethyl-1,2-benzanthracene is a stronger donor but a weaker acceptor than 1,2,5,6-dibenzanthracene. We found monomethyl-benzanthracenes to have very similar electron-donating capacities, but in measurements of electron-accepting capacity Pointeau[7] has found differences from which he concludes: "On constate immediatement qu'a l'exception du 5-Me et pour les deux premières vagues, les composés les plus concérigènes possèdent des potentiels de réduction plus bas que les autres".

(4) The Pullmans concede that steric effects can be invoked to explain the inactivity of the large hydrocarbons and of derivatives containing long substituent chains. This, incidentally, removes several of the compounds with low k values from their Table 2. However, the Pullmans feel that steric factors cannot account for differences in carcinogenicity of methyl-derivatives of 1,2-benzanthracene, or among hydrocarbons composed of 5 or 6 fused benzene rings "where the active and inactive molecules are frequently of a very comparable size and shape". In other words, substitution of a methyl group here or there, or even a benzene ring here or there, makes very little difference to the shape of a molecule. Such statements seem to have little contact with chemical or biological reality. Numerous examples could be quoted of small substitutions in hormones or drugs having profound effects on biological activity. With carcinogens there is somewhat greater latitude for modification, but obviously this has limits. Although Huggins and Yang's[8] frame must be deepened in order to accommodate 9,10-dimethyl-1,2-benzanthracene and œstrogenic steroids, which are non-planar, it serves to remind us that only certain configurations are permissible in carcinogens, which might have been expected *a priori*. Many non-carcinogenic hydrocarbons with low k values might be excluded on this basis alone.

(5) The Pullmans overlook our argument about the stability of hydrocarbons under physiological conditions. As we mentioned, polarographic experiments show an interesting feature that could not have been predicted theoretically, namely, that in some series of compounds, only carcinogens were found to undergo single-electron oxidation. This is true of 3,4-benzpyrene (using the classical terminology) whereas pyrene itself was oxidized by a two-electron step and 1,2-benzpyrene by a three-electron step, with the formation of insoluble products. Anthracene and 1,2-benzanthracene undergo multi-electron oxidation whereas carcinogenic benzanthracene derivatives do not. Thus the products of oxidation of carcinogens are likely to be more stable than those of non-carcinogens. Other such differences were found among amines and will be discussed elsewhere. With regard to acenes, we are not aware of any experiments showing that they are "far better electron donors and electron acceptors than the isomeric phenes" but if this is so it is highly improbable that they would be stable in the body.

(6) The Pullmans apparently agree with our statement that among steroids only œstrogens are strong electron donors, but they ignore our experimental results showing that solvated œstrogens (including stilbœstrol) are easily measurable electron acceptors. As we said, the only steroids that are efficient carcinogens are œstrogens, which reproducibly induce tumours in a variety of experimental situations[9]. This is certainly not true of the androgens[9]. The alleged carcinogenic effects of testosterone in the paper quoted by the Pullmans[10] are based on trivial differences from controls which do not even approach statistical significance at the 5 per cent level. If the androgens are carcinogenic at all, the fact that high doses were used for

long periods makes it difficult to exclude the possibility that the small effects observed are due to metabolites.

(7) The Pullmans' statement about heterocyclic *N*-compounds seems to arise from their failure to appreciate that such compounds, and also amines, are better electron donors as measured polarographically than is evident from charge-transfer absorption spectra (Fig. 1 and Tables). We can only repeat that the carcinogenic dimethylbenzacridine was a stronger electron donor than the non-carcinogenic acridine and benzacridine, and that the highly carcinogenic benzpyridocarbazoles were among the strongest donors in all the compounds tested (Table 1 of the second paper). Although carbazole was also a strong donor, it had no measurable acceptor property and is in any case known to be rapidly metabolized. The case of indole, also a strong donor, illustrates the difficulties that can arise when attempting to make any simple comparison of chemical and biological properties. The experiments of Ehrhart *et al.*[11] demonstrate at an acceptable level of significance the carcinogenicity of indole. However, since certain metabolites of indole, notably 3-hydroxy-anthranilic acid, are themselves carcinogenic, they might be responsible for the effects observed with indole itself. Some amines are activated by metabolic changes such as *N*-hydroxylation, and inactivated by other metabolic changes. Hence rejection of our hypothesis out of hand because the *prima facie* correlations between electronic properties and carcinogenicity are imperfect shows lack of appreciation of the complexity of the biological situation.

(8) We believe that our hypothesis was well worth developing. The Pullmans merely recapitulate what they have already written many times. There are difficulties about the *K–L* region hypothesis (for example, the difference in carcinogenicity of 3- and 4-fluoro-substituted 10-methyl-1,2-benzanthracenes[12]) which seem to imply that it requires still further qualification. There is danger in stagnation; we have attempted to let in some fresh air. Others had previously considered separately the electron-donating and electron-accepting properties of certain carcinogens. We showed that this view could be generalized to bring out common features in carcinogens of very different chemical structure (hydrocarbons, steroids, aromatic amines and heterocyclic *N*-compounds). We believe that our hypothesis is more useful than the Pullmans' because it is at once more specific and more general: more specific because it is based on particular and objectively measurable properties of molecules and not mathematical abstractions; and more general because it is applicable without any *ad hoc* assumptions to a wide range of compounds. Furthermore, our hypothesis has greater predictive value, both in suggesting new carcinogens and in suggesting specific possibilities as to modes of action rather than the "numerous chemical potentialities of the *k* region" which the Pullmans seem unwilling to specify more precisely. Our hypothesis could change the emphasis of research in this field. Until now it has been customary to examine molecules to ascertain what makes them carcinogenic. We think that the problem should be reversed, examining other compounds with the right electronic properties in order to ascertain why they are not carcinogenic: and this suggests numerous experiments.

(9) The Pullmans criticize us for attempting to offer a unique concept for the mode of action of different types of chemical carcinogens. They fail to notice that we explicitly excluded alkylating agents, so that in fact we offered two independent carcinogenic mechanisms. When virtually nothing is known about the mechanisms involved, it seems to us common sense to follow one or two likely leads and see how much can be explained on this basis. We make no apology for adhering to William of Ockham's precept. The analogy with mutagenesis is more pertinent than the Pullmans realize. It is now generally accepted that mutagenesis can occur in one of two ways: by substitution or chemical modification of a nucleotide base (through alkylation, de-amination, etc.), or by intercalation of a molecule of a mutagen such as proflavine between two base pairs, so that during replication the base sequence is changed. Charge-transfer is almost certainly involved in the latter mechanism, which is in fact very similar to our 'sandwich' model.

A new phase of experimental work on chemical carcinogenesis is beginning, and it will soon become apparent whether the Pullmans' hypothesis, or ours, or something quite different, proves more useful in predicting and explaining new observations.

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TRANSVERSE SWELLING AND SHRINKAGE OF SOFTWOOD TRACHEIDS

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THE aqueous swelling and subsequent shrinkage during drying of softwood tracheids in their pulped state is of basic importance to an understanding of the relationship between fibre morphology and properties, and technologically is of interest in the elucidation of the properties of paper made from them.

This subject has been covered adequately by macroscopic techniques, for example, by the estimation of the specific volume of swollen pulps from measurements of hydrodynamic properties[1] and by the measurement of the retention of water by pulps subjected to centrifugation[2]. Little direct microscopic evidence is available, however, and only in the work of Weidner[3] can reference be found to the measurement of dimensional changes of paper-making fibres during drying. The theory put forward by us for the shrinkage, structure and properties of paper[4], based largely on microscopic observation of paper structure and structural changes during drying and tensile straining.

has required confirmation by measurements of tracheid shrinkage and this has led to the work described here.

A few tracheids were collected from aqueous suspension on a wire mesh and microscopic observation was carried out on regions of tracheids lying over the grid openings. Micrographs were taken of the tracheids in their wet state, after drying to equilibrium with 20 per cent relative humidity and again after re-wetting. Due to their natural irregularity along their length, the shrinkage of the tracheids was measured by planimetry of areas of known lengths. A typical series of micrographs is shown in Fig. 1. Only commercial pulps which had been once dried were used, to ensure that dimensional changes caused by the initial collapse of the cell were eliminated. Results obtained from two pulps, a Norway spruce, sulphite pulped, and a Scots pine, sulphate pulped, at various degrees of beating are given in Fig. 2.

It should be borne in mind that the tracheids, being collapsed and ribbon-like in cross-section, tend to position themselves on the grid in such a way that their largest cross-sectional dimension is measured and therefore these results apply only to that dimension.

There are three aspects of interest arising from the results:

(*a*) Although the mean values of shrinkage are in the region of 20–25 per cent of the original tracheid width, individual values ranged from about 5 to 50 per cent, the standard deviation being about 10 per cent. This wide distribution of values does not appear to arise from experimental error and must be accepted as a real indication of the changes in width of tracheids dried under these conditions. This implies that the loss of thermodynamically bound water is not the sole factor in determining tracheid shrinkage and that other mechanisms, such as the loss of capillary water with collapse of the capillaries or the rearrangement of the cellulosic structure under the internal tension, must contribute.

(*b*) The irreversibility of swelling is appreciable, only about 70 per cent of the shrinkage being recovered on re-wetting. This is a finding of importance to the structural theory of paper[4], which states that the irreversibility of shrinkage of paper on re-wetting is attributable largely to the irreversibility of tracheid shrinkage.

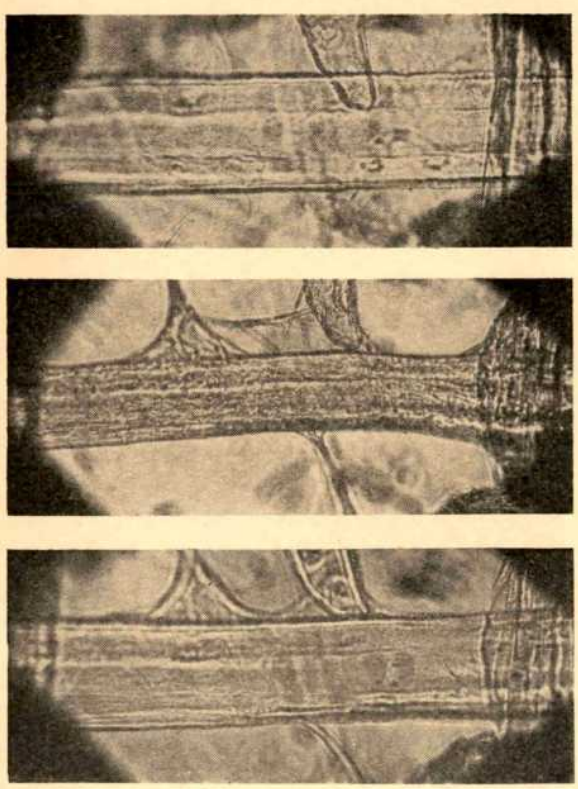


Fig. 1. Scots pine tracheid in the wet, dried and re-wetted state (× 300)

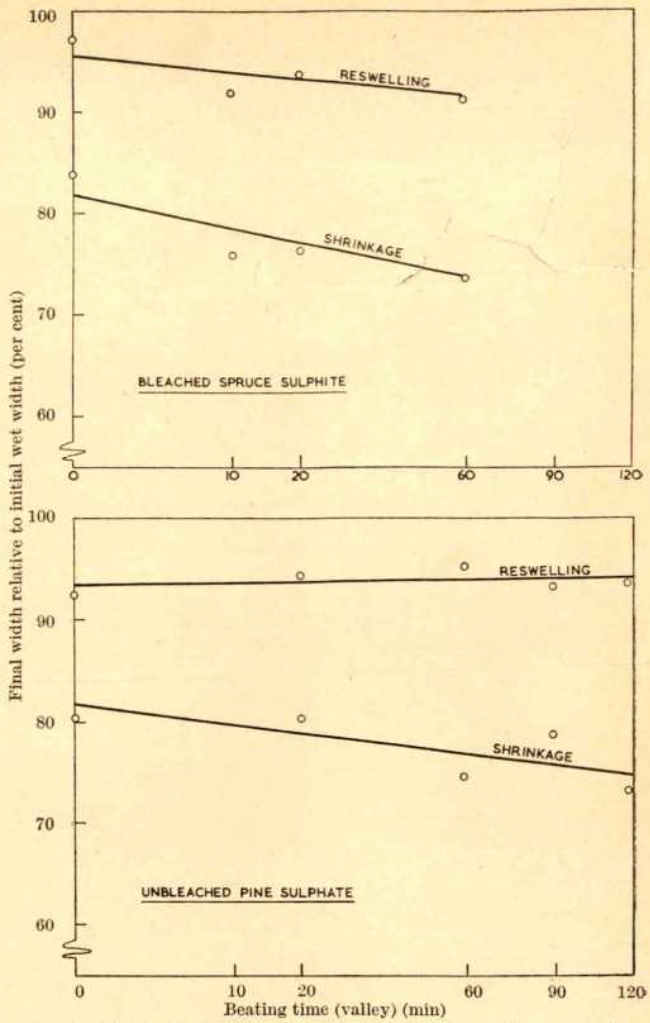


Fig. 2. Effect of beating on the transverse shrinkage and recovery of tracheids. Each point is the mean of measurements on about 15 tracheids

(*c*) The shrinkage is larger for the pulps in the more heavily beaten state. (The results are adequate for both pulps to give statistical confidence to this statement at the 1 per cent level.)

This is a finding of considerable importance technologically and yet is in opposition to the work of Weidner, who found the reverse effect. We believe that the discrepancy can be explained from the difference in experimental conditions between Weidner's work and our own. In his apparatus tracheids were dried on to glass slides. It is now known that the adhesion of fibres to glass during drying precedes shrinkage[4] and will restrict it. Furthermore the areas over which adhesion occurs is considerably larger at higher beating times[5]. These effects would combine to give an apparent decrease in shrinkage for the higher beaten pulp. In our work the use of a wire mesh circumvented this difficulty.

We thank Miss I. Emery for carrying out the measurements and Dr. J. P. Weidner for a private communication in which he agrees with our explanation of the aforementioned discrepancy.

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LETTERS TO THE EDITOR

ASTRONOMY

Terrestrial Oceanic Ridges and Lunar Mare Ridges

OCEANIC ridges show striking similarities to lunar wrinkle, or mare, ridges. The following points of comparison may be noted: (*a*) Both the terrestrial and lunar ridges are serpentine and furcate in places. (*b*) The smoothed form of the profiles of oceanic ridges[1] and lunar wrinkle ridges[2] is similar. (*c*) Central rifts characterizing parts of the oceanic system of ridges are also found in several mare ridges. The discovery of rifts capping the lunar ridges was made recently by Kuiper[3] and further announcements have been made by me[4] and by Arthur[5]. (*d*) In those instances where oceanic, and mare, ridges abut on the terrestrial, and lunar, continents, respectively, related lineaments continue the general trend for some distance into the continent in question. (*e*) Some of the terrestrial ridges terminate by wedging out and others end in major strike-slip faults[6]. Precisely these properties describe the terminal stages of lunar wrinkle ridges. For example, the furcating wrinkle ridge in Sinus Aestuum slices the Apennines in strike-slip faults as far as the ruined ring Marco Polo.

Set against these points of similarity, it must be noted that the actual linear dimensions of typically well-developed mare ridges are but one-tenth of those of the oceanic ridges, and the same scaling factor applies to the widths of the rifts in the ridges. (The depths of the lunar rifts have not been measured. These rifts should not be confused with rilles, of known depth, which are also associated with mare ridges.)

The strike-frequency diagram[7] for the segments of lunar wrinkle ridges suggests quite strongly that many of these ridges originated in concealed strike-slip faults. Others, particularly those with a north-to-south trend, probably arose in tension fractures. The anomalously high frequency of occurrence of small craters perched on the crests of the lunar ridges, and their relatively high albedo[8], support the hypothesis that wrinkle ridges are extrusion phenomena. The flows evidently solidified before travelling more than about 10 km across the Moon's surface.

If, as I believe, wrinkle ridges are more recent than the fractures that controlled their gestation, offsets would not be well preserved in the presently observed topography because of the obscuring effects of the extruded lavas. Offsets are still preserved, however, in the lunar continents, where masking and re-moulding through extrusion have been less.

Strike-slip faults that are transverse to the terrestrial oceanic ridges are now well known, yet no longitudinal strike-slip faults have been reported. By analogy with wrinkle ridges, there may be lava flows covering any independent topography on the ocean floors; but longitudinal offsets could be looked for via magnetic anomaly charts. This is, of course, not easy because the anomalies tend to parallel the ridges[9]. On the other hand no one, to my knowledge, has looked for offsets indicating movement along an oceanic rift. Discovery of such movement would bring the analogy between lunar and terrestrial ridges still closer, and would indicate where selenology might supplement geological work.

The analogy may not be complete. Oceanic ridges are now widely explained on the basis of convection currents in the mantle. The currents are rising beneath the ridges, carrying up heat and tearing them apart laterally. Are we to believe that the complex of lunar wrinkle ridges, connecting as they do with the low walls of ghost craters in maria, were all raised by convection currents?

There is no evidence for recent continental drift on the Moon. Indeed, there is a marked uniformity of faulting in set directions[10], and this speaks against very large-scale movements. The Russian photographs of the averted hemisphere of the Moon demonstrate the asymmetric distribution of the lunar maria and continents; and I (ref. 11) have shown that this picture has remained unchanged since Mesozoic time, when the maria were formed. The continental drift theory of the Earth would bring the Afro-Asian countries and the Americas together into one land mass at essentially the same time.

This result may be explained if both the Earth and Moon were heated up by the decay of the long-lived radioactive isotopes. Whereas the lunar continents have not moved appreciably since the Mesozoic, however, the terrestrial continents have moved apart since that time. It would therefore appear that the history of the heating process has been different in the Earth and Moon and, specifically, that the pattern of convection in the two planets has been of a different order—at least in recent times—as Runcorn[12–14] has maintained. It is likely that, in the case of the Earth, complex, high-order patterns of convection have set in, but that such a state of development had not been realized in the Moon.

The wrinkle ridges may possibly be associated with local currents arising during the cooling of the mare lavas; this might help to explain their concentric arrangement in some maria; but, as there is evidence for their association with strike-slip faults, I feel that it would be important to investigate oceanic ridges bearing in mind the possibility of associated longitudinal strike-slip faults rather than purely tensile fractures.

I thank Imperial Chemical Industries, Ltd., for a research fellowship.

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Radio-carbon Dates and the Mayan Correlation Problem

IN two papers published in 1956 and 1960[1], Linton Satterthwaite, jun., presented his interpretation of radio-carbon dates obtained from wood from Temples I and IV and Structure 10 at Tikal, Guatemala. He concluded that the Goodman–Thompson correlation of the Mayan and Christian calendars, or a variant thereof, should be accepted. In this communication, I propose to point out certain weaknesses in his basic assumptions, and to offer alternatives which, in my opinion, are as likely to be valid as his.

To simplify matters for the reader, I shall elect to refer only to the 1960 paper. In this, with Dr. Elizabeth Ralph, Satterthwaite presented radio-carbon dates obtained at the

University of Pennsylvania for samples of wood taken from ten beams in Temple IV and six beams of Temple I, and offered for comparison radio-carbon dates obtained at other institutions. For the ten beams in Temple IV dated at the University of Pennsylvania, an average date of A.D. 743 ± 48 y. is offered. This may be compared with a date of A.D. 751 May for 9–16–0–0–0 according to the Goodman–Thompson correlation. All dates given in this note are in the Julian Calendar.

Satterthwaite quotes four dates obtained at other institutions for Temple IV and Structure 10, the latter assumed to be within ten years of the former, an assumption I am willing to accept. They are, with one Pennsylvania date added, as follows:

			A.D.	
1951	Kulp *et al.*	Columbia University	481 ± 120	weight 3
1954	Libby	University of Chicago	433 ± 170	,, 2
1954	Libby	University of Chicago	469 ± 60	,, 6
1958	Broeckway *et al.*	Columbia University	456 ± 60	,, 6
1960	Ralph	University of Pennsylvania	465 ± 85	,, 4

The final date listed is one associated with the University of Pennsylvania sample number 229, which Satterthwaite elected to discard, saying the beam from which it came must be "an older re-used beam". He did not offer any evidence in favour of this assumption, no carving left over from a previous use, only the fact that it appeared to be older.

The weights I have assigned above to the five dates are approximately inversely proportional to the standard deviations of the dates. With them, one finds a weighted average of the five dates, A.D. 463 ± 10 y. Here, the standard deviation is a measure only of the scatter of the five values used. The average date obtained may be compared with dates as follows: 473 A.D., March 19 for 9–16–0–0–0 according to the Smiley correlation[2], 490, November 4 for the Makemson correlation[3] and 491, July 8 for the Spinden correlation[4].

I now suggest that the earlier date, A.D. 463, is the one which should be assigned to Temple IV and Structure 10 at Tikal, and that the University of Pennsylvania date, A.D. 743, represents beams which were installed at a later date approximately 280 years (perhaps 260 years, or five calendar rounds) after the original construction. Satterthwaite himself directs attention (p. 177) to damage to beams due to termites. If the University of Pennsylvania samples were carefully chosen from the best-preserved beams in the temples, they might easily have been taken from beams which had replaced earlier ones in the original structure. If the Mayans considered zapote (sapodilla) wood to be termite-resistant, it would explain why at least one old zapote beam remained in the structure, and other beams, perhaps of other wood less resistant to termites, had been replaced by zapote beams.

There is, of course, a weakness in the radio-carbon approach to the problem in that the presence of a date on a beam does not assure one that the wood belongs to that epoch. The beam might be a part of a re-used beam, or it might be of wood that was cut early and allowed to season before use, or it might represent timber that was later cut and carved to replace a beam damaged by termites, fire, storms, or other ravages of time. There is also the question as to whether the dates, when carved on beams, represented predictions for the future, or records of observations made in the past. I am inclined to accept the latter point of view, but this matter is not important here.

It now appears that the matter of the correlation of the Mayan and Christian calendars will be settled by astronomical means, perhaps with help from dendrochronology.

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METEOROLOGY

Atmospheric Hydrogen Sulphide Concentrations during the London Fog of December 1962

Very few figures have been published concerning the hydrogen sulphide content of the atmosphere. Information on this subject was required in connexion with investigations on the tarnishing of silver, and determinations have been made at the Post Office Research Station, London, N.W.2, at about weekly intervals for the past 2·5 years. The method used was to suck, by means of a small electric pump, a measured volume of pre-filtered air through filter paper impregnated with silver nitrate and to compare the resultant silver sulphide stain with standards. The site is on a hill 240 ft. above sea-level in north-west suburban London. Immediate surroundings are residential and open space with typical suburban mixed residential, light industrial, railway and power station development beyond.

The range of results may be broadly summarized as follows:

Season and weather	Approximate hydrogen sulphide content of atmosphere Parts per thousand million by volume (p.p.t.m.)
Summer, very clear, fresh conditions with wind from a westerly quarter	0·1 or less
Summer, average conditions	0·1–0·5
Winter, average conditions	0·5–1
Winter, fog; drift of air from the East	1–5

These figures are only intended to give some indication of the values generally to be expected under given conditions since many factors, including, in particular, wind direction and velocity, exert a great influence. An easterly wind brings polluted air from London to the site, whereas wind from a westerly quarter has passed over regions much less built up and industrialized and the latter is therefore associated with lower hydrogen sulphide contents.

During the foggy weather early in December 1962 measurements of atmospheric hydrogen sulphide were made with an automatic apparatus that had just become available.

Table 1

Date 1962	Time of start of test	Appearance of fog	Wind strength	Direction shown by wind-vane	Hydrogen sulphide content of atmosphere (p.p.t.m.)
Dec. 6	10.40	Thick	Calm	E	11
	11.12	,,	,,	,,	7·5
	11.44	,,	,,	,,	5
	12.16	,,	,,	,,	5
	12.48	,,	,,	,,	7·5
	13.20	,,	,,	,,	10
	13·52	,,	,,	,,	8·5
	14.24	,,	,,	,,	9·5
	14.56	,,	,,	,,	9·5
	15.28	,,	,,	,,	10
	16.00	,,	,,		15
	16.32	,,	,,		20
	17.04	,,	,,		25
	17.36	,,	,,		27
	18.08	,,	,,		33
	18.40	,,	,,		15
Dec. 7	09.33	Slight—moderate	,,	S	7
	10.05	Misty; weak sunshine	,,	,,	5·5
	10.37	,, ,,	,,	,,	4·5
	11.09	,, ,,	,,	,,	4
	11.41	,, ,,	,,	,,	4·5
	12.12	,, ,,	,,	,,	3·5
	12.44	Sunshine	,,	,,	4·5
	13.16	Slight fog	,,	,,	2·5
	13.48	Misty; cloudy	,,	S.W.	2·5
	14.20	Clear; cloudy	Very light breeze	,,	1·5
	14.52	,, ,,	Light breeze	,,	1·3
	15.24	,, ,,	,, ,,	,,	1·0
Dec. 10	10.53	,, sunshine	Moderate breeze	N.W.	0·25
	13.54	,, ,,	,, ,,	,,	0·25

The principle used was the same as in the method for intermittent determinations previously mentioned, the stains being formed on a strip of impregnated paper which was automatically advanced at the end of each test period. Tests, each lasting 32 min, followed each other without intervals on December 6, 1962, a day of continuous thick fog, several preceding days also having been calm and foggy. 32-min tests were continued on December 7 when the fog was clearing; but on December 10, after a week-end of strong westerly winds, when a moderate north-west breeze was still blowing, it became necessary to extend the test periods to 3 h to obtain stains of measurable intensity.

Although it remained apparently calm near ground-level on December 6, the rise in atmospheric hydrogen sulphide between 15·28 and 18·08 may have been caused by a slight air drift from the south-east bringing more heavily polluted air from the central London area, this being indicated by the extension of the area of dense fog in a north-westerly direction from this Station during the period concerned. Some air movement occurred during the night of December 6–7, altering the direction of the wind-vane on top of the building from east to south, reducing the fog density, and lowering the hydrogen sulphide content of the atmosphere. The time of the change in direction is unknown, but it might well have been associated with the marked fall in hydrogen sulphide that occurred at 18.40 on December 6. The wind-vane was invisible at this time.

The morning of December 7 remained calm and after an initial fall in hydrogen sulphide corresponding to the clearing of the fog the content was substantially constant until the afternoon when a light south-west breeze caused progressive reduction. The strong westerly winds of the week-end December 8–9, continuing as a moderate north-west breeze on December 10, resulted in lower atmospheric hydrogen sulphide contents than usual for the time of the year.

The figures for the period 16.00 onwards on December 6 are the highest ever recorded in our tests. Previously, the maximum observed at this Station was 5 p.p.t.m., although in certain other areas subject to heavy, specific, industrial pollution, values up to 10 p.p.t.m. have occasionally been found under normal weather conditions.

I thank the Engineer-in-Chief of the Post Office for permission to use the information contained in this communication.

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Ice Nucleation by Metaldehyde

During recent years ice-nucleation by some organic substances has been investigated with increasing interest in fundamental, as well as in practical, fields. Some steroids can nucleate ice crystals at temperatures as high as − 1° C when prepared by thermal recrystallization[1,2]. One of the amino-acids[3] is reported to nucleate ice at −4·5° C. Although many of them have complicated molecular and crystal structures and are not common, they may be useful in understanding heterogeneous ice-nucleation. For application to cloud-seeding, phloroglucinol[4], α-phenazine[5] and a few others have been found among the common organic compounds to overcome the faults of silver iodide as a practical seeding material, such as photolytic inactivation and high price. The wide variety of organic compounds gives some hope of finding improved cloud-seeding materials.

Metaldehyde $(CH_3CHO)_4$, a cyclic tetramer of acetaldehyde, was recently found to have remarkable properties in ice-nucleation, producing ice-crystals at temperatures as high as −0·4° C under suitable conditions.

Ice-nucleating ability depended on the method of preparation, dispersal and particle size.

Prepared by recrystallization from ethanolic solution by cooling, the crystals were ground and dispersed into the air. After sedimentation, those smaller than 13μ in diameter were collected on an inactivated metal tray and cooled on the top of an aluminium plate in a cold chamber. The temperature of the plate was measured by a copper-constantan thermocouple. Water vapour was supplied for 10 min and the crystals which had formed were detected by the addition of a supercooled aqueous solution of polyvinyl alcohol (1 or 5 per cent, depending on the temperature), after which they grew to visible size. 1 g of crystals treated in this way produced 10^4 ice crystals at −0·6° C. At −2° C a large proportion of the crushed crystals (10^6/g) was observed to nucleate ice. For comparison, the onset of ice-formation of ground silver iodide using the same method was −4° C, and of ground α-phenazine −3·5° C.

10^4 ice-crystals were generated at −0·4° C from 1 g of sublimed metaldehyde crystals. At −10° C the sublimed metaldehyde crystals gave as many nuclei per gram as silver iodide, when detected in a cold chamber with a supercooled 5 per cent solution of polyvinyl alcohol at the bottom, but the ice-crystals were produced more slowly. The soap film technique[6] yielded slightly different temperatures for the onset of nucleation by crushed crystals between − 1° and −2° C. For nucleation at warm temperature, metaldehyde therefore seems to be preferable to silver iodide.

It is known that silver iodide smoke released in the atmosphere decays due to the action of sunlight. This is important in cloud-seeding applications, so that the solar effect on metaldehyde was tested. Ground particles on the metal tray were placed in sunlight on a fine day in April, in Sydney, for 2 h and tested at −2° C, and no significant change of ability to nucleate was observed. However, when they were placed under the sunlight for more than 1 h at a temperature higher than 55° C, the nucleating property disappeared completely. Metaldehyde sublimes at 112° C but decomposes partially at more than 80° C giving paraldehyde, a cyclic trimer of acetaldehyde. Therefore the observed inactivation could be either evaporation or decomposition. As long as the nuclei were kept under 33° C for less than 2 h the activity at −2° C remained unchanged. It therefore seems that the use of metaldehyde in cloud-seeding operations should be seriously considered, for it is both more efficient and more economical at the temperatures where natural ice nuclei are very rare.

A possible reason for its nucleating ability is suggested by its molecular and crystal structure, determined by Pauling and Carpenter[7]. The tetragonal crystal of metaldehyde recrystallized from absolute alcohol has a body-centred unit of structure with $a_0 = 10{\cdot}40$ Å and $c_0 = 4{\cdot}11$ Å containing two molecules of the tetramer

Table 1

Metaldehyde	Ice					
Plane	Orientation *A*		Orientation *B*		Orientation *C**	
	Plane	Metaldehyde molecules matching to ice	Plane	Metaldehyde molecules matching to ice	Plane	Metaldehyde molecules matching to ice
(110)	(0001)	½	(0001)	½	$(10\bar{1}0)$	½
$(1\bar{1}0)$	$(01\bar{1}0)$	½	$(1\bar{2}10)$	½	$(\bar{1}2\bar{1}2)$	½
(001)	$(\bar{2}110)$	1	$(10\bar{1}0)$	½	$(1\bar{2}16)$	½
Direction	Misfit		Misfit		Misfit	
$[1\bar{1}0]$	6·1%		8·7%		4·7%	
[110]	0·2%		0·2%		6·1%	
[001]	9·1%		5·0%		5·0%	

* $< (\bar{1}2\bar{1}2) - (1\bar{2}16) = 87°$.

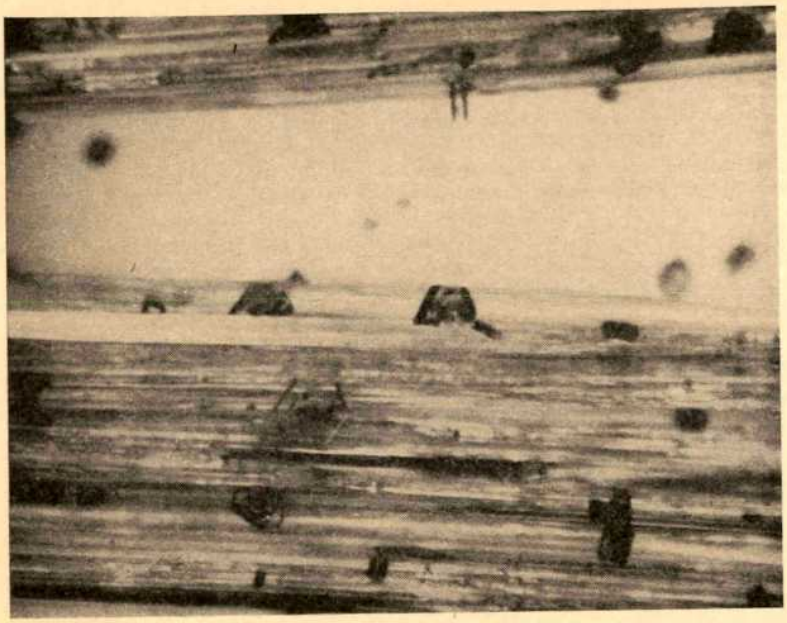


Fig. 1. Ice crystals growing on the prism plane of metaldehyde at −15° C

$(CH_3CHO)_4$. It has a pronounced prismatic cleavage due to its dipolar molecular structure and the resulting planes are parallel to its c-axis. For a tetragonal unit $(\sqrt{2}\,a_0)^2 \times 2c_0$, taken from the metaldehyde lattice, three possible orientations of ice were estimated to match in three directions of the unit, [1$\bar{1}$0], [110] and c-axis with reasonable misfits (Table 1). Observation with a microscope of ice nucleation on metaldehyde crystals obtained from ethanolic solution showed that these three orientations appeared very often; however, some others were also seen. Fig. 1 shows some epitaxial growth of ice on long needles of metaldehyde crystals at −15° C. A smooth prism plane gave few nucleation sites and ice crystals were more likely to be observed at the large steps and crushed parts. Although ice-formation by sublimation was easily seen at these temperatures, freezing of condensed water was predominant at higher temperatures.

I thank Dr. E. K. Bigg for suggestions on ice-crystal detection and the Commonwealth Scientific and Industrial Research Organization for a fellowship which enabled this work to be carried out while on leave from the Chemistry Department, Nagoya University, Japan.

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PHYSICS

Units in Vacuum Measurements

Along with the growing unification of the various branches of physics there has been a trend toward unifying our systems of units. Originally the various branches developed systems of units peculiar to each; but as the branches have overlapped and merged some special units have been discarded in favour of those related in a simple way to the units selected as fundamental. The joule, for example, is beginning to replace the calorie as a heat unit, and meteorologists are changing from the inch of mercury to the bar for barometric pressures.

In the light of this general trend, one may ask why we continue in vacuum work to use the millimetre of mercury, and the closely related torr, which are not simply related to the fundamental units, but are specific to the field of pressure measurements. Why do we not use the dyne/cm², for example? There is a historical reason, of course. Early measurements of pressure were made with the mercury manometer, which is a reliable absolute instrument, or with instruments calibrated against it. For pressures too low for the simple manometer we have the McLeod gauge, which is still an absolute instrument. Although we now have ionization gauges to measure pressures which the McLeod gauge cannot measure, these gauges are not absolute, and we calibrate them against the McLeod gauge. It is quite natural, then, to express their calibrations in the same units as we use for the standard gauges. The typical user of one of these ionization gauges may not be impressed by this argument, however, since he may never have occasion to calibrate his gauge against a manometer, in which case the millimetre of mercury loses its special claim.

These arguments are something of a digression from the main question, however. There is a more fundamental point to raise about vacuum units. Let us consider what vacuum gauges actually measure. A mercury manometer measures pressure directly. However, an ionization gauge counts a sample of ionized molecules, so in the operational sense it measures molecular density. Although in most circumstances prevailing in vacuum work molecular density and pressure are simply proportional, we can show from the ideal gas law that the general relation is $n \sim P/T$. This has been discussed in more detail elsewhere[1]. It is sufficient here to point out that an ionization gauge does not measure pressure directly. It should also be pointed out that whereas molecular density has a clear and unequivocal meaning at any pressure, the term 'pressure' begins to lose its conventional meaning somewhere in the very high vacuum region.

Perhaps we are in our present state of confusion on these matters because ionization gauges are calibrated against pressure gauges. If we could construct an ionization gauge of absolutely known sampling efficiency it would be an absolute gauge. It might then be clearer that the gauge counts molecules, rather than measuring pressure. There would then be little argument against calibrating it to read in molecules/cm³, or some such unit. From the fact that at a pressure of 760 torr there are $6{\cdot}02 \times 10^{23}$ molecules/mole, it is a straightforward calculation to show that 1·00 molecule/cm³ corresponds to a 'pressure' of $2{\cdot}83 \times 10^{-17}$ torr, or, in the mks system, 1·00 molecule/m³ corresponds to $2{\cdot}83 \times 10^{-23}$ torr.

Because of the wide range of densities encountered in vacuum work these days, a further change is worth considering. In vacuum work one really thinks more in logarithmic than in linear terms. A constant-speed pump reduces the pressure in an ideal chamber at a rate proportional to e^{-at}, which plots as a straight line on a log pressure versus time plot. There are now available on the market electrometers and gauge controls with logarithmic scales covering as many as six decades. Would it not be reasonable to have a logarithmic density unit, much like the chemists' pH scale? If the new unit were to be defined as the logarithm of the number of molecules per m³, the zero would be at 1·00 molecule/m³, or $2{\cdot}83 \times 10^{-23}$ torr. A pressure of $1{\cdot}00 \times 10^{-6}$ torr would correspond to a

density of 16·55 logarithmic units. With the suggested choice of a zero point, it is not likely that we would be using negative logarithms very much. Indicators with a logarithmic response could use a simple linear scale on the indicating meter, and linear paper could be used on recorders. The indicator could be made to take care of varying gauge constants by incorporating a zero-suppression circuit.

With these advantages, there is one disadvantage of a logarithmic unit that must be admitted. In residual gas analysis it would not be convenient to calculate total densities from partial densities in logarithmic units. Whether or not this would be a serious drawback depends on how important such calculations are relative to the entire vacuum field. It might be desirable to have both a logarithmic and a linear unit of density. Whichever the choice, of either or both, it seems that the basic vacuum unit in high-vacuum work should be one of molecular density, rather than pressure.

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* Operated with support from the U.S. Advanced Research Projects Agency.

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Inhomogeneous Problems in Elasticity or Heat Conduction

N. LOUAT[1] has recently described a method of solving two-dimensional problems in elasticity by the replacement of free surfaces by two mutually independent arrays of edge dislocations chosen so as to produce zero stress at each free surface. M. A. Jaswon[2] has described a similar procedure in which prescribed boundary displacements may be achieved by means of boundary distributions of point forces chosen to satisfy a set of Fredholm integral equations. The attraction of each method lies in the fact that the effect of a point force within an infinite medium may be calculated from a simple formula. Furthermore, the use of digital computer techniques allows the superposition of a large number of such forces.

It may be worth while to note that the technique of summation of the effect of point forces is also a useful one for treatment of inhomogeneous problems in elasticity or heat conduction. Such problems, although of considerable practical importance, have received little attention in the literature.

Suppose that a solution of the equations of elasticity has been obtained for homogeneous elastic material subject to given boundary conditions, boundary forces, and body forces. Let v_1, v_2, v_3 denote the components of the displacement at a general point, let e_{ij} denote $\frac{1}{2}(\partial v_i/\partial x_j + \partial v_j/\partial x_i)$, and let θ denote $e_{11}+e_{22}+e_{33}$. It may be shown that if the elastic parameters λ and μ suffer a small change from constant values of λ_0 and μ_0 to become: $\lambda = \lambda_0 + l(x_1,x_2,x_3)$, $\mu = \mu_0 + m(x_1,x_2,x_3)$, then each displacement v_1,v_2,v_3 is increased by an amount w_1,w_2,w_3 which may be found as the result of applying to the homogeneous material an internal force distribution of components[3]:

$$g_i = 2\sum_j \partial(me_{ij})/\partial x_j + \partial(l\theta)/\partial x_i \qquad (1)$$

The resulting stress distribution may readily be found in terms of the total displacement $v_i + w_i$.

The form of equation (1) is interesting in that it may be expressed as a sum of some terms such as $e_{ij}\partial m/\partial x_j$ which become large when m changes abruptly, and some terms such as $m\partial e_{ij}/\partial x_j$ which become large whenever m differs from zero in a region of high stress. Thus, if an elastic material contains intrusions of material of different elastic constants the effect of the intrusions is equivalent to a set of forces distributed over their surfaces and another set distributed throughout their volume. In many instances this second set of forces is small so that the intrusions may be replaced by forces on their surfaces.

The analysis outlined above is not restricted to two-dimensional problems.

A similar treatment may be given of the equation of heat conduction through inhomogeneous material. The equation of heat conduction in a solid is:

$$\nabla k.\nabla T + k\nabla^2 T = g\partial T/\partial t \qquad (2)$$

where T is the temperature, k is the conductivity, and $g = \rho c$, where ρ is the density and c is the specific heat. Suppose that for constant parameters k_0 and g_0 a solution is $T_0(x,y,z,t)$. If the parameters k and g change to become: $k = k_0 + Ak_1(x,y,z)$, $g = g_0 + Ag_1(x,y,z)$, then equation (2) may be satisfied by a solution $T(x,y,z,t)$ of the form:

$$T(x,y,z,t) = \sum_{n=0}^{\infty} A^n T_n(x,y,z,t)$$

where the T_n are found in succession as solutions of the equations:

$$k_0\nabla^2 T_n - g_0\partial T_n/\partial t = g_1\partial T_{n-1}/\partial t - \nabla(k_1\nabla T_{n-1}) \qquad (3)$$

The constant A is chosen to specify the scale of the inhomogeneities and has no other physical significance.

Each of equations (3) represents a temperature distribution T_n in a homogeneous material that contains a distribution of heat sources or sinks corresponding to the terms on the right-hand side. Thus, the temperature distribution within inhomogeneous material may be investigated in terms of that within homogeneous material containing a suitable distribution of heat sources.

An advantage of the form of equations (1) and (3) is that it is not necessary to choose a co-ordinate system to fit the shape of the inhomogeneities; thus the technique may be applied to treat the effect of irregularities of general shape. Some preliminary investigation has shown that there are many instances where use of equations (3) leads to a solution the terms of which may be found by algebraic recurrence relations. The technique is now being applied to several problems of practical interest.

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Use of Long Cavities for Aqueous Samples in an Electron Spin Resonance Spectrometer

IN a comment on the electron spin resonance spectrometer designed especially for aqueous samples which has been described recently by Cook and Mallard[1], Stoodley[2] has calculated that the method of using a multimode microwave cavity tends to degrade the sensitivity rather than improve it. I would like to point out that support for Stoodley's result is provided by a spectrum recorded by Varian Associates on their aqueous sample spectrometer and published in some recent advertising material[3]. The signal-to-noise for this spectrum is some thirty times better than for that presented by Cook and Mallard. Further, although the sensitivity was said to be comparable with that obtained by Commoner *et al.*[4], in fact these workers claim a figure of 2·5 to 5×10^{-7} molar of Mn^{++} while that of Cook and Mallard is 3×10^{-6} molar. The sensitivity of the Varian spectrometer expressed in these terms is 1×10^{-7} molar. The difference in sensitivity between that achieved by Cook and Mallard and by Varian can be attributed to three causes. First, sensitivity is proportional to the square root of the microwave power and, while Varian quote 180 mW, Cook and Mallard appear to be using a 723 *A/B* klystron which gives 20 mW. Hence the sensitivity drops by a factor of three. Secondly,

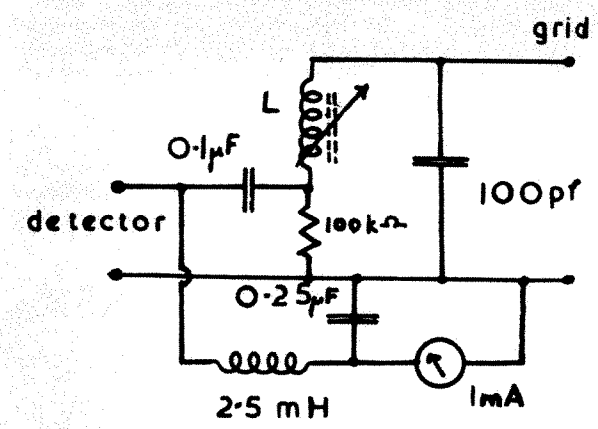


Fig. 1. Improved input circuit for cascode or pentode pre-amplifier giving a near-ideal noise figure (L_1 = 1 mH, one section of 'Radiospares' 465 kc/s tuned transformer)

according to Stoodley, the cavity design should give a sensitivity which is 4·4 times worse than optimum. The remaining factor of 2·2 is due to deficiencies in the pre-amplifier used by Cook and Mallard.

The pre-amplifier, which uses the cascode circuit, can be criticized in two respects. First, when a cascode is used as a radiofrequency amplifier it should be operated with an anode load resistance of the same order as the anode slope resistance of the valve[5] (10 $K\Omega$). To use a higher value results in an increase in gain from approximately μ to μ^2, where μ is the amplification factor of the valve, but also causes the gain of the first stage to rise from unity to μ, thus causing instability and failure to realize the original idea behind the cascode circuit. The design published by Ingram[6] is also subject to this criticism. In both cases a resistance of the order of 10 $K\Omega$ should be shunted across the output tuned circuit which otherwise would typically have an effective resistance of the order of 300 $K\Omega$. The other, equally serious, criticism is that Cook and Mallard have not matched the input of the amplifier to the crystal. A complete treatment of this point is given by Moxon[7], and a practical input circuit following Moxon's analysis is shown in Fig. 1. Assuming an equivalent noise resistance of 600 Ω for the 12AT7 and a crystal impedance of 400 Ω and neglecting regeneration, the calculated noise figure for Cook and Mallard's circuit should be 4 db. With matching applied and assuming a Q factor of 100 and a capacity of 100 pF, this figure is reduced to 0·35 db for the cascode, and 0·5 db for an EF89 pentode with an equivalent noise resistance of 1 $K\Omega$. Thus on theoretical grounds there is no objection to the cascode circuit provided that the output is suitably damped and an input matching circuit is used. It should be noted, however, that a conventional pentode circuit gives a noise figure which is also nearly ideal and no significant advantage is obtained by using the cascode at such low frequencies (465 kc/s).

In support of these calculations the appropriate circuits have been constructed and noise figures measured for a source impedance of 400 Ω. The results are quoted below.

Cook and Mallard's circuit as published	7 db
Cook and Mallard's circuit with input match	1 db
EF89 pentode with input match	1 db

The first of these results is worse than that calculated and may be explained as being due to regeneration. The others agree within experimental error.

One remaining comment is that in Cook and Mallard's circuit there is a 10 $K\Omega$ resistor in series with the d.c. path of the crystal. It is generally recognized that this deteriorates the sensitivity of a crystal detector and the resistor should therefore be replaced by a choke. Cook and Mallard's remark that choke decoupling caused oscillation confirms the view that the circuit as presented is inherently unstable. A choke could have been used satisfactorily if the output had been suitably loaded.

It may therefore be concluded that the sensitivity of the spectrometer described by Cook and Mallard can be improved by reverting to the conventional cavity design described by Stoodley and used by Varian and also by adopting the input circuit indicated in the figure for the pre-amplifier. Further, no deterioration in sensitivity will result if the cascode circuit is discarded in favour of a conventional pentode.

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GEOLOGY

High-temperature Minerals in Non-metamorphosed Sediments in Israel

An unusual mineral assemblage was recently found in a sedimentary rock sequence of Maastrichtian to Palæocene age, the so-called 'Mottled Zone', in Israel and adjoining parts of Jordan[1]. This formation is known to occur in at least four widely separated places: the Hatrurim basin west of the Dead Sea, the Beersheba valley in the northern Negev, the Ramlah area in the coastal plain, and at Ma'aleh Adumim in the Jordanian part of the Judean desert.

The most characteristic rock of this formation is largely composed of calcite and spurrite, the latter locally dominant, and minor amounts of pyrite, apatite, spinel, a dicalcium silicate, gehlenite, and a mineral identical with, or closely related to, brownmillerite. (We thank Mr. E. Sass, of the Hebrew University, Jerusalem, who helped in the determination of this mineral.) This rock is cut by numerous small veinlets filled by rare minerals, including bayerite, vaterite, portlandite, hydrated calcium silicates of the tobermorite group, and thaumasite. Ettringite is sufficiently abundant to be considered rock forming. These minerals are associated with calcite, aragonite, gypsum, hæmatite, and many others at present under investigation.

Anhydrous calcium silicates, such as spurrite, are so far known only from limestones, contact-metamorphosed by igneous intrusions. The accompanying hydrous calcium silicates are generally explained by the action of hydrothermal solutions on the high-temperature silicates. Mineral assemblages of this kind occur in the classic localities of Crestmore, California[2] (gehlenite, ettringite, thaumasite, tobermorites); Scawt Hill, Northern Ireland[3,4] (gehlenite, spurrite, larnite, ettringite, portlandite); and Ballycragy, Northern Ireland[5,6] (vaterite, larnite, tobermorites, thaumasite). Ettringite was recently also found in the high-temperature zinc deposit of Franklin, New Jersey[7]; its type locality is Ettringen, Germany[8], where it occurs together with portlandite. Thaumasite is known also in magmatic sulphides (Sweden, Norway, Utah) and in basic lavas (New Jersey, Hungary, Northern Ireland)[9]. Portlandite has been reported from calcareous xenoliths on Mount Vesuvius[10]. Brownmillerite has hitherto not been found in nature, but is an essential constituent of Portland cement clinker. Thus, the mineral assemblage discussed here occurs either in magmatic rocks or in highly metamorphosed sediments of the sanidinite facies; but more than any other natural occurrence it resembles Portland cement and its alteration products.

The unique feature of the 'Mottled Zone' mineralization is the occurrence of high-temperature minerals and of secondary minerals derived from them in a sedimentary sequence unaffected by either contact or regional metamorphism. Contact metamorphism is excluded by field evidence, as well as by the constant stratigraphic position of these deposits at various places; the tectonic position of the formation in areas where folding is absent or very slight excludes regional metamorphism. Moreover, the calcite–spurrite formation in one of the localities here described, though heavily eroded, extends over an area of 5 × 8 km and reaches a thickness of up to 80 m, in contrast with the occurrence so far known where rocks carrying these minerals are generally confined to narrow contact zones.

The mechanism by which these minerals developed in the sedimentary environment of the 'Mottled Zone' is now being examined. It is tentatively suggested that the energy required for the formation of the high-temperature silicates was in this case supplied not externally from magmatic sources, but internally by subsurface oxidation of organic matter and sulphides, both of which were abundant in the original rock. This process might be compared to the spontaneous combustion of artificial slag heaps, also known as coal tips.

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Ngurumanite, a New Hypabyssal Alkaline Rock from Kenya

In June 1959, E. P. Saggerson and B. H. Baker of the Geological Survey of Kenya discovered a number of small alkaline intrusions in upper Tertiary lavas on the Nguruman Escarpment, which forms the western wall of the Rift Valley about seventy miles south west of Nairobi. During a detailed examination of these intrusions in 1960, we found the alkaline rocks to be emplaced in a flow of ankaratrite (biotite-olivine melanephelinite), that is evidently intercalated in more common olivine basalts. The intrusions, which are probably of upper Tertiary age, are closely controlled by two north–south vertical fault zones in the ankaratrite. Narrow dykes feed sills about 2 ft. thick and these thin rapidly into an intricate network of veins.

The medium- to coarse-grained intrusive rocks consist largely of pyroxene and altered nepheline and are thus somewhat similar to many melteigites: they differ radically, however, in containing an iron-rich mesostasis and numerous vug-like segregations of zeolites and calcite. The combination of these unusual features has led us to propose a new rock name, 'ngurumanite', given after the Nguruman Escarpment where the rock was discovered.

Although most of the ngurumanite is distinctly red-brown, thin green veins of similar composition also occur, field relationships showing that the greenish intrusions were emplaced after red varieties. The close spatial relationship and the similarity in mineral and chemical composition of the ngurumanites and host ankaratrite strongly suggest that all the rocks were derived from a common magma and that ngurumanite can be considered a hypabyssal equivalent of the ankaratrite. Lacroix has described other hypabyssal equivalents of ankaratrites, though the Kenya rocks lack the felspar, biotite, hornblende and olivine of tamaraïte[1] and felspar, biotite and olivine of fasinite[2].

A full description of the rocks and their petrogenetic significance will appear elsewhere. Meanwhile, we thank the Commissioner of Mines and Geology, Kenya, for permission to publish this article.

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GEOCHEMISTRY

Comparative Uranium and Thorium Analyses of Basic and Ultra-basic Rocks

Recently a number of new analyses using neutron activation and isotope dilution techniques have been reported for uranium and thorium in certain basic rocks (that is, eclogites) and ultra-basic rocks (that is, peridotites and dunites) which are variously considered to represent materials forming the Earth's upper mantle[1,2]. In view of the significance of these results with respect to the radioactive generation of heat in the upper mantle, it is of some importance to establish the interrelation of the results reported by the various workers interested in the problem of the determination of low-level uranium and thorium abundances in rocks.

Tilton *et al.* have variously reported uranium abundances (using both isotope dilution and neutron activation techniques) and thorium abundances (using only isotope dilution techniques) in both basic and ultra-basic rocks[3–4]. Through the courtesy of Dr. Tilton and Dr. Reed aliquots of the samples of four of the rocks analysed by them were made available to us for the simultaneous determination of uranium and thorium by a neutron activation technique described elsewhere[5]. Of the four rocks analysed here, two are of basic composition (the Hualalai basalt and the eclogite from Salt Lake Crater) and two are of ultra-basic composition (the Twin Sisters dunite and the Gila dunite nodule).

Basalt, Hualalai, Hawaii. Probably as a result of the relatively high levels of uranium and thorium existing in this rock, together with the high state of homogeneity of the sample, there is extremely good agreement between values reported previously and our results.

Pyroxene-rich Eclogite, Salt Lake Crater, Hawaii. Tilton and Reed[2] have reported that the rock used for this sample was contaminated with carbonate. However, they removed this carbonate by leaching the rock powder in hot 4 N hydrochloric acid for 30 min and determined uranium in the leachings as well as uranium and thorium in the leached rock. The rock samples analysed in the work recorded here were similarly leached with hot 4 N hydrochloric acid, and 4·8 and 4·0 per cent respectively of each sample were found to be soluble. The thorium content of the leachings was determined but the uranium analyses were unfortunately lost. The uranium content of the leached rock was found to be identical with that reported by Tilton and Reed, but our thorium figure is some 26 per cent higher than their value. We have previously analysed an olivine eclogite nodule (*R*419) also from the Salt Lake Crater and found 0·042 p.p.m. uranium and 0·10 p.p.m. thorium[1]. At that time it was pointed out that the thorium/uranium ratio for this oceanic eclogite was 2·4 and lower than the range for eclogites

Table 1. Comparative Uranium and Thorium Analyses of Basic and Ultra-basic Rocks

Rock	Locality	Sample	Uranium (p.p.m.)			Thorium (p.p.m.)		Th/U
			Isotope dilution	Neutron activation		Isotope dilution	Neutron activation	This work
			Previous work	Previous work	This work[e]	Previous work	This work[e]	
Basalt	Hualalai, Hawaii	Total rock	0·50[a]	0·46[b]	0·455 ± 0·012 0·453 ± 0·012	1·60[d]	1·59 ± 0·04 1·62 ± 0·04	3·5
Pyroxene-rich eclogite nodule	Salt Lake Crater, Oahu	Soluble in dilute HCl (μg/g rock) Total rock minus HCl-soluble fraction	0·015[a] 0·030[a]	— —	— 0·0303 ± 0·0007 0·0297 ± 0·0008	— 0·102[a]	0·00549 ± 0·00016 0·129 ± 0·003 —	0·37 4·3
Dunite nodule	Gila, Arizona	Total rock	0·003[a] 0·005[a]	0·01[a]	0·00414 ± 0·00020 0·00505 ± 0·00023	—	0·0115 ± 0·0006 0·0147 ± 0·0005	2·8 2·9
Dunite	Twin Sisters, Washington	Total rock	0·016[a]	< 0·0004[a] 0·0012[b] 0·0008[c]	0·00127 ± 0·00050 0·00757 ± 0·00055	0·05[d]	0·00102 ± 0·00042 0·00535 ± 0·00351	0·80 0·71

[a] Tilton and Reed (ref. 2). [b] Hamaguchi, Reed and Turkevich (ref. 3). [c] Goles (personal communication). [d] Tilton (ref. 4). [e] Precisions calculated from counting statistics.

from deep-seated pipes in the continental regions (3·5–5·8)[1]. The ratio for the carbonate-contaminated rock (using Tilton and Reed's analyses) is also 2·4 but the ratio for the leached rock (on our analyses) is 4·2, which is identical to the average ratio calculated previously for the continental pipe eclogites. It may well be that some carbonate contamination in our previous sample *R*419 reduced the thorium/uranium ratio to the low value observed, since (on the basis of Tilton and Reed's uranium and our thorium analyses) the thorium/uranium ratio of the leachings is only 0·37.

Dunite, Gila, Arizona. The previously reported analyses for uranium are unusual in that the activation results are 2–3 times larger than the isotope dilution results. Our uranium determinations show a relatively small variability (suggesting sample homogeneity) and are consistent with the lower isotope dilution results. Our thorium results also show little variation.

The rock is a dunite nodule associated with basaltic rocks in Arizona and is of interest because it has been suggested that these nodules are derived from upper mantle material[6]. The thorium/uranium ratios for the samples are 2·8 and 2·9 and are close to the range for upper mantle materials discussed elsewhere[1].

Dunite, Twin Sisters, Washington, United States. The previously reported uranium results show more than an order of magnitude variation between isotope dilution and activation methods and more than a factor of three variation between different samples analysed by the same activation method (Table 1). Dr. G. Goles has kindly supplied us with a previously unpublished uranium analysis for the same rock sample using another activation technique, and his result agrees quite well with the highest activation values reported by Tilton and Reed. The two uranium determinations from this investigation show an unprecedented variability with the lower value close to the highest activation results reported previously while the other value is some six times larger. It is significant that the thorium values for each sample (which are both very much less than a previous isotope dilution result) show a corresponding variability so that the thorium/uranium ratios on each sample are 0·8 and 0·7, respectively. This would seem to constitute good evidence that the variability is real and is a result of inhomogeneities in the original sample. In view of the variations observed, it is difficult to assign meaningful uranium and thorium abundances to this rock; but it does seem as if the isotope dilution results reported previously are too high.

The Twin Sisters dunite is generally regarded as an alpine-type peridotite which may represent upper mantle material which has been intruded into the crust during an orogenic episode[7]. However, the very low thorium/uranium ratio in the rock is far outside the range calculated on other evidence for possible upper mantle material. This does not rule out an upper mantle origin for this peridotite but it would seem to indicate that if it is so derived then it must have had its original thorium/uranium ratio modified during or subsequent to its intrusion into the crust.

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CHEMISTRY

Evidence for New Phase occurring in Portland Cements

The X-ray powder diffraction patterns normally obtained from Portland cements contain only lines which can with confidence be said to be due to the presence of the four major phases, alite (modified C_3S), C_2S, C_3A^*, a ferrite phase in the solid solution series C_6A_2F–C_4AF–C_2F and minor constituents such as CaO, MgO and $CaSO_4$. Recently, however, I have obtained from a few cements diffraction patterns in which a number of unusual lines were present. The new lines, from their position and intensity, appear to be produced by material having a structure closely similar to perovskite, that is, orthorhombic, pseudo-cubic, $a' = 3{\cdot}8$ Å. This material cannot be true perovskite ($CaO.TiO_2$) because the TiO_2 content of the cements is too small (Table 1). The structure of C_3A bears some resemblance to that of perovskite, being cubic with a cell-side four times that of the pseudo-cell of perovskite, and careful consideration of the intensities of reflexions suggests that these cements may contain little or no C_3A and that it has been replaced by a similar phase with the lower symmetry of perovskite.

One possibility is that the new phase is NC_8A_3, described by Brownmiller and Bogue[1], or KC_8A_3, described by Suzukawa[2], or more likely, since the two compounds are isomorphous, a solid solution $\binom{N}{K}C_8A_3$; but for the available alumina to be combined as this compound rather than C_3A, nearly all the alkali in the cements would be required. The usual view[3] is that the alkali in the cements is preferentially combined as Na_2SO_4 and K_2SO_4

and the remainder as $KC_{23}S_{12}$ and NC_8A_3, and it is perhaps significant that in the three clinkers, A, B and C, the SO_3 content is low and the alkali may thus have been free to form $\binom{N}{K}C_8A_3$.

Unfortunately, chemical analyses of residues which have been enriched in the new phase by selective hydrolysis show that, while in the two cements *B* and *E* rather more than half of the Na_2O and of the K_2O is retained in the new phase, in cement *D* much less, about 15 per cent, is retained, the quantities in this case being similar to those retained in normal C_3A in other cements. It seems, therefore, that sodium and potassium are not the only substituting ions which will produce the new phase. A large number of cations have been shown to be capable of replacing calcium or titanium in perovskite and giving either cubic or pseudo-cubic structures; in particular, replacements of the type $A^{II}(B^{II}_x B^{III}_y B^{V}_z)O_{2\cdot3-3\cdot0}$, where $x+y+z=1$, have been reported[4], which suggests possibilities such as $CaO(MgO_xAl_2O_{3y}P_2O_{5z})$ in cement.

The only unusual feature which the five cements have in common is a rather high MgO content; but as some MgO is detectable as periclase in the residues from selective hydrolysis, the presence of MgO in the chemical analysis cannot be taken as evidence of its presence in the 'perovskite' phase.

Tentatively, therefore, I conclude that the new phase must be regarded as a modified C_3A in which additional oxides, which may be Na_2O, K_2O, MgO or even Fe_2O_3 or traces of TiO_2, have lowered the symmetry from cubic to orthorhombic.

The X-ray evidence for the existence of the new phase is presented in Table 2. This evidence could not be obtained on a direct X-ray examination of the complete cement, only the line at $d=2\cdot71$ Å being distinguishable. The process of selective hydrolysis which was evolved is carried out in two stages. Acetic acid is added slowly, while stirring, to a 1·0 per cent suspension of cement in water, to maintain *p*H 4·5–5·0 for about 10 min. The residue is filtered off, washed with methanol and dried at 105° C. This treatment breaks down the silicate phases into products that are either soluble or amorphous, and no lines for these phases are detectable in the X-ray patterns of the residues.

A similar treatment at *p*H 3·5–3·8, for about 20 min, will leave only the ferrite phase, the quartz and some of the periclase unattacked.

Table 2 gives the observed *d* values and intensities (peak heights on diffractometer chart record) for a residue from the first stage (*p*H 4·5–5·0) of selective hydrolysis of cement *E*. The third column indicates to which compound each line may be attributed, lower case letters being used when the compound has only a very weak line at any *d* value.

It will be seen that most of the lines in the pattern which are not attributable to C_4AF match the patterns of NC_8A_3, and/or perovskite. The intensity relationship of the two lines at 2·72 and 2·695 is constant in the patterns of the residues from all five cements; this suggests that they are both produced by one material not by a mixture of C_3A and another compound. If this is the case then the six strongest lines of the new phase may be regarded as a splitting of the three strongest lines of C_3A: C_3A (440) at $d=2\cdot70$ (I = 100) becomes 2·72 and 2·695; C_3A (008) at $d=1\cdot908$ (I = 32) becomes 1·920 and 1·890; C_3A (844) at $d=1\cdot558$ (I = 24) becomes 1·564 and 1·550. This is characteristic of the change from a cubic to a pseudo-cubic structure, and it seems much more likely that the cement contains one lower-symmetry polymorph of C_3A than a mixture of C_3A with a material which closely resembles it both in composition and structure.

The immediate interest in the formation of this compound is that it renders measurement of the C_3A content of cements by X-ray diffraction more difficult, but its occurrence is of wider interest in the study of the high temperature phase chemistry of cements generally.

Examination of a number of cement clinkers suggests that the X-ray pattern described above is characteristic of the 'dark prismatic interstitial phase' which has been detected in some microscopic studies of polished clinkers. No clinker so far examined has shown either phenomenon without the other.

Table 1. Minor Element Content of Five Ordinary Portland Cements Found to Contain 'Perovskite' Phase

	Cement clinkers			Cements ($CaSO_4$ added)	
	A	*B*	*C*	*D*	*E*
MgO	1·21	3·96	4·18	1·64	1·07
SO_3	0·44	0·07	0·00	2·57	2·25
Na_2O	0·30	0·80	0·50	0·10	0·21
K_2O	0·81	0·41	0·25	1·61	0·70
TiO_2	0·21	0·21	0·21	0·24	0·23
P_2O_5	0·13	0·21	0·23	0·04	0·14
Mn_2O_3	0·07	0·02	0·02	0·06	0·05

Table 2. X-Ray Powder Data for Residue from Selective Hydrolysis of Cement *E*

d (Å)	*I*		*d* (Å)	*I*	
7·62	3		2·428	4	*a n p*
7·31	4	*F*	2·398	2	
6·81	3	*a*	2·376	4	*n*
5·12	4	*a f*	2·310	3	*P*
4·42	6	*a*	2·282	3	*P*
4·217	11	*A N*	2·270	3	*N*
4·062	3	*A*	2·205	12	*A N P*
3·850	3	*P*	2·107	2	*n P*
3·694	3	*n*	2·081	1	
3·652	3·5	*F*	2·050	4 (d)	*F P*
3·427	8	*N P*	2·032	3	*N*
3·348	14	*a N P Q*	1·960	3	*a N*
3·055	7	*a N*	1·920	24	*F N P*
2·900	3	*a n P*	1·905	7	*A*
2·813	5		1·890	13	*N P*
2·773	6·5	*A F n*	1·828	4	*a F n*
2·717	47	*P*	1·750	2	*n p*
2·695	100	*A N P*	1·564	18	*N P*
2·647	18	*F*	1·550	11	*N P*
2·576	2	*a F p*	1·358	4	*N P*
			1·347	8	*A N P*
			1·214	4	*N P*

C_3A, *A a*; C_4AF, *F f*; NC_8A_3, *N n*; perovskite, *P p*; quartz, *Q*.

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* Cement chemist's notation: $C_3S = 3CaO.SiO_2$; $C_3A = 3CaO.Al_2O_3$; $C_4AF = 4CaO.Al_2O_3.Fe_2O_3$; $NC_8A_3 = Na_2O.8CaO.3Al_2O_3$; etc.

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Surface Charge Density and Sorption of Water Vapour on Lithium Kaolinite

Keenan *et al.*[1] in an examination of adsorption of water vapour on several monoionic derivatives of kaolinite from Bath, South Carolina (Peerless No. 2, Vanderbilt Co., New York), found that—hysteresis being absent for all samples—lithium kaolinite showed the lowest water adsorption at any relative vapour pressure. Prior to the adsorption measurements (at 20° C), the samples were degassed under high vacuum at 70° C. Expressing the amount of water adsorbed per unit surface, these authors found identical values at any relative pressure for two samples of lithium kaolinite with different granulometric composition. They concluded that the adsorbed lithium ions have no effect on adsorption of water, and attributed this behaviour to the small ionic size of lithium, which permits the unhydrated ion to fit into the surface structure or to penetrate in the vacant octahedrally co-ordinated sites,

thus becoming sterically hindered from interaction with water vapour.

R. T. Martin[2], however, observed hysteresis in the water adsorption–desorption cycle on lithium kaolinite, prepared from a sample of clay with the same origin and submitted to the same pretreatment as in the work of Keenan *et al.* Moreover, this author ascribed the hysteresis to ionic hydration and deduced from loop scanning experiments that the hydration of lithium ions occurred from a relative pressure of 0·8 onwards.

J. J. Jurinak[3] investigated sorption of water vapour on kaolinite from Dry Branch, Georgia (Georgia Kaolinite Co.), and observed that the lithium-saturated sample, degassed under high-vacuum, exhibited reversible adsorption–desorption when the degassing temperature did not exceed 70° C. Like Keenan, Jurinak suggested absence of ionic hydration in lithium kaolinite.

In experiments carried out by me[4] on kaolinite from Whitehill, Plymouth, England (Continental China Clay, Brussels), the characteristics of which have been described previously[5], different monoionic derivatives (H^+, Na^+, Li^+, Ca^{2+}, Mg^{2+}) were also prepared and the isotherms determined at 20° C by a high-vacuum technique similar to that used by Keenan. Not only hysteresis was exhibited by all samples over nearly the whole relative pressure-range; but, whereas the general trend in succession of the isotherms was Li^+ kaolinite $< Na^+$ kaol. $< H^+$ kaol. $< Mg^{2+}$ kaol. $< Ca^{2+}$ kaol., the amount of water retained by the lithium derivative slightly outweighed the amount retained by the sodium one up to $p/p_0 = 0{\cdot}18$ in both branches of the adsorption–desorption cycle.

This behaviour can only be explained by assuming widely differing values in the binding energies of the lithium ions to the mineral lattice, one part of ions being so loosely bound that hydration occurs from low relative pressure onwards, another part being so strongly fixed that hydration—if occurring—only takes place at pressures near saturation.

That a single cation may be fixed by a clay mineral with a wide range of bonding energies is generally accepted[6] and can be accounted for with the aid of the following considerations: (1) The charge deficiencies on the mineral surface may originate from geometrically and energetically differing levels: substitution in the tetrahedral or in the octahedral layer, broken bounds around the edges, tetrahedrically co-ordinated aluminium[7], probably around the edges too. (2) If only, or predominantly, one mode of cationic binding is displayed by a particular clay mineral, for example, isomorphic substitution, a non-homogeneous distribution of negative charges may lead to local concentrations of adsorbed cations to the extent that repulsion forces become operative between them. Although a disturbance of the electrical neutrality in some part of the lattice must have a repercussion on the whole structure it is obvious that a cation repelled from the locus of positive charge deficiency should be less tightly held. It is logically premised that the probability of heterogeneous distribution increases with increasing charge density. When the negative charges are located around the edges (as is claimed by several authors for kaolinite) a high charge density will also induce interaction between ion-lattice attraction and ionic repulsion, especially if the ratio edge surface to planar surface of the particles is small.

Applying these considerations to dehydrated Li^+-kaolinite systems, it can be conclusively stated that the geometric and energetic status of the lithium ions versus the lattice and the subsequent behaviour upon contact with water vapour are ruled by the size and shape of the mineral particles, the nature of the charge deficiencies and, to a major extent, by the charge density. The different behaviour displayed by the lithium kaolinites in three of the four cases which are referred to should be understood in these terms.

The values of the surface charge density calculated on the four samples conform to the foregoing premises.

Absence of ionic hydration, resulting from a strong and probably single-valued Li^+-lattice binding energy, is observed on samples with a surface charge density of 0·6 (ref. 3) and 1·0 (ref. 1, fractionated sample) electronic charge per $m\mu^2$ respectively. For the lithium kaolinite undergoing ionic hydration at high relative pressure (ref. 2) and for which an average weaker binding energy has to be invoked, the value amounts to 1·3 $e/m\mu^2$. Finally, the strong heterogeneous distribution of Li^+ ions displayed by the sample of reference 4 corresponds to a surface charge density as high as 1·6 $e/m\mu^2$.

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In situ Treatment of Kieselguhr for Gas Chromatography

The deleterious effects of adsorptive activity in supporting media for gas chromatography are well known; broad, long-tailed peaks are obtained, making area measurements in quantitative work more difficult, and obscuring small peaks due to impurities following a major peak. In so far as adsorptive activity may be due to metallic impurities, some amelioration is commonly effected by acid or alkali washing. However, the surface even of pure silica possesses active adsorption sites, presumably due to free OH groups, and several authors have indicated that adsorption due to this cause can be greatly reduced by treatment of the support with certain reactive silicon compounds.

The use of dimethyldichlorosilane for this purpose appears to have been first suggested in 1958 by Kwantes and Rijnders[1], and it has been used since by several other workers[2–4]. Since it is a difunctional compound, it would appear to be by no means ideal; if only one chlorine atom reacts with the surface of the support, a polar group still remains, while the reaction of both chlorine atoms requires a felicitous distribution of hydroxy groups on the original surface. Trimethylchlorosilane has been proposed for the same purpose by Kiselev[5], and would appear to be preferable, but hexamethyldisilazane, first proposed by Purnell[6], has become the most widely used reagent.

We have found that, although treatment by Purnell's method produces a marked improvement in the inertness of the column support, the friable nature of kieselguhr leads to the production of a considerable proportion of fines during the operation, the loss in weight on screening through a 100-mesh sieve amounting to 50 per cent. Moreover, fracture of the kieselguhr particles during sieving will, of course, generate new reactive surfaces.

It occurred to us that these disadvantages might be avoided by treatment of the column packing *in situ*. The procedure consists simply in injecting a small quantity of hexamethyldisilazane into the column at a temperature of 80° C, and with the carrier gas flowing at a reduced rate. A typical column 0·4 cm × 185 cm packed with 5 per cent 'Apiezon *L*' on kieselguhr requires an injection of 50 μl. of the reagent, at a carrier gas flow-rate of 5 ml./min. The reagent is eluted in about 4 min, whereupon the column is ready for use.

The effect in peak shape is illustrated in Fig. 1, which shows the peaks obtained with aniline on untreated and on treated columns.

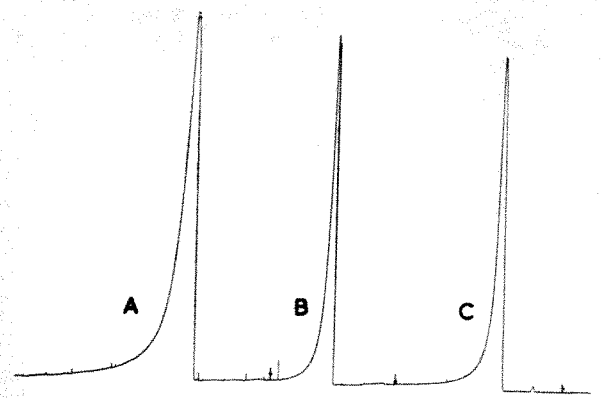


Fig. 1. Chromatograms obtained with aniline on packing prepared from (*A*), untreated 'Embacel'; (*B*), packing (*A*) treated *in situ* with hexamethyldisilazane; and (*C*), pre-treated 'Embacel'. Conditions: 0·4 cm × 185 cm column packed with 10 per cent 'Apiezon *L*' on 'Embacel', operated at 125° C with nitrogen carrier gas at flow-rate of 20 c.c./min; flame ionization detector

The superiority of the *in situ* treatment is more clearly indicated by a comparison of the ratio of peak height to width at 2 per cent of height. This ratio is 3·86 for the untreated column (*A*), 5·68 for the pre-treated column (*C*), but reaches the value of 9·18 for the column (*B*) treated *in situ*.

The *in situ* treatment of a more actively adsorbing support is even more striking in its effect. A column packed with 10 per cent 'Apiezon *L*' on 'Sterchamol' and operated under the same conditions as in Fig. 1 retained aniline and *o*-cresol indefinitely (more than 2 h); *in situ* treatment with hexamethyldisilazane allowed normal elution in 32 and 38 min respectively.

Naturally the new method can only be used when the stationary phase is itself free from polar groups, but this is not a serious limitation in practice, since adsorption by the support is less serious when a polar stationary phase is present; presumably the latter is preferentially adsorbed at the active centres.

Finally, the *in situ* use of hexamethylsilazane is also of value in reconditioning a column which has become contaminated with sample residues; such contamination often gives rise to a noisy baseline, and otherwise necessitates replacement of the column.

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Electron Affinity of Tetracyanoethylene

The compound tetracyanoethylene (TCNE) has frequently been reported to be one of the strongest known Lewis acids. We have examined the electron affinity of TCNE using a modified form of the magnetron previously described[1].

A copious ion current was observed in the temperature-range investigations (1,500°–1,750° K), and at the lower temperature this ion current was some 20 times as large as the electron current. A preliminary analysis of the results indicates that the electron affinity is in the region of 150 kcal (6·5 eV). We attribute this extremely high value to the interaction of the electron with the octupole formed by the four cyanide groups.

This result is interesting in that the value found is greater than the work function of most metals. It is, however, to be stressed that the figure reported here is only a preliminary one, although it is correct as to order of magnitude.

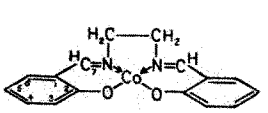
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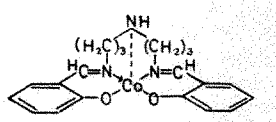
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Five-Co-ordinate Compounds of Cobalt

Most complex compounds of cobalt are of oxidation state +3 and six-co-ordinate, or of oxidation state +2 and four- or six-co-ordinate. Until recently, only a few apparently five-co-ordinate complexes had been reported[1]. These were the anions $[Co(CN)_5]^{3-}$ and $[Co(CN)_5]^{2-}$, and the oxygen-carrying Schiff's base compounds (*A*) and (*B*).



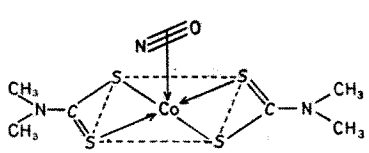
(*A*)



(*B*)

Formula (*A*) shows the planar four-co-ordinate configuration suggested by Calvin *et al.*[2], but Diehl[1] believed that the true structure was five-co-ordinate with a water molecule bridging two cobalt atoms. However, tetrahedrally disposed bonds around the bridging oxygen atom would prevent the parallel arrangement of molecules of (*A*) deduced from X-ray evidence[2]. In addition, Ueno and Martell[3a] do not report any infra-red absorptions due to water in the compound and this is supported by other investigations[3b]. There is no direct proof that the secondary amino group in (*B*) is co-ordinated to the cobalt atom. The cobaltocyanide ion, in the solid state, has been found to be dimeric, with cobalt–cobalt bonds[4], and in aqueous solution is probably $[Co(CN)_5H_2O]^{3-}$. The compound first reported to be $Ag_2Co(CN)_5$ (ref. 5) is now believed to contain bridging cyanide groups[6]. So perhaps none of these compounds contains five-co-ordinate cobalt.

Many other five-co-ordinate compounds of cobalt have now been reported, but a detailed X-ray examination has been carried out only on nitrosyl(dimethyldithiocarbonato) cobalt (I) (ref. 7), which has the square pyramidal structure (*C*) with the N–O bond inclined at 139° to the pyramidal axis and forming an unsymmetrical π-complex with the cobalt atom.



(*C*)

Other apparently five-co-ordinate compounds are: $Co^{III}(PEt_3)_2Cl_3$ (ref. 8)*, $Co^{II}MeAs[(CH_2)_3AsMe_2]_2I_2$ (ref. 9*a*)*, $Co^{II}MeAs[(CH_2)_3AsMe_2]_2Br_2$ (ref. 9*b*)*, $Co^{II}(CNR)_5^{2+}$ (ref. 10), $Co^{I}(CNR)_5^{+}$ (refs. 10, 17), $Co^{I}(PR_3)_2(CO)_3^{+}$ (ref. 11), $Co^{II}Br_2[PH(C_6H_5)_2]_3$ (ref. 12), $Co^{II}X_2(CO)(PEt_3)_2$,* $Co^{I}X_2(NO)(PEt_3)_2$,* X = F, Cl, Br[13], Co^{I}(acetylacetone)$_2$(NO) (ref. 14)*, and $[Co^{III}$(8-aminoquinoline)$_2(NO)]X_2$ (ref. 15)*. Their likely configurations are square (or tetragonal) pyramidal, and trigonal bipyramidal[16]. Compounds marked with an asterisk have been assigned pyramidal structures. No detailed account of the first compound in the list has appeared, and the last is unusual in containing cobalt (III) and co-ordinated NO^-.

Compounds such as cobalt carbonyl hydride $HCo(CO)_4$ and its methyl-substituted derivative $CH_3Co(CO)_4$ (ref. 18*a*) are formally five-co-ordinate. In the former the carbonyl groups are tetrahedrally disposed with the H atom buried[18b] in the electron density of the metal and making little contribution to the stereochemistry of the molecule. Even the methyl compound appears to have essentially a tetrahedral configuration[18a].

We have found that compound (*A*) and several substituted forms (Table 1) will form mononitrosyls. These give non-conducting solutions in nitrobenzene, and molecular weight determinations show the parent, the 5,5′-dichloro, and the 7,7-dimethyl compounds to be monomeric in nitrobenzene. They have magnetic moments of about 0·5 Bohr magnetons at room temperature, and that of the 5,5′-dinitro compound increases linearly with $\sqrt{T}$. It therefore arises from temperature independent paramagnetism, no unpaired electrons are present, and electron pairing has occurred between the cobalt (II) atom ($3d^7$) and the NO molecule. From the magnetic results the compounds may be regarded as containing cobalt (III) and co-ordinated NO^-, or cobalt (I) and co-ordinated NO^+. However, their infra-red spectra show absorptions at the lower end of the range[19] ascribed to co-ordinated NO^+ (Table 1). These compounds are isoelectronic with (*C*), which has a similar nitrosyl absorption[20] at 1,626 cm^{-1}, and we believe them to be of the same structure. The planar nature of the parent compound would favour the formation of a square or, more accurately, tetragonal structure on five co-ordination.

Temperature-independent paramagnetism of the above magnitude could contribute largely to the fractional magnetic moment of 0·87 Bohr magnetons at room temperature reported for Co(acetylacetone)$_2$ NO. It is not necessary, then, to postulate[14] an equilibrium between a diamagnetic compound containing cobalt (I) and NO^+, and a paramagnetic compound containing cobalt (II) and neutral NO. The infra-red band at 1,675 cm^{-1}, typical of co-ordinated NO^+, indicates the former structure.

Table 1

Substituent	NO stretching frequency (cm^{-1})
None	1,624
5,5′-dimethyl	1,614
7,7′-dimethyl	1,618
3,3′-dimethoxy	1,634
5,5′-dichloro	1,638
3,3′-dinitro	1,667
5,5′-dinitro	1,696

The increase in the infra-red absorption frequencies with the substitution of electron-attracting groups (Table 1) shows that electron removal from the cobalt atom increases the N–O bond order, presumably by decreasing the extent of $d_\pi - p_\pi$ double bonding from the metal to the co-ordinated nitric oxide.

If no bridging water molecule is present in the parent compound (*A*) then the pyridine adduct of (*A*), and its oxygenated form in which an oxygen molecule bridges two cobalt atoms[2], contain five-co-ordinate cobalt. The oxygenated form has been considered to contain a peroxide bridge[2], but if the axis of the oxygen molecule were at right angles to the line joining the cobalt atoms to give a π-bonded complex, as has now been found for other cobalt compounds containing peroxide bridges[21], the bonding of NO and O_2 would be analogous. There is some evidence that this type of bonding occurs in the nitric oxide compound of hæmoglobin[22] and in oxyhæmoglobin[23].

The readiness of cobalt to form five-co-ordinate compounds can be related to the tendency of the metal to achieve (cobalt (I), $3d^8$) or approach (cobalt (II), $3d^7$) the effective atomic number of the next inert gas[24].

We have found that other Schiff's base complexes of cobalt (II), including the oxygen-carrying compound (*B*), will react with NO. This is also true for similar complexes of other transition metals, and we are investigating the nature of the products.

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Thermal Decomposition of Certain Benzyl Esters

The early investigations of Hurd and Blunck[1] on the mechanism of the pyrolysis of esters have shown the existence of several decomposition pathways. In recent years, the degradation has been thoroughly examined, and the principal mechanism of the reaction has been shown to be a *cis* elimination[2]. However, at the present time, the nature of the secondary reaction mechanisms is not clearly understood.

An early investigation into the nature of these reaction pathways was reported by Hurd and Bennett[3] on the decomposition of benzyl benzoate. They indicated that the reaction affords toluene, benzaldehyde, benzoic acid, and benzoic anhydride. In recent years this ester and related compounds have been investigated by Jones and Ritchie[4]. They examined the decomposition at both 300° C and 500° C; the lower temperature pyrolysis gave results that were similar to those reported by Hurd and Bennett—however, the higher temperature pyrolysis gave complex decomposition products. Although details of the mechanism have not been postulated, both research groups have suggested that an ether intermediate may be involved in the reaction. In an attempt to isolate such an intermediate, the pyrolysis was examined at both 250° C and 675° C.

Benzyl acetate and benzhydryl acetate were pyrolysed in the following manner. The apparatus used for the decomposition consisted of an electrically heated 'Vycor' tube 28 cm long and 2 cm in diameter packed for a distance of about 20 cm with 'Pyrex' glass beads of about 6 mm diameter. The tube was heated to 675° C, and then 5 ml. of the ester was allowed to drop into the tube at a rate of about one drop per second. In the benzyl acetate experiment, the pyrolysate was analysed directly

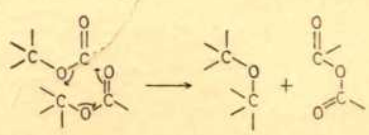
by gas chromatography. The analysis was conducted at a column temperature of 195° C, 'Apiezon *L*' as the liquid phase, and a helium flow rate of about two bubbles per second. At these conditions three peaks were recorded that had retention times of 4, 8 and 26 min. These peaks corresponded to toluene (15 per cent), benzaldehyde (23 per cent) and benzyl acetate (62 per cent), respectively. The benzhydryl acetate products were analysed by elution chromatography (alumina). This analysis disclosed the presence of diphenylmethane (0·347 g), benzhydryl acetate (2·63 g) and benzophenone (0·401 g).

In an attempt to isolate intermediates in the decomposition, pyrolyses of benzyl benzoate and benzhydryl acetate were conducted at 250° C. The degradation was done in the following manner: 1 g of the ester was placed in a 'Pyrex' tube, and then the tube was heated to a temperature between 245°–250° C, and kept in this range for 24 h. The pyrolysate was then cooled to room temperature, dissolved in benzene, and chromatographed on alumina. In the benzyl benzoate experiment, a compound sublimed from the reaction mixture; this compound was identified as benzoic acid (0·120 g). Chromatographic analysis of the residue gave toluene and benzaldehyde (0·071 g; gas chromatography disclosed 40 per cent toluene and 60 per cent benzaldehyde), benzyl benzoate (0·016 g) and benzoic anhydride (0·045 g).

On chromatographic separation the benzhydryl acetate pyrolysis afforded diphenylmethane (0·220 g), benzhydryl ether (0·176 g), benzhydryl acetate (0·101 g), and benzophenone (0·193 g). Since the isolation of the ether was accomplished in the decomposition of benzhydryl acetate, the pyrolyses of benzyl benzoate and acetate were repeated. All attempts to isolate such an intermediate in the pyrolysis of these esters have failed. These results may be attributed to the instability of the ether under fairly low pyrolysis temperatures[5].

A low-temperature decomposition of fluorenyl acetate was also investigated in an attempt to isolate the ether. In this case, as in the benzyl ester experiments, the ether could not be isolated. The products that could be identified were fluorene (0·104 g), fluorenyl acetate (0·223 g) and fluorenone (0·128 g).

The formation of ethers and anhydrides in the decomposition may be easily explained in terms of a bimolecular elimination via a *quasi* six-membered intermediate:



The carbonyl and hydrocarbon components are formed from the breakdown of the ether[6]. This mechanism is also supported by the investigations of Coffin[7,8] on the decomposition of ethylidene acetate and related compounds. This pyrolysis affords the aldehyde and anhydride; the reaction mechanism has been postulated to be a concerted elimination[9].

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ENGINEERING

Hydrostatic Extrusion

Bobrowsky and Stack[1] have suggested the possibility of using the hydrostatic extrusion process as a means of fabricating wire. That this is a proved fact is shown in Fig. 1. This shows five stages in the production of



Fig. 1

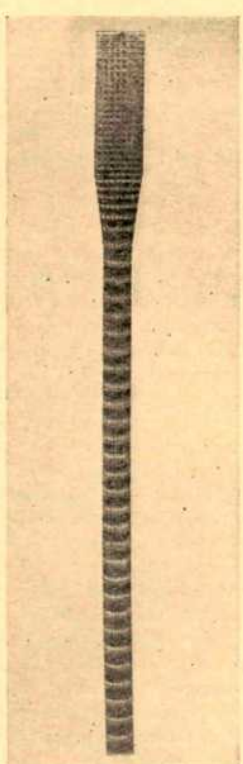


Fig. 2

aluminium wire from a ½-in. diam. bar. For illustration purposes in showing all reductions on one specimen, extrusion has been stopped at an earlier point on each successive pass, a fact which also points to the smoothness of this particular operation.

In their cold extrusion of aluminium, Bobrowsky and Stack found no irregularity on the back face of their partially extruded billet. This is typical of hydrostatic extrusion, and the fact that a state of homogeneous deformation exists until well into the die aperture is particularly evident in the specimen shown in Fig. 2. The photograph is that of the back end of a partially extruded long copper billet which has been split longitudinally and inscribed with a grid before extrusion in order that the flow pattern subsequent to extrusion may be observed. It will be seen that the transverse grid lines remain straight until about half-way into the die. Another interesting feature is that the uniform curvature of the grid lines across the extruded length indicates uniform mechanical properties throughout the product.

These experiments on conventional materials were part of incidental work supporting basic research into a high-pressure extrusion process at the Reactor Fuel-Element Laboratories, U.K. Atomic Energy Authority, Springfields, Preston, Lancs.

This communication has been submitted by permission of the Managing Director of the Authority.

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BIOCHEMISTRY

Release and Breakdown of Sialic Acid from Human Salivary Mucin and its Role in the Formation of Dental Plaque

Sialic acid is a constituent of human salivary mucin obtained by precipitating the mucin from saliva with either ammonium sulphate, weak acids, acetone or alcohol. Dried salivary mucin contains 2 per cent sialic acid[1]. In man, so far, the only sialic acid to be found from over a dozen different sources[2] has been *N*-acetylneuraminic acid (NANA), and it is reasonable to assume that it exists in this form also in human salivary mucin. Sialic acid, estimated as NANA by Svennerholm's method[3], is found in freshly collected, human, wax-stimulated saliva. There was a wide variation between different individuals and between the same individuals on different occasions. The mean of 40 determinations gave a value of 45 μg/ml. with a standard deviation of 12 μg/ml. and a range of 19–85 μg/ml. Dental plaque, however, which is believed to contain mucin[4], contained zero or trace amounts of NANA in over 40 determinations. The sialic acid must be lost from the mucin either when present in the saliva or else very rapidly during formation of the dental plaque.

Freshly collected saliva was incubated at 37° and the total and free sialic acid estimated at various times by the methods of Svennerholm[3] and Warren[5], respectively. The difference between these two values at any time represents the sialic acid that was still bound to the mucin. In over 30 samples of saliva the sialic acid was rapidly released and metabolized. In Fig. 1 is shown typical results obtained with pooled saliva during 6 h incubation. The addition of dental plaque up to a concentration of 2 per cent had no effect on the rate of release or breakdown of the sialic acid. After incubation for 24 h, only trace amounts of NANA were present in the saliva. NANA, when added to the saliva, was rapidly metabolized. Antibiotic ('Ledermycin', Lederle Laboratories) or heating in boiling water for 5 min prevented both the release of sialic acid and its subsequent destruction. It is concluded that the enzyme systems responsible for the release and breakdown of sialic acid from salivary mucin are bacterial in origin, are mainly present in the saliva, and correspond in action to the enzymes neuraminidase and aldolase, the action of which on other mucins and sialic acids respectively have been described elsewhere[2].

Addition of neuraminidase (receptor-destroying enzyme from *Vibrio cholerae*, Wellcome Research Laboratories) to either heated saliva or saliva containing antibiotic released the sialic acid from the mucin.

Dried salivary mucin contains 6 per cent hexosamine[1]. The hexosamine content of fresh saliva, estimated as glucosamine by the method of Boas[6], varied in a similar manner to NANA, there being a regular weight ratio of hexosamine : sialic acid of $3{\cdot}0 \pm 0{\cdot}3 : 1$. The metabolism of hexosamine was prevented by either antibiotic or heat, but on incubating fresh saliva alone for 24 h at 37°, 25 per cent of the hexosamine remained and some 90 per cent of this was not dialysable, indicating that it remained bound to some large molecule. In contrast to its content of sialic acid, dental plaque contains appreciable amounts of hexosamine. The mean hexosamine content of 13 samples of freshly collected plaque was 0·91 per cent with a mean deviation of 0·18 per cent. This corresponds to 3·5 per cent of the dry weight of the plaque. It is apparent that the sialic acid of the mucin is more amenable as a source of carbohydrate than is the hexosamine.

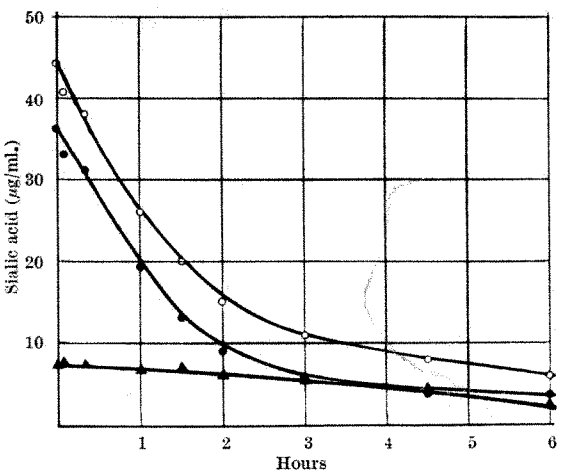


Fig. 1. Total (○), bound (●) and free (▲) sialic acid in human, pooled, wax-stimulated saliva on incubating at 37°

Gottschalk[7] has shown that the specific enzymatic release of sialic acid from ovine submaxillary mucin by neuraminidase causes the mucin molecule to change from a rigid rod form to that of a more random form without depolymerizing the molecule, and produces a concomitant decrease in the viscosity of the solution. It is tempting to speculate that a similar type of mechanism occurs in saliva, the viscosity of which decreases on standing unless arrested by enzyme poisons or penicillin[8]. There is very little direct evidence that the observed decrease in viscosity of standing saliva is due to depolymerization of the mucin, although this mechanism is extensively quoted[4]. The relationship, if any, between the sialic acid content of saliva and its viscosity is at present under investigation in this laboratory.

The strongly charged carboxyl groups of sialic acid (pK_a 2·6) impart a negative charge to the mucin molecule under all conditions that are experienced in the saliva. This charge is probably the main factor responsible for the low *p*H (*p*H 2–3) of minimum solubility of salivary

mucins[9]. The release of sialic acid from mucin should raise its *p*H of minimum solubility towards neutrality and under the conditions experienced in saliva would tend to cause the resultant mucin to precipitate out of solution. It has been found in this laboratory that there is a significant increase in the turbidity of saliva containing antibiotic on the addition of neuraminidase compared with a control saliva to which heat denatured enzyme has been added.

Reference to a typical oral organism that is known to produce neuraminidase could not be found in the literature. This may be because the enzyme is induced (adapted) by the organisms in the saliva because of the paucity of carbohydrate present. Recent work suggests that the neuraminidase of *Vibrio cholerae* is an induced enzyme[2]. Some indication that the enzyme is induced in saliva is shown by the fact that the sialic acid is released much more slowly from salivary mucin in the presence of an added 1 per cent buffered glucose. After 90-min incubation of fresh saliva containing glucose, the sialic acid content of the mucin was, in the majority of cases, more than 90 per cent its original value and in some instances over 50 per cent remained after 24 h incubation. This is in striking contrast to the results obtained in the absence of glucose (Fig. 1). The *p*H of the solutions in all instances were kept above 6 by the addition of phosphate buffer.

It is thought in this laboratory that the reaction involving the release of sialic acid from salivary mucin leading to its precipitation under mildly acid or neutral conditions plays an important part in the formation of dental plaque, and the lack of sialic acid and the presence of bound hexosamine in the plaque substantiate this view.

I thank Mrs. Mary Griffiths for assistance, Dr. A. J. Milne Gall and all members of the hospital staff who collected plaque and saliva, and the Medical Research Council for financial support.

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Isolation of Phosvitin from the Plasma of the Œstrogenized Immature Pullet

The presence of phosphoprotein in the plasma of the laying hen is widely held to be the result of the action of œstrogenic substances produced by the growing ova of the mature bird[1], though the precise nature and quantities of œstrogenic substances present in the blood stream have not yet been determined. Recently it has been shown that the phosphoprotein in the plasma of the laying hen is phosvitin, the principal protein of egg yolk[2], and it seemed desirable to examine the plasma of œstrogen-treated non-laying birds to determine whether or not such treatments could, in fact, lead to the formation of phosvitin.

Immature pullets 12–14 weeks old were injected intramuscularly with œstradiol benzoate (2 mg/day) on alternate days for 10 days. During this time the plasma phosphoprotein concentrations increased markedly. The birds were then bled, the plasma collected and the phosphoprotein precipitated as the calcium complex before working up as described by Heald and McLachlan[2]. The partially purified fraction was further purified by stepwise elution from diethylaminoethyl cellulose with sodium chloride at *p*H 8·6[3]. Two main fractions containing phosphorus were obtained: a major fraction eluted with 0·30 M sodium chloride amounting to 54–60 per cent of the phosphorus, and a second fraction eluted with 0·25 M sodium chloride amounting to 30 per cent of the phosphorus added. The remaining 10–15 per cent of the phosphorus was found in minor fractions eluted with 0·15 M and 0·20 M sodium chloride respectively. The major fraction contained 9·5 per cent phosphorus and 9·75 per cent nitrogen, yielding a nitrogen/phosphorus ratio of 2·22. It contained 3·66 moles serine/10^4 g protein yielding a serine/phosphorus ratio of 1·0. These properties are identical with those of phosvitin from egg yolk[4] and from the plasma of the laying hen[2]. The second peak contained 6·3 per cent phosphorus and 10·9 per cent nitrogen, yielding a nitrogen/phosphorus ratio of 3·83. It contained 2·03 moles serine/10^4 g protein, giving a serine/phosphorus ratio of 1·0.

The results clearly establish that administration of œstrogen to the immature pullet results in the appearance of phosvitin in the plasma. Since, in birds of this age, the ovary is not developed and largely non-functional[1] the results also provide strong evidence that the synthesis of phosvitin takes place elsewhere in the body. The second component, which contains phosphorus and serine in a ratio indicative of their existence as phosphorylserine, was not found in any quantity in the plasma of the laying bird[2]. Since the immature pullet has no developing ova capable of removing plasma phosphoprotein for yolk formation it is considered that this second component is either an accumulated intermediate of phosvitin synthesis or a product of its breakdown.

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Inhibition of Biological Synthesis of Acetylcholine by Triethylcholine

Bowman and Rand have suggested that the mechanism by which the triethyl analogue of choline causes failure of neuromuscular transmission is by interfering with synthesis of acetylcholine in the nerve endings[1]. If so, triethylcholine may be like HC-3 (hemicholinium), which has been found to inhibit the synthesis of acetylcholine by organized nerve tissue[2,3].

The experiments reported here were carried out to determine the effect of triethylcholine on synthesis of acetylcholine by sub-cellular fractions of rabbit brain homogenates.

The choline acetylase (acetyl-Co*A*: choline-*O*-acetyltransferase, EC 2.3.1.6) incubation system used was that described by Hebb, 1963[4]; acetyl-Co*A* is produced from acetylphosphate and coenzyme *A*, by phosphotransacetylase. The ACh synthesized was assayed biologically using the frog rectus abdominis muscle. Triethylcholine has been shown to be inactive on this assay preparation[1] and it was confirmed that triethylcholine does not affect contractions due to acetylcholine.

Table 1 shows the effect of triethylcholine on the synthesis of acetylcholine by the large granule or 'mitochondrial' fraction of rabbit brain (P_2 fraction) (ref. 4). When the concentration of choline (0·8 mg/tube) was optimal an equimolar concentration of triethylcholine (1·04 mg/tube) inhibited synthesis of acetylcholine by 11 per cent; doubling the triethylcholine concentration almost doubled the inhibition, and at the largest concentration of triethylcholine (10·4 mg/tube) the inhibition was 40 per cent.

Table 1. Inhibiting Action of Triethylcholine (TEC) on Synthesis of Acetylcholine by Mitochondrial Fraction of Rabbit Brain

Choline Cl Added to the system	Acetylcholine synthesized $\mu g/g$ $P2$ fraction	% inhibition produced by TEC 1·04 mg	TEC 2·08 mg	TEC 10·4 mg
0·2 mg	103	21	32	53
0·8 mg	195	11	20	40
3·2 mg	175	2·5	4·5	3·0

0·8 mg choline Cl equimolar with 1·04 mg TEC Cl. Final volume of system on incubation = 0·1 ml. enzyme + 0·5 ml. Pre-incubation mixture = 0·6 ml.

At a reduced concentration of choline (0·2 mg/tube) the same amounts of triethylcholine produced a greater inhibition of synthesis of acetylcholine. However, when the concentration of choline was increased 4-fold, triethylcholine had no significant effect on enzymatic synthesis of acetylcholine.

Treatment with ether is known to activate choline acetylase in brain homogenates[5], possibly by breakdown of membranes enclosing the enzyme. Comparisons have been made of the effect of triethylcholine on synthesis of acetylcholine by P_2 fractions before and after treating them with ether, which caused a 4-fold increase in the activity of the fraction (Table 2). The results show that while triethylcholine still inhibited enzymatic synthesis of acetylcholine the effect, expressed as a percentage, was much smaller than that obtained with untreated particulates.

Table 2. Inhibiting Action of Triethylcholine on Synthesis of Acetylcholine by Ether and Non-ether Treated Mitochondrial Fractions of Rabbit Brain

Choline Cl added	Acetylcholine synthesized ($\mu g/g$) $P2$ fraction: No TEC	TEC (4·16 mg)	% inhibition
No ether treatment 0·4 mg	108 μg	69 μg	36
Ether treatment 0·4 mg	438 μg	377 μg	14
Activity			

Ether treated : non-ether treated fraction—438 : 108, approx. 4 : 1.

Similar experiments using tissue slices obtained from rabbit caudate nucleus also demonstrated the inhibition of synthesis of acetylcholine by triethylcholine in the presence of low choline concentrations.

These results indicate that triethylcholine inhibits synthesis of acetylcholine more readily when the tissue binding of the choline acetylase is not disturbed and that the inhibiting action of triethylcholine can be antagonized by using higher levels of choline. This suggests that triethylcholine is acting like hemicholinium by competing with choline for access to the enzyme through some membrane. It may also possess a slight direct inhibiting action on choline acetylase since in ether-treated particulates synthesis of acetylcholine was still impaired to some extent by large concentrations of triethylcholine, although here again the inhibition could be overcome by increasing the choline concentration.

Taken as a whole the results described here provide an explanation of the effects of triethylcholine *in vivo* and support the hypothesis advanced by Bowman and Rand that this is due to impairment of synthesis of acetylcholine.

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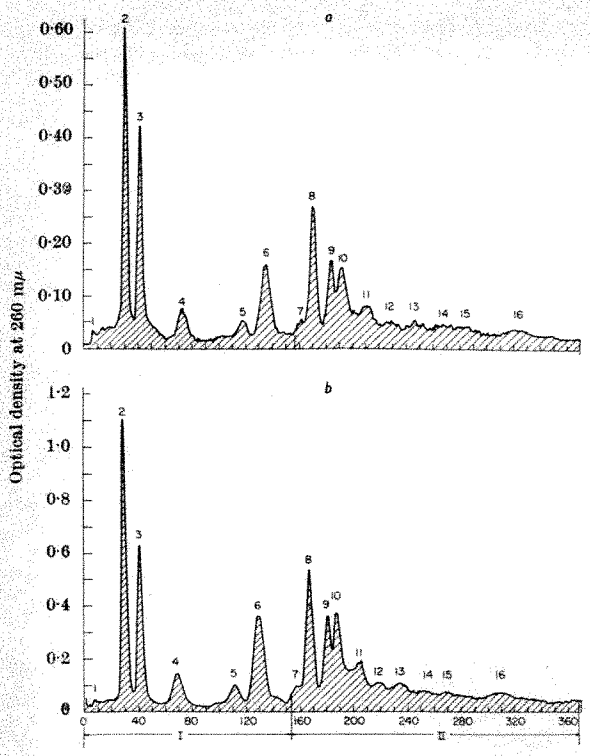
Nucleotide Sequence Investigations in Microsomal Ribonucleic Acids of Normal and Rous Sarcoma Virus-infected Tissues

A characterization of nucleotide sequence in ribonucleic acids (RNAs) in tumours would be of importance to the understanding of the structure of these molecules in neoplastic tissues. Methods for the separation of mono- and oligo-nucleotides liberated with ribonuclease digestion by ion exchange chromatography have been developed[1] and applied to amino-acid acceptor RNAs with promising results[2]. This communication describes an investigation of some nucleotide sequences in microsomal RNA extracts from normal and Rous sarcoma virus-infected tissues. These are initial studies of a programme designed to analyse the structure of the nuclear and cytoplasmic RNA's of normal and tumour cells.

Chorio-allantoic membranes of 10-day old chick embryos were infected with 0·2 ml. of a $2{\cdot}5 \times 10^{-2}$ dilution of standard preparations of Rous sarcoma virus supplied by Dr. W. R. Bryan, of the National Institutes of Health. Eggs were incubated for 7 days, and tumour as well as normal membranes were afterwards collected. Histological examinations showed that virus-infected tissue contained more than 200 sarcoma cells for each normal cell present.

Tissue was homogenized in an ice-cold solution of 0·30 M sucrose and 0·001 M magnesium chloride with a 'Vir Tis 45' high-speed homogenizer. Nuclei, mitochondria and unbroken cells were separated by centrifugation at 8,500g for 15 min. Microsomal fractions were isolated by centrifugation at 105,400g (average) for 2 h. To extract RNA, pellets were suspended in 0·01 M potassium phosphate buffer, pH 7·0, containing 0·01 M disodium ethylenediamine tetraacetate (EDTA) and homogenized briefly at low speed. The solution was brought to room temperature, 0·5 per cent sodium lauryl sulphate was added, and stirred for 10 min. An equal volume of phenol (88 per cent) was then added and stirring was continued for 1 h. The preparation was centrifuged, the aqueous layer carefully pipetted off and the phenol extraction was repeated once more. Phenol was removed by shaking with ether at 2° C and ether was expelled by bubbling with nitrogen. The RNA was dialysed overnight (all dialysis tubing was pre-soaked in buffered 0·01 M EDTA for 2 days) against several litres of distilled water and vacuum-dried. RNA was determined by the Mejbaum orcinol reaction as described by Schneider[3]. Ultra-violet absorption spectra showed an average 260 mμ/280 mμ absorption ratio of 2·1 and 260 mμ/230 mμ of 1·6 for normal and tumour extracts.

Samples of RNA were resuspended in 0·01 M potassium phosphate buffer, pH 7·0, pancreatic ribonuclease (RNAse) was added (0·1 mg/mg RNA) with 1·0 per cent chloroform and the solution was incubated for 16 h at 37° C. The pH at the completion of the experiment was unchanged. Normal and tumour preparations showed the same rate of increase in optical density at 260 mμ with time and maximum values of 20–30 per cent increase. To separate nucleotide and oligonucleotide fragments, the digests were added to DEAE-'Sephadex' columns and eluted with ammonium carbonate. Mono- and oligo-nucleotides were identified by ultra-violet absorption spectra with pH changes[1]. The results are shown in Fig. 1. A comparison of the amount of material in each peak is given in Table 1. The last column of Table 1 indicates the degree of variation obtained in duplicate chromatograms of digests from tumour tissue RNA from an identical extraction where half the extract was chromatogramed a week after the other half. No gross differences in chromatographic pattern of RNAse digests of the normal and tumour tissue were detected. No significant changes were evident when the data were subjected to Friedman's rank sum test at the 30 per cent level. Fine differences in the amount of nucleotide in any given peak, which would be important biologically, probably cannot be detected in view of the



Figs. 1*a* and 1*b*. Chromatography of pancreatic ribonuclease digests of 3·3 mg of microsomal RNA from normal tissue (*a*) and 6·3 mg microsomal RNA from Rous sarcoma tissue (*b*). DEAE-'Sephadex *A*-25' (60 cm × 0·5 cm) exchanger was eluted with a gradient of increasing concentration of ammonium carbonate using a Varigrad. Region I, 100 ml. each of 0·01 M, 0·24 M, 0·01 M, 0·20 M, 0·20 M, 0·40 M, in six Varigrad chambers; Region II, 150 ml. each of 0·40 M, 0·50 M, 0·40 M, 0·90 M, 0·70 M in five chambers. Volume of fractions collected was 3·5 ml.

limits of analysis. Rous virus RNA, which is present in the microsomal RNA fraction from tumour tissue, is too small in amount compared to the cellular RNA to be detected in this analysis.

In view of the findings that RNA can induce differentiation and transformation in malignant cells[4–7], it is important to determine the structure of the various nucleic acids of malignant and normal tissues and to determine which, if any, have an altered base sequence. One may predict that the informational RNAs may be altered in tumours, although the functions of microsomal and parts of the nuclear RNA are still obscure. The results reported here show no significant differences in certain microsomal RNA base sequences of Rous sarcoma and normal tissues and depict the type of reproducibility that

Table 1

Peak No.	Proposed composition*	Ratio: amount of nucleotide in normal/tumour for each peak†	Ratio of amount of nucleotide in duplicate experiments with tumour RNA
1	Unidentified	—	—
2	C_p	1·01	1·19
3	U_p	1·21	1·06
4	A_pC_p	0·87	0·98
5	A_pU_p	1·10	1·08
6	G_pC_p	0·81	0·92
7	$(A_pA_pC_p)$	0·97	0·77
8	G_pU_p	0·99	1·16
9	$(A_pA_pU_p)$	0·88	1·06
10	$(G_pA_pC_p)$	0·78	0·88
11	$(A_pG_pC_p)$	1·03	
12	$(G_pA_pU_p)$	1·12	
13	$(A_pG_pU_p)$	0·83	
14	$(G_pG_pC_p)$	0·98	
15	$(G_pG_pU_p)$	0·91	
16	Beginning of tetranucleotides	0·92	

* The sequences in parentheses are provisional.

† The ratio of milligrams of normal to Rous sarcoma tissue added to column has been normalized to 1·00.

has been obtained from a study of sequences using ion exchange chromatography with DEAE–Sephadex.

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Stimulation by Rat Liver Ribonucleic Acid of Incorporation of Amino-acid into Rat Liver Ribosomes

The discovery[1] that ribonucleic acid (RNA) and synthetic polyribonucleotides stimulated the incorporation of amino-acids into protein-like material in cell-free system from *E. coli* has been followed by many reports[2–6] of similar findings with systems from the same and other cells. Rat liver RNA was shown[5] to stimulate amino-acid incorporation when added to an *E. coli* system and rat liver RNA and polyuridylic acid caused increased incorporation of phenylalanine in a rat-liver microsomal system[6]. We wish to report that polyuridylic acid and rat liver RNA stimulate incorporation of amino-acid with rat liver ribosomes.

Table 1

	Ribosomes	C.p.m./mg protein
(*A*)	Prepared normally	
	Control	1,109
	+ RNA (exp. 1)	1,550
	+ RNA (exp. 2)	1,730
(*B*)	Prepared without salts	
	Control	488
	Pre-incubate 10 min	86
	Pre-incubate 10 min in presence of RNA (exp. 1)	382
	Pre-incubate 10 min in presence of RNA (exp. 2)	594
	Pre-incubate 10 min in presence of *s*RNA	74
	Pre-incubate 10 min + ^{14}C phenylalanine	164
	Pre-incubate 10 min in presence of 25 μg poly + ^{14}C phenylalanine	1,296

Rat liver RNA, prepared by the lauryl sulphate–phenol method[7], in the presence of bentonite[8] and in the cold to reduce breakdown of the RNA, caused stimulation (see Table 1) of incorporation of DL-(1-^{14}C)-leucine when added to a rat liver ribosomes system prepared as previously described[9]. When the ribosomes were washed and suspended in 0·05 M *tris* buffer *p*H 7·8 without added salts, instead of a medium containing salts, incubation at 37° for 10 or 15 min drastically reduced the ability of these ribosomes to incorporate amino-acids into protein when cell-sap, ATP, ^{14}C-amino-acid, GTP and sufficient salts were added to achieve optimal conditions[9]. The addition of RNA during the pre-incubation caused a greater percentage stimulation in the subsequent amino-acid incorporation and, in some experiments, stimulated the incorporation of amino-acids to above that shown by the ribosomes which had not been pre-incubated (see Table 1). Although the percentage stimulation was greater with the attenuated ribosomes, addition of RNA to ribosomes which had not been pre-incubated caused a greater absolute increase in the radioactivity incorporated.

It could be argued that the added RNA is protecting the ribosomes (or the soluble RNA (*s*RNA) subsequently added in the cell-sap) from breakdown by ribonuclease known to be associated with ribosomes[10]. We have, in fact, observed breakdown of added, labelled RNA during pre-incubation but it seems unlikely that all the effect of added RNA on amino-acid incorporation can be ascribed to protective action since we have observed that some preparations of RNA did not, for some still unknown reason, exhibit any stimulatory effect when added during incubation. We have also found that addition of 25 μg of polyuridylic acid to the ribosomes during pre-incubation caused a 6–8-fold stimulation of incorporation of phenylalanine into perchloric acid-precipitable material. It seems unlikely that such a small amount of synthetic polymer could exert much protective action.

It is also unlikely that the RNA added is causing stimulation because of the *s*RNA contained in it since addition of *s*RNA of equivalent amount to the stimulatory RNA used caused no stimulation of incorporation of amino-acid (Table 1).

A possible explanation of these results is that messenger RNA[11] (*m*RNA) exists in rat liver and is needed for incorporation to occur and that the added RNA contains *m*RNA. Pre-incubation may be removing messenger RNA from the ribosomes so that any added *m*RNA has a greater chance of sticking to the ribosomes, thus accounting for the greater percentage stimulation. Alternatively, pre-incubation in the absence of magnesium may have enhanced the ability of the *m*RNA added to combine with the ribosomes since, in the absence of magnesium, RNA will not form inter- and intra-molecular bonds.

Experiments are in progress to distinguish between the messenger and the protective function of the added RNA and to examine the properties of the stimulatory RNA.

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Microbiological Dehydroxylation of Cholic Acid

The transformation of steroids by micro-organisms has been extensively applied to the steroid hormones and their derivatives[1]. More recently this technique has been extended to other compounds[2]. However, the dehydroxylation reaction of steroids by micro-organisms has not been reported.

In continuing our examination about microbiological degradation of bile acids[3], it was found that *Corynebacterium simplex* (I.F.O. 3530), cultured in a medium containing cholic acid as the sole source of carbon, produced 12α-hydroxy-3-oxo-$\Delta^{1,4}$-choladienic acid, which corresponds to the dehydroxylation derivative of cholic acid.

The following physical constants of the methyl ester of this acid indicated that this ester may be methyl 12α-hydroxy-3-oxo-$\Delta^{1,4}$-choladienate: $C_{25}H_{36}O_4$ (found: C, 75·11; H, 9·13); m.p. 146–148°; $[\alpha]_D^{23}$ +29·4 ± 2° (dioxane); $\lambda_{max}^{alc.}$ 246 mμ (ε = 15,400); $\nu_{max}^{CHCl_3}$ 3,481 cm^{-1} (OH), 1,732 cm^{-1} (ester) and 1,607, 1,624 and 1,663 cm^{-1} ($\Delta^{1,4}$-3-ketone). The identity with an authentic methyl 12α-hydroxy-3-oxo-$\Delta^{1,4}$-choladienate, prepared from methyl 12α-hydroxy-3-oxo-Δ^4-cholenate by selenium dioxide dehydrogenation, was determined by infra-red comparison and mixed melting point.

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Micro Method of Haptoglobin Typing using Acrylamide Gels

The value of haptoglobin typing for human sera in relation to population investigations is well known[1]. The usual method of haptoglobin typing following electrophoresis on starch gels[2] is reliable but rather slow and cumbersome so that a quick and reliable method is of considerable advantage when large numbers of sera are to be examined.

The purpose of this communication is to describe a micro-method which utilizes electrophoresis in acrylamide gels on microscope slides and can be completed in 50 min. This method also offers a better resolution of haptoglobin types than the starch gel method and only 0·1 μl. of serum is required for examination. Recording of the results is done on photosensitive paper by trans-illumination of the slides in a condenser-type enlarger.

Microscope slides 75 mm × 25 mm are placed on a flat 'Perspex' tray the slides of which project uniformly 1·5 mm above the surface of the slides. A solution of acrylamide (supplied as chemical grout AM9 by Cyanamid. Australia Pty., Ltd.) of the following composition is prepared and is immediately poured into the tray.

AM9	7·5 g
Catalyst DMAPN	0·5 ml.
Ammonium persulphate	0·5 g
Water	to 100 ml.

The solution is then sealed from air with a flat 'Perspex' cover slid over the liquid. Polymerization occurs within 4 min at room temperature. The cover is then removed by sliding it off the gel and the gel is cut with a razor-blade along the edges of the slides, which are then immersed in a solution of *tris* (2-amino-2-(hydroxymethyl)-1 : 3-propanediol) of the following composition.

tris	9·2 g
Citric acid	1·0 g
Distilled water	to 2 l.

Sets of twelve slides are washed with 500 ml. of the *tris* solution for 6 h and then with a fresh 500 ml. of the solution overnight, following which the slides can be used, or left in the solution until required for use. The slides are placed in an open electrophoresis tank, as no special precautions are required to prevent evaporation, and

slits are made in the gel with a razor-blade 15 mm from the cathode end of the gel. After hæmoglobin has been added in the usual fashion[2] to the sera to be examined strips of cellulose acetate membrane or Whatman paper No. 1, approximately 1 mm × 5 mm, are immersed in the sera and after careful blotting are placed in the slits. The slides are then connected to the buffer tanks containing borate buffer *p*H 8·9, 0·0115 M, by filter paper wicks moistened with the buffer solution and are subjected to electrophoresis for 30 min at 20 V cm^{-1}. At the completion of the electrophoresis run the slides are immersed in the staining solution consisting of:

Saturated solution of 0-dianisidine (ref. 3) in ethanol	20 ml.
Ethanol	40 ml.
Acetate buffer, *p*H 4·6, 2·7 molar	20 ml.
Distilled water	60 ml.

After 10 min, 2 ml. of 30 per cent hydrogen peroxide are added for each 100 ml. of the staining solution and the slides are left for a further 10 min. The slides are then placed in tap-water for 30 min, after which they should be read or photographed within 12 h as the colour, although initially quite intense, gradually fades.

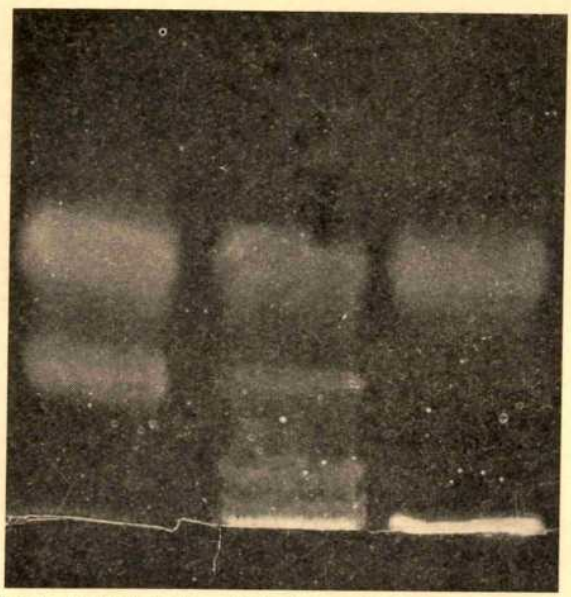


Fig. 1. Anode at the top of the photograph. Left to right: haptoglobin type 1–1, 1–2 and 2–2. Typing was confirmed by starch gel electrophoresis

With this technique the haptoglobin 1–1 band closely follows the hæmoglobin band, while the type 2–2 bands penetrate into the gel for only a short distance from the origin. The type 1–2 haptoglobin bands are particularly well displayed in the intervening zone. Because acrylamide gels are completely clear the contrast provided by the stained zones is extremely sharp. The extremely small amount of serum necessary to perform the analysis may have some importance in forensic applications of this method.

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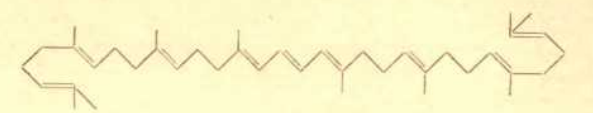
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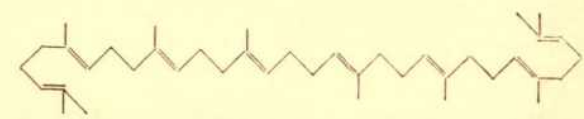
Comparison of the Reaction of *N*-bromosuccinimide with Phytoene and Lycopersene

It is now well established that phytoene (I) is a precursor of the coloured carotenoids such as ζ-carotene and lycopene[1,2]. If the biosynthesis of carotenoids were exactly analogous to that of sterols then lycopersene (II), the 40-C analogue of squalene, would be the first 40-C precursor formed. The consensus of opinion is that this compound is probably not a naturally occurring material and is not a carotenoid precursor[1,3–5]; the opposite view is, however, held by Grob *et al.*[7,8]. Zechmeister and Koe[9] showed in 1954 that *N*-bromosuccinimide dehydrogenates phytoene to phytofluene (III) and ζ-carotene (IV), compounds now known to be intermediates between phytoene and lycopene (V). It was of importance to know what action *N*-bromosuccinimide, which imitates nature in its reaction with phytoene, would have on lycopersene under similar conditions.

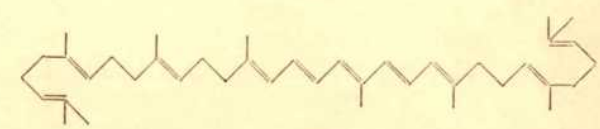
In a typical experiment with phytoene (ex carrot oil) 20 mg were treated with *N*-bromosuccinimide according to the method of Zechmeister and Koe[9] for 8–10 min at 80–85° under carbon dioxide. The reaction mixture was chromatographed on a column of either CaO/'Celite' or alumina (Brockmann, grade V). In addition to unreacted phytoene, phytofluene and ζ-carotene were identified. Table 1 indicates the amount of each component formed; the phytoene unaccounted for must have been broken down in side-reactions; no trace of the unsaturated polyenes, neurosporene and lycopene, was obtained.



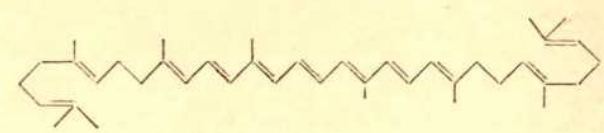
I



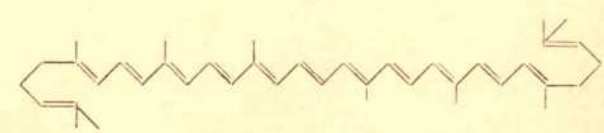
II



III



IV



V

Lycopersene (50 mg) was treated in the same way, and on chromatography of the reaction products, a number of slightly fluorescent zones were obtained; none of these exhibited the characteristic greenish fluorescence or absorption spectrum of phytofluene; furthermore, no coloured bands corresponding to ζ-carotene, neurosporene and lycopene were obtained. A red band was observed tightly bound to the top of the column; it did not have the characteristic appearance of a carotenoid; but it was eluted with ethyl acetate and examined

Table 1. Production of Phytofluene and ζ-Carotene from Phytoene by the Action of *N*-bromosuccinimide*

Compound	Amount (mg)
Residual phytoene	4·90
Phytofluene	0·44
ζ-Carotene	0·045

* Reaction carried out according to ref. 9.

spectrophotometrically. It showed only general absorption in the visible region of the spectrum and no bands characteristic of carotenoids.

Because it was possible that lycopersene reacted more rapidly with *N*-bromosuccinimide than phytoene did, the experiment just described was repeated and samples of the reaction mixture were taken at 10-min intervals over a period of 1 h. These samples were then examined by thin-layer chromatography with 'Kieselgel *G*' as adsorbent[3-5] and light petroleum (b.p. 40–60°) as developing solvent; markers of lycopersene and phytoene were always included as references. The positions of the spots after development were revealed by exposing the plates to iodine vapour when compounds possessing unsaturated centres are revealed by a brown stain[4,5]. In no case could a spot corresponding to phytoene be obtained, although the method employed would reveal the presence of 0·50 μg of this compound. A large spot corresponding to unchanged lycopersene was observed in every case.

The results indicate that under the experimental conditions which allow the conversion of phytoene into phytofluene and ζ-carotene by *N*-bromosuccinimide, lycopersene is not dehydrogenated to phytoene.

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Synthesis of an Aromatic Ring in Insects

It is now generally accepted that oxidation products of aromatic compounds are involved in the hardening of the skeletal proteins of insects[1]. The strong circumstantial evidence that, in blowfly larvæ, tyrosine gives rise to 'tanning' phenols[2] has recently been substantiated by the finding that injected tyrosine labelled with carbon-14 is converted to *N*-acetyl-3,4-dihydroxyphenylethylamine, the natural tanning agent of *Calliphora*[3].

In the cockroaches, *Blatta* and *Periplaneta*, 3,4-dihydroxybenzoic (protocatechuic) acid, after oxidation, serves to harden the proteinaceous egg capsule[4]. This dihydroxyphenol is produced in relatively large amounts. It has been shown that it is secreted by one of the sexual accessory glands—the left colleterial gland—in the form of the 4-O-β-D-glucoside[5]. This stable precursor is enzymatically hydrolysed at the time of egg-laying to give free protocatechuic acid, which is then oxidized to its quinone[6]. In view of the importance of 3,4-dihydroxyphenols to insects, and the sparsity of information as to their origins, a preliminary attempt has been made to discover the metabolic pathway to protocatechuic acid. In the main this has involved injecting substances labelled with carbon-14 which could be suspected of being metabolic precursors of protocatechuic acid, and afterwards assaying egg-capsules and colleterial gland for radioactive protocatechuic acid and its precursors.

In order to examine the possibility that synthesis of protocatechuic acid would occur from tyrosine, the aromatic ring remaining intact, approximately 0·4 μc. uniformly labelled tyrosine carbon-14 was injected into female cockroaches (*Periplaneta americana* (L.)) which were due to start egg-laying within two days. After egg-laying had taken place, extracts of the egg capsule, run as partition paper chromatograms, in butanol/acetic acid/water, 4: 1: 1, indicated that a radioactive substance was present corresponding with protocatechuic acid. Conversion of tyrosine to this substance was in excess of 6 per cent. Likewise, if left colleterial glands were assayed 24 h after injection, a substance corresponding with the glucoside of protocatechuic acid was strongly radioactive. After injection with tyrosine-α-carbon-14, no radioactive protocatechuic acid was detectable in egg-capsules.

These results can be interpreted as indicating that tyrosine is converted in the insect body to protocatechuic acid, and that partial degradation of the side-chain occurs.

In a second set of experiments, 1 μc. glucose-1-carbon-14 was injected into cockroaches. It was afterwards found that, not only was the glucoside, as would be expected, radioactive, but also free protocatechuic acid.

The attribution of radioactivity to protocatechuic acid and its glucoside was based on the detection of radioactivity in appropriate positions on partition paper chromatograms. For added support, the substances were eluted and run in a second solvent. A further elution was carried out, and the free acid esterified with methanol; the R_F value of the ester corresponded with authentic methyl ester of protocatechuic acid. In addition, the suspected protocatechuic acid, from animals that had received injections of glucose-1-carbon-14, was eluted from the chromatogram and co-crystallized with authentic protocatechuic acid. After three crystallizations the product retained its specific activity, while specific activity rapidly fell off at each recrystallization with *p*-hydroxybenzoic acid. Protocatechuic acid derived from the glucoside by acid or enzymatic hydrolysis behaved in the same way.

The evidence shows that both tyrosine and glucose can serve as precursors of protocatechuic acid, but the metabolic pathways appear to be distinct. Inasmuch as glucose can give rise to protocatechuic acid, and since tyrosine can, after ring-cleavage, be used in part to form glucose, a pathway from tyrosine to protocatechuic acid by way of glucose is conceivable; but were this the case, the conversion ratio from glucose to protocatechuic acid would be expected to be greater than (certainly not less than) the conversion ratio from tyrosine. The latter is, however, ten times the former. It appears, thus, that protocatechuic acid can be synthesized by two independent routes: (*a*) directly from tyrosine; or (*b*) from glucose.

The conversion of glucose to aromatic compounds by several paths is known to occur in micro-organisms and plants. One pathway involves condensation of acetates to form the ring[7], but does not lead to benzene compounds hydroxylated in the 3 and 4 positions; another, however, involving condensation of phospho-enol-pyruvate with erythrose-phosphate is known to lead to synthesis of both tyrosine[8] and protocatechuic acid[9]. It can be reasonably supposed that the second of these is the one involved in insects. At the time of these experiments there was no report of the ability of a metazoan to synthesize an aromatic ring, but there is evidence now that perhaps such a synthesis occurs in nematode worms[10], and there is circumstantial evidence that it must occur in echinoderms[11].

It appears possible that the glucose pathway to aromatic compounds in cockroaches is a result of the activity of symbiotic micro-organisms: there is some corroborative evidence, for example, mealworm beetles freed of their symbionts have a lighter coloration than normal[12], which could be taken to indicate that dietary aromatic compounds are insufficient and must be supplemented by symbiotic products before an adequate amount of phenolic pigment can be synthesized. It is interesting to find that, with one exception, all investigations of the dietary requirements of insects have included as essential either tyrosine or phenylalanine[13]: the exception is the case provided by the cockroach *Blatella* which is closely related to *Periplaneta*. Unfortunately, no information about the requirements of *Periplaneta* is availale.

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PHYSIOLOGY

Behaviour of Lysosomes in Hæmorrhagic Shock

In a series of detailed publications[1–5], Fine *et al.* have maintained that the probable cause of death in traumatic shock is the irretrievable collapse of the circulation caused by a bacterial toxin which intensifies vasoconstrictor activity so as to injure the splanchnic viscera, with resulting decline in vascular reactivity to catechol amines and functional damage in key organs, especially the liver and intestine. In the absence of any obvious systemic or local infection, these workers have considered that the most likely source of the toxin is an endotoxin derived from the intestinal flora. The difference between that type of shock which responds to resuscitative measures, that is, 'reversible shock', and the irreversible form would appear to depend on the ability of the reticulo-endothelial system, particularly in the liver and spleen, to inactivate this toxin. This ability declines if the animal is in shock for 2 h or longer. There are indications that this detoxication process is concurrent with an esterase-like protein in spleen and liver that releases phosphate. Abolition of vasoconstrictor activity in the splanchnic area by cœliac blockade preserves this process as well as the vascular integrity of the portal and systemic circulation. Under these circumstances the animals are able to survive an otherwise lethal exposure to shock. It is noteworthy, however, that while functional differences in the spleens and livers of normal and shocked animals have been observed, no histological abnormalities have been detected.

It appears, therefore, that the prime cause of traumatic shock is a defect in the detoxicating powers of the reticulo-endothelial system. It has been shown recently that the lysosomes do not act merely as 'suicide particles', but show reversible 'activation' indicative of a physiological function in cellular detoxicating mechanisms[6,7]. Moreover, not only is the characteristic enzyme of these particles an acid phosphatase, but also the apparent activation, and even liberation, of the enzyme has been found to be a very early concomitant of the shock of operative trauma[6].

It seemed reasonable, therefore, to examine the condition of the acid phosphatase in lysosomal particles in the spleen of rabbits subjected to both reversible and irreversible hæmorrhagic shock. This has become possible in tissues because of the development of the controlled temperature freeze-sectioning procedure[8] and of a modified procedure for this enzyme[10]. The preparative procedure has been shown not to alter the permeability of the lysosomal membrane to β-glycerophosphate which was used as the substrate, so that apparent activation of this enzyme within the particle can be detected. In the histochemical method the lysosome is subjected to a degree of disruption so controlled as to modify the membrane of normal lysosomes only sufficiently to yield a weakly positive particulate reaction (the fragility test of Bitensky[7]). If the lysosomes have become 'activated' by the previous conditions within the body, the membrane will be less capable of withstanding this degree of disruption and a strong reaction will ensue, possibly with some diffusion of stain out of the particles. With more extreme *in vivo* damage, the controlled degree of disruption may produce either diffuse staining or even complete loss of staining due apparently to release of the enzyme from the particles into the cell, or even entirely out of the section.

A study has now been made in which the condition of the acid phosphatase in the spleen of normal rabbits and of those subjected to reversible or irreversible hæmorrhagic shock has been investigated. For the reversibly shocked animals, rabbits were exposed to severe hæmorrhagic shock for up to 2 h; some were resuscitated by being transfused with all their shed blood and were observed for up to a further 2 h to ensure the reversibility of this condition, while others were killed immediately. For irreversibly shocked animals the time of exsanguination was prolonged for at least 6 h, after which time they were shown not to respond to treatment. Sections were prepared by the freeze-sectioning procedure and stained for acid phosphatase by the method described by Bitensky[10].

The fragility test performed on sections of spleen from normal animals demonstrated that acid phosphatase was contained within discrete particles in large cells of the pulp which appeared to be macrophages. In their response to acid conditions, and in the behaviour of the enzyme, these particles corresponded to lysosomes[9]. In all the rabbits subjected to reversible hæmorrhagic shock these particles were more fragile. Thus, for a given incubation period, they showed greater apparent activity for acid phosphatase than did normal particles, or were even disrupted by the incubation, allowing local diffusion of the reaction. However, in each of a series of experiments, the effect in the animals in irreversible shock was more extreme, varying from considerable diffusion of the reaction to almost total loss of staining.

The ability of lysosomal enzymes to perform a physiological, as distinct from a 'suicidal', role in the cell must depend on the structural integrity of the organelles. Toxic conditions have been shown to cause such reversible modification of this membrane as to increase its permeability, as evinced by this intraparticulate response to β-glycerophosphate, without necessarily permitting the escape of the enzymes[6,11]. It has now been shown that in the spleen of rabbits subjected to reversible hæmorrhagic shock, the membrane of these organelles is indeed so modified. However, in the animals suffering irreversible hæmorrhagic shock, the altered location of the acid phosphatase indicated disruption of the structural integrity of these organelles. Hence, it has been shown that one difference between the reversible and irreversible condition of hæmorrhagic shock is the change in the state

of lysosome-like particles in the large cells of the pulp of the spleen, as would be indicative of a breakdown in the ability of such organelles to function in detoxication.

It may thus be permissible to speculate as follows: it is known that many conditions, including the trauma of surgical operation, cause modification to lysosomes[6,7]. Moreover, lysosomes seem to have some role in the detoxicating mechanism of normal cells[7]. It has now been shown that the condition of irreversible shock may be characterized by the disruption of lysosome-like particles. Thus, it seems conceivable that the primary effect in irreversible hæmorrhagic shock is loss of the integrity of lysosome-like organelles. Consequently they can no longer detoxicate endotoxin, which is thus able to circulate and affect vital sensitive target organs in the body.

Without the courteous assistance of H.M. Customs, this collaborative study would not have been possible.

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Assessment of Functional Competence in Isolated Muscular Tissues

In order to test for functional competence in thin pieces of isolated tissue containing muscular elements, such as the skin of the rat (which contains smooth muscle and a sheet of skeletal muscle known as the panniculus carnosus), a bath was devised in which the muscles could be excited directly or indirectly by transmural stimulation[1]. The response of the stimulated muscle provided a measure of functional competence in the surviving tissue. This bath and also our method of recording the pressure changes developed in it are described here.

A circular piece of the tissue under investigation was first cut out and then clamped between two 'Perspex' rings to form a diaphragm. To achieve rigid clamping of the tissue between the rings, the upper surface of the lower and the lower surface of the upper ring were lined with glass paper. This assembly was then placed on a thin rubber washer in the small 'Perspex' bath specially made to accommodate it (Fig. 1). The bath was made in two sections, the lower half with an internal ledge on which the rings rested and an internal thread above the ledge and the upper half with an external thread so that it could be screwed into the lower and would thereby serve to hold the rings and the tissue firmly in place. This created an upper and a lower compartment separated from one another by the tissue; both compartments were filled with Krebs's solution (of the following composition in g/l.: sodium chloride 6·9; potassium chloride 0·35; potassium dihydrogen phosphate 0·16; crystalline calcium chloride 0·37; crystalline magnesium sulphate 0·29; glucose 1·0 and sodium bicarbonate 2·1) and a coiled platinum electrode projected into each.

The upper compartment (approximate capacity 10 ml.) was connected with a reservoir containing Krebs's solution which could enter this compartment at controlled rates: the solution in both this compartment and the reservoir was bubbled continuously with 95 per cent oxygen: 5 per cent carbon dioxide. Although continuous oxygenation was provided only through the Krebs's solution of the upper and not the lower compartment, the results have shown that this was sufficient to prevent any deterioration of response in thin muscular tissues such as rat diaphragm or rat panniculus carnosus over a period of hours.

The lower compartment (approximate capacity 2·5 ml.) was also filled with Krebs's solution and was connected to another reservoir, the height of which could be varied. Adjusting the height of this reservoir changed the pressure in the lower compartment and on the under surface of the tissue diaphragm. When the reservoir was raised above the level of the bath, positive pressure was applied to the lower surface of the tissue and pushed it upwards into the shape of a dome. This had the effect of stretching the fibres and thus putting a load on the tissue; the extent of the load depended on the height of the reservoir. Very little load (reservoir heights of 5 cm or less) was required to give maximal contractions. Transmural stimulation flattened the dome as the muscular elements contracted and increased the pressure in the lower compartment.

Changes in pressure in the lower compartment were transmitted to the diaphragm of a variable reluctance pressure transducer which was connected to the compartment by means of a small glass funnel, the rim of which just fitted over the circumference of the transducer, and

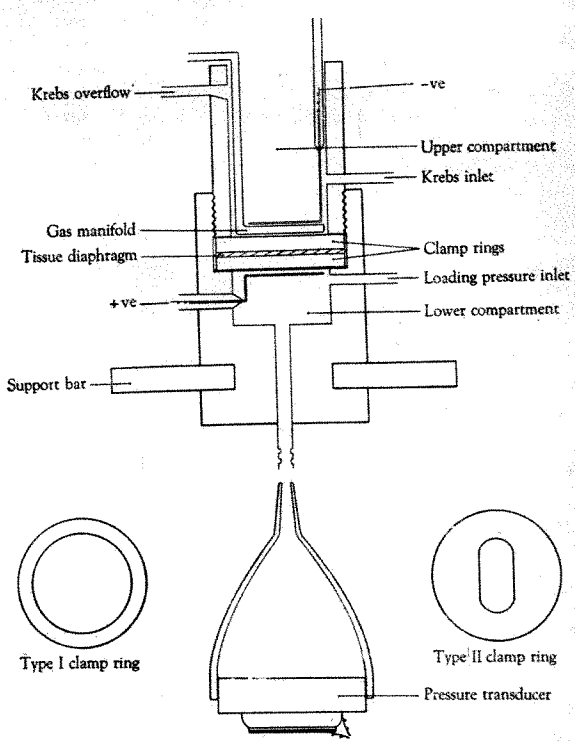


Fig. 1. Diagram of bath assembly with tissue-diaphragm in place ready for stimulation. Type I clamp rings are for tissues which can be obtained in adequate amounts; where less is available Type II clamp rings may be used

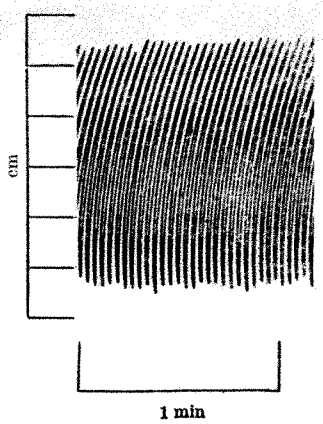


Fig. 2. Record of pressure fluctuations produced by contractions of the rat panniculus carnosus preparation as the tissue-diaphragm. Stimulation with supramaximal square-wave shocks of 0·04 msec duration. Frequency 0·5 c/s. Pen recorder

the stem was attached by polythene tubing to the lower compartment. The whole system, lower compartment, the connecting tube and funnel were filled with Krebs's solution so that pressure changes in the lower compartment were transmitted via the fluid in the system to the transducer.

The transducer formed one arm of an a.c. bridge the output from which was amplified, demodulated and recorded on a moving-coil pen recorder. Rapid fluctuation of pressure corresponding to the contractions and relaxations of the tissue-diaphragm and giving one full-scale deflexion per sec were well seen in this way (Fig. 2), though slower changes, such as those resulting from smooth muscle contraction, gave less satisfactory records.

This system of sensing pressure changes is essentially similar to that using differential inductive pressure transducers. We have also used these transducers in conjunction with an ultra-violet recorder (New Electronic Products, Ltd.), and with this method of recording excellent results were obtained with both slow and fast contractions. The great sensitivity and speed of recording of this method showed that with the rat panniculus carnosus preparation[2] the relaxatory phase was not merely the mirror image of the contractile phase as we had been led to suppose from pen recordings, but showed two distinct waves along its course. These are more fully described elsewhere[2].

The temperature of the solution in the upper compartment was controlled by running the Krebs's solution through glass coils in a constant-temperature water bath.

Transmural stimulation with coaxial electrodes[3], mostly of guinea pig ileum, has been fairly widely used since it was introduced by Paton, but coaxial disposition of electrodes can only be used in tubular tissues such as intestine or those which can be prepared as tubes such as tail skin[4,5]. The method described here permits application to a wider variety of problems.

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Biomicroscopic Observations on Vasoconstrictive and Thrombogenic Effects produced by Long-chain Saturated Fatty Acids within the Vasculature of the Wing of an Unanæsthetized Bat

Poole[1] reported in 1955 that when certain fatty acids in low concentration are added to plasma the clotting time is shortened. In 1959 he[2] modified Chandler's[3] system to make and to study artificial thrombi. In 1961, with Connor[4], he showed that when straight-chain saturated fatty acids with 16 or more carbon atoms were added to mixtures of blood in Chandler's system thrombus formation was accelerated and the thrombi so formed larger. Short-chain fatty acids, C_6 and C_7, had no activity and unsaturated acids were inactive, or very nearly so.

That these *in vitro* effects occur *in vivo* were shown by Connor[5] and Soloff and Day[6], who found massive arterial and venous thrombosis in dogs after a single intravenous infusion of palmitic or stearic acid.

Soloff and Day found that dogs which survived infusions of the saturated fatty acids had shorter silicon clotting, recalcification, and stypven times and lower platelet and polymorphonuclear cell counts than those dogs given unsaturated fatty acids. Kaolin produced thrombi and death similar to that seen after infusions with saturated fatty acids.

However, they could not obtain blood for coagulation studies from the dogs which died because the needle almost invariably became impaled upon a clot or upon gelatinous-like blood.

In an attempt to visualize the dynamic morphological changes culminating in thrombosis produced by intravascular injections of fatty acids, we have used Nicoll and Webb's[7] technique to examine the vasculature in the wing of the unanæsthetized bat.

As little as 0·01 c.c. of 0·01 per cent suspension of the sodium salt of stearic or palmitic acid injected into a major artery or vein in the unanæsthetized bat's wing produced unique vascular effects and thrombogenic effects which cannot be duplicated by much larger volumes of oleic or linolenic acid, inorganic solutions of comparable *p*H, glucose, saline or kaolin in concentrations of 0·001–0·1 per cent. Even amounts of kaolin sufficient to cover the entire circumference of the intima and occupy half the length of the major vessel and reduce its lumen to one-half or less fail to change the normal flow and characteristics of the bat's blood.

Immediately after the initial flush, saturated fatty acids injected intra-arterially produced an intense arterial vasoconstriction and thrombi. Palmitic acid appears to produce a more widespread vasoconstriction but the general characteristics of the vasoconstriction are identical for both substances. On the other hand, stearic acid is more thrombogenic.

Vasoconstriction is diffuse and occupies both the major vessel and all its branches and occasionally vessels other than those injected. Superimposed upon this diffuse constriction are more intense focal constrictions which have no recognizable relationship to architectural differences in the character of the vessel. It is, of course, possible that these focal constrictions may be at the origin of the vaso vasorum. The combination of diffuse and superimposed focal vasoconstrictions gives the vessel a unique appearance. When multiple focal constrictions are present, the artery may assume a beaded and rarely a violin-like shape. Vasoconstriction may persist for several hours to as long as several days (in one bat for 11 days). During this time, vasoconstriction may remain unaltered or wax and wane slightly due to vasomotion. Both the diffuse and focal constrictions are independent of nervous control because they can be produced in the denervated vessel. However, part of the persistence of the vasoconstriction is under nervous control because constriction that has persisted for hours or

days can be partly released by sectioning of the arterial nerve supply.

Blood within the constricted vessels oscillates back and forth except in that region where an unobstructed branch permits its exit.

At the same time, platelet thrombi are seen in the major arterial vessel and its branches. They are usually small and are quickly swept through the A/V pathways into the major vein and finally out of the field of vision. Some platelet thrombi may become adherent to the inner surface of the arterial wall. These adherent platelet thrombi most commonly are situated at the origin of bifurcations of the major vessels or their branches. These adherent platelet thrombi may persist for hours or days. Some platelet thrombi develop tails of white- and red-cell aggregates or such aggregations may surround the free margins of the platelet thrombi. Similarly, platelet thrombi which are swept into the major vein may also become adherent to the venous wall. Platelet thrombi in the vein may be much larger than those seen in the artery. An occasional platelet thrombus may be actually 2–3 times the diameter of the constricted artery. This large size is partly due to impaction of platelet thrombi on others upstream. Platelet thrombi, therefore, enlarge not only by aggregation of individual platelets, but also by embolization of one platelet thrombus on another. A platelet thrombus which is adherent to the internal surface of the vascular wall is rocked back and forth until it is finally, after hours or days, dislodged and swept forward again either to disappear into the central circulation or to join platelet thrombi upstream.

However, when the thrombus obstructs flow or when blood oscillates back and forth within the constricted vessel, white and red cell tails may also develop.

It is noteworthy that many more white blood cells are seen adhering temporarily to the vascular endothelium after an injection of an unsaturated fatty acid than after the injection of a saturated one, even after its vasoconstrictive effects have disappeared.

Intravenous injections of saturated fatty acids do not produce the vasoconstrictive effects seen after arterial injection. Platelet thrombi are, however, produced and are both more abundant and larger. When the platelet thrombus is large enough to obstruct or almost completely obstruct the lumen of the vein, the collateral circulation enlarges and takes over the flow of blood. These platelet thrombi may also develop a white and red cell tail.

Heparin prevents both these vasoconstrictive and thrombogenic effects.

The fact that saturated fatty acids are thrombogenic in the living dog and bat suggests that this property is common to the mammalian species and tends to unite Duguid's[8] hypothesis that atherosclerosis is a transformation of thrombosis with the more commonly accepted hypothesis that atherosclerosis is a fault of fat metabolism[9]. However, this work indicates that saturated fatty acids also produce vasoconstriction of arteries. This effect may be equally or more important than its thrombogenic effect in the etiology of atherosclerosis, which is an arterial disorder.

All biomicroscopic observations are being documented by cinephotomicrography.

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HISTOCHEMISTRY

Histochemical Demonstration of Monoamine Oxidase in the Earliest Phase of Wound Healing

A dynamic pathological process, such as the local response to injury, brings about the production of numerous substances by the injured cells. According to the prevalent view, directly vasodilative forces are augmented in the inflammation (for example, leucotaxin[1] or histamine[2]). To the best of my knowledge there has been no previous report of the other possible mechanism, inactivation of vasoconstrictive factors. The latter could indirectly contribute to the vasodilatation.

Monoamine oxidase deaminates serotonin (5-hydroxytryptamine), released by platelets[3]. In addition, catecholamines are involved. An increase in monoamine oxidase activity might, thus, lead to a decrease in these vasoconstrictive substances.

In the present experimental investigation on 27 male albino rats, circular skin wounds, 5 mm in diameter, were cut in a shaved dorsal area. The skin inside the circle was excised and the wounds were neither sutured nor dressed. The animals were decapitated at intervals of 1, 2, 4, 8, 16, 24, 48, 72 and 96 h after the injury. Skin flaps containing the wounds were removed immediately and frozen with isopentane, chilled to $-70°$ C.

Sections were cut in a cryostat at 20μ, incubated for 60 min at $+37°$ C in a solution described by Glenner[4], and further processed according to his method. Tryptamine was used as the substrate in all experiments, but in place of 'Nitro-*BT*', '*TNBT*' (2,2′,5,5′-tetra-*p*-nitrophenyl-3,3′-(*p*-biphenylene)-ditetrazolium chloride[5], Dajac Laboratories) was used as an electron acceptor in alternate sections. For controls, phenylhydrazine hydrochloride (0·01 M) served as a specific inhibitor of monoamine oxidase. In addition, alternate sections were stained with hæmatoxylin and eosin.

Activity of monoamine oxidase was indicated by formazan deposits, 'Nitro-*BT*' yielding blue and '*TNBT*' brownish granules. In the uninjured skin farther away from the wound the basal and spinous layers of the epidermis, and the outer root sheaths of the hair follicles showed intense enzyme activity. The most striking colouring was demonstrated in the sebaceous glands. The dermal fibroblasts exhibited only a faint monoamine oxidase reaction.

The first increase in monoamine oxidase activity could be seen in the wound periphery 1 h after the injury. The increase became more distinct after 2 h (Fig. 1). Monoamine oxidase active dermal fibroblasts could be demon-

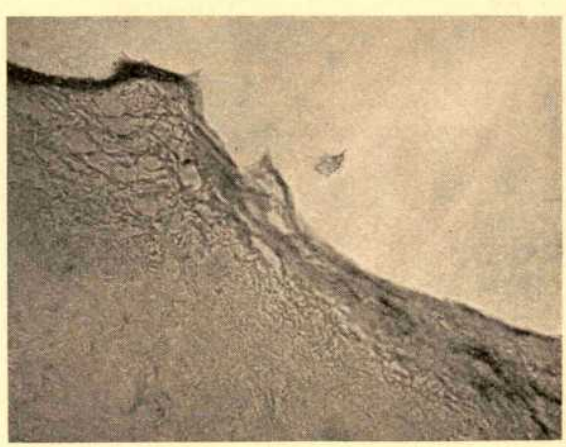


Fig. 1. Photomicrograph (× *c.* 83) of a 2-h injury. Monoamine oxidase activity appears peripherally to the edge of the wound. (Method of Glenner[4], by using 'Nitro-*BT*').

strated in a peripheral area, 200–500μ beyond the edge of the wound, whereas in the immediate vicinity of the edge the activity began decreasing. In a 4-h wound, the monoamine oxidase pattern resembled that observed after 2 h, the descent in the nearest surroundings of the injury being, however, more pronounced. In an 8-h wound, the activity had already diminished even in the peripheral fibroblasts. After 16 h, no increased activity of monoamine oxidase could be demonstrated in the wound periphery. Four hours after the injury, leucocytes began to invade the peripheral area, but no distinct leucocytic zone could be seen until 8 h had elapsed. Afterwards, the peripheral zone with increased cellularity took up a non-specific weak staining which resisted the pre-incubation inhibition. By contrast, both the increased monoamine oxidase activity demonstrable in 1-, 2-, 4-, and 8-h wounds, and the activity of the uninjured skin disappeared in the control sections, pretreated in the inhibitor solution. The enzymatic pattern observed by using 'Nitro-*BT*' was similar to that of '*TNBT*'. The latter tetrazolium salt, however, appeared to be more effective in detecting a relatively faint activity, for example, that observed in a 1-h wound.

In conformity with other enzyme reactions in wounds[6] the continuous decrease in monoamine oxidase activity in the nearest surroundings of the injury should be considered as an early sign of imminent necrosis. Even here, the hair follicles seem to be more resistant than the dermal elements.

In the wound periphery monoamine oxidase activity was increased from one to eight hours after the injury, disappearing thereafter. During the very first phase monoamine oxidase may, thus, contribute to the inflammatory vasodilatation which subsequently seems to be maintained by other factors.

One to eight hours post-operatively an initial increase in the activity of several hydrolytic and oxidative enzymes[6–9] and of transglucosylases[10] has been observed in the peripheral area of healing wounds. A defensive role, for example, in connexion with phagocytosis, has been ascribed to many of these enzymes[6–9]. The injury affecting the connective tissue cells in the peripheral wound zone seems, thus, to serve as a stimulus, activating local defence forces after a short mobilization time. Monoamine oxidase apparently joins these defence mechanisms by contributing to the initial vasodilatation, that is, the vascular response to injury.

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Carbonic Anhydrase and Formation of the Hen's Egg Shell

Mainly because of the observed inhibitory effects of sulphonamides on egg shell formation[1–3] and partly because the enzyme carbonic anhydrase is present in the reproductive tract of the laying hen[4], it has been generally assumed that this enzyme plays an important part in egg-shell formation by making available the carbonate ion. Nevertheless, while some workers[4,5] have attempted to correlate egg-shell formation with increased carbonic anhydrase activity of the uterine tissue of laying hens, little attention has been focused on the role the shell membrane might play during shell deposition. The keratin-like shell membranes[6] which enclose the egg-white are believed to be attached to the major part of the shell by a mammillary layer, so called because of the presence of numerous aggregates (the mammillæ) of a substance with the staining characteristics of a mucoprotein[7].

This preliminary report of work using histological staining techniques indicates that the mammillary layer can be considered both as a special layer of the outer shell membrane and as an initiator of shell deposition. Mammillary layer preparations were made by decalcifying pieces of shell for 60 min (or whole shell eggs for 24 h) in aqueous solutions of ethylenediamine tetraacetic acid (5 per cent w/v) adjusted to *p*H 8·5 with a 10 N sodium hydroxide solution. To aid microscopic observations the mammillæ and membranes were stained with aqueous alcian blue (1 per cent w/v) and acid fuchsin ($\frac{1}{2}$ per cent w/v) solutions respectively.

Fig. 1 is a surface view of the outer shell membrane obtained by decalcifying the shell of a whole egg. The inner shell membrane was discarded and the four layers of the outer shell membrane were exposed by tearing away an edge of the membrane with a pair of forceps. The outermost layer of the membrane to which the mammillæ are attached (Fig. 2) represents a part of the outer shell membrane additional to the three layers described by Moran and Hale[8]. Treatment for 1 h at 32° C of fresh unfixed samples of the mammillary layer with a solution containing cobalt sulphate and sodium bicarbonate according to Hausler's[9] modification of Kurata's[10] method (see ref. 11) has shown that the enzymatic activity of carbonic anhydrase is concentrated at the mammillæ.

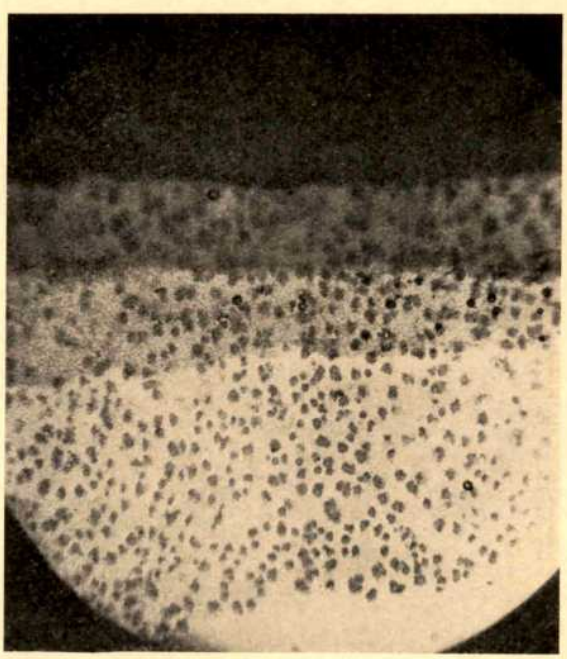


Fig. 1. Surface view of the outer shell membrane showing four layers. The mammillæ appear as dark spots on the outermost layer of the membrane. (× *c.* 30)

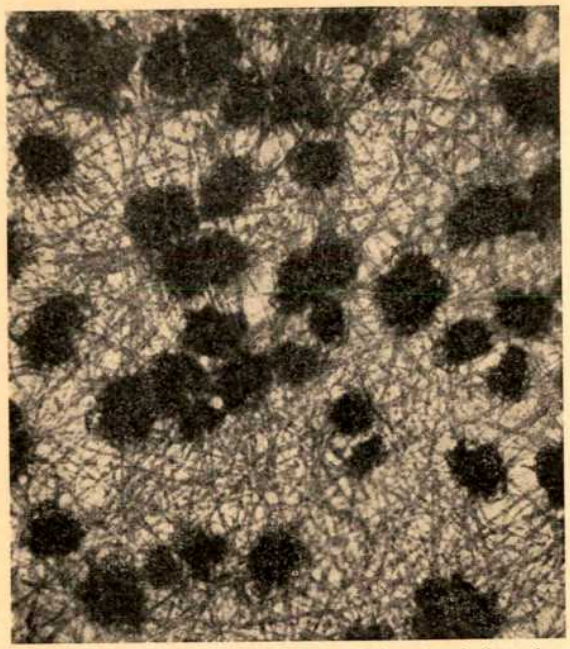


Fig. 2. Surface view of the outermost layer of the outer shell membrane showing the distribution of the mammillæ. (× *c.* 240)

Failure of membranes to retain cobalt either after boiling for 15 min or in the absence of bicarbonate ions and the observed inhibition of the staining reaction by 4×10^{-2} M 'Diamox' (2-acetylamino-1,3,4-thiadiazole-5-sulphonamide sodium) confirms the presence of carbonic anhydrase.

Studies with shell-less eggs removed from the uteri of laying hens confirm the presence of the fourth layer of the outer shell membrane before deposition of the shell, and the presence of carbonic anhydrase at the sites of the mammillæ. The enzymatic activity in the membranes of eggs removed from the uterus seems to be greater than that observed with membranes isolated from complete egg shells and in the former case an incubation period of 15 min was sufficient to demonstrate the enzymatic activity.

Although the overall mechanism of shell formation involving the transfer of calcium ion from the uterine fluid to egg shell is unknown, it seems likely that carbonic anhydrase situated at the mammillæ aids shell deposition by catalysing the decomposition of H_2CO_3, a component in the equilibrium reaction $2HCO_3^- \rightleftharpoons H_2CO_3 + CO_3^=$, and thus increases the amount of available carbonate ion at the sites of calcification. It is assumed that bicarbonate ions are readily supplied by the blood stream of the laying hen during shell formation.

We thank Cyanamid of Great Britain for a gift of 'Diamox' (sodium salt).

Note added in proof. Terepka[12], from studies with polarized light, has proposed that the shell consists of structural units extending radially from the mammillary projections. The identification of carbonic anhydrase activity at the mammillæ affords a possible mechanism for the initiation of such a development.

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PATHOLOGY

Experimental Production by 2-ethyl-2-phenyl Butyramide of Intrahepatic Cholestasis with Bile Plugs in Dogs

Oral administration of 2-ethyl-2-phenyl butyramide in dogs produces intrahepatic cholestasis with bile plugs in bile canaliculi. Intrahepatic cholestasis with bile plugs is observed in a small percentage of patients receiving chlorpromazine, C-17 alkylated steroids, or several other chemically unrelated drugs. Histologically[1], this condition is characterized by bile plugs in bile canaliculi and deposition of bile pigment in liver and Kupffer cells. Changes in hepatic parenchymal cells, usually in the form of feathery degeneration and more rarely in the form of focal necrosis, are regarded as secondary to the biliary stasis. Infiltration of portal tracts was reported occasionally. Electron microscopic examination[2] of these livers showed dilation of some bile canaliculi and alteration in the microvilli of practically all; the microvilli were either absent or stunted and irregular in shape. Although functional[3] as well as histochemical and ultrastructural[4] changes were demonstrated in the livers of rats dosed with C-17 alkylated steroids, typical intrahepatic cholestasis with bile plugs has not been produced by a drug in normal animals[1].

The compound 2-ethyl-2-phenyl butyramide—$C_3H_5(C_6H_5)(C_2H_5)CONH_2$ was described in 1926[5] as a hypnotic with severe hepatic toxicity, but no histological data were reported at that time.

Dogs receiving 50 mg/kg/day of this compound for 4 days showed in hæmatoxylin–eosin stained paraffin sections typical bile plugs in bile canaliculi without significant changes in hepatic parenchymal cells or infiltration of portal tracts. The combined *BSP–PSP* liver and kidney test showed significant *BSP* retention and normal *PSP* elimination[6].

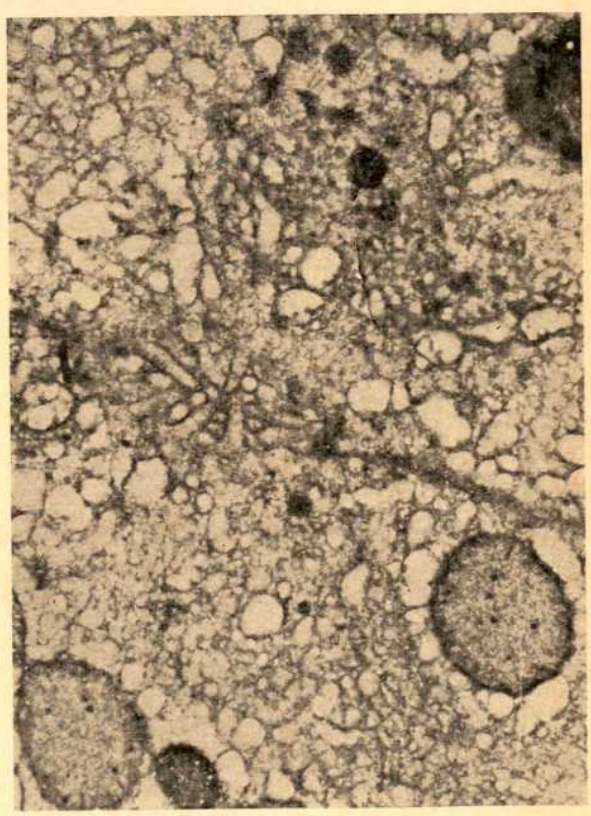


Fig. 1. Electron photomicrograph of liver from dog dosed orally for 4 days with 50 mg/kg/day 2-ethyl-2-phenyl butyramide. Canaliculus with distended interlacing microvilli is shown in the centre. Above and to the left of the canaliculus electron dense material is present in the Golgi vesicles. Uranyl acetate stained section. (× 18,600)



Fig. 2. Electron photomicrograph of same case as Fig. 1. Canaliculus distended with bile plug. (× 8,800)

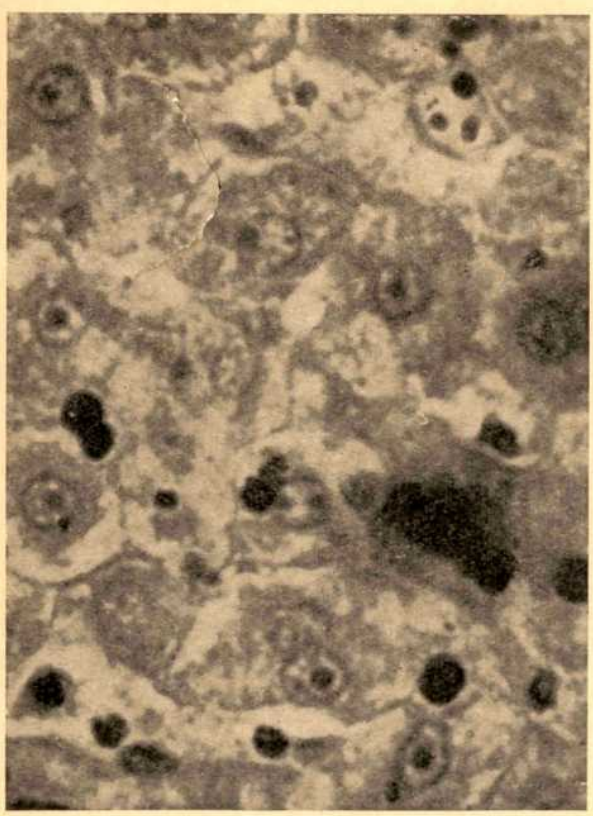


Fig. 3. Hæmatoxylin and eosin stained liver section from dog dosed orally for 11 days with 50 mg/kg/day 2-ethyl-2-phenyl butyramide. Bile plugs in canaliculi and degenerative changes in liver cells

The electron microscope revealed some canaliculi filled with dense material which obscured the microvilli; these corresponded to the bile plugs seen in light microscopic sections. More commonly we found canaliculi with long slightly distended interlacing microvilli and indistinct lumina with little or no bile. Adjacent to many of these altered canaliculi electron-dense material occupied the vesicles associated with the Golgi apparatus (Fig. 1). In other instances a canaliculus filled with bile was seen which appeared ready to burst into an adjacent space between two hepatic cells (Fig. 2). In this space microvilli appeared relatively normal.

The administration of the same dose for 11 days showed in hæmatoxylin–eosin stained paraffin sections severe intrahepatic cholestasis with feathery degeneration, single cell necrosis, and acidophilic bodies (Fig. 3).

Red blood cell survival times studied with Squibb 'Chromitope Sodium' in control dogs and in dogs dosed with 2-ethyl-2-phenyl butyramide showed no significant differences between test and control animals; this means hæmolysis had not occurred.

The availability of a simple chemical substance which will produce within a very short time intrahepatic cholestasis in dogs appears to be a worthwhile tool for the investigation of the mechanism of bile secretion and the pathogenesis of intrahepatic cholestasis. We are at present investigating the effect of 2-ethyl-2-phenyl butyramide on the morphology and histochemical reactions of the rat liver.

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Homograft Antigens: Isolation of Fractions of Normal Cells which retain Activity after Freeze-drying

In previous reports[1], we have described the isolation from splenic and lymph node cells of homograft antigens which required immediate bioassay, because they lost antigenic activity rapidly on standing and were almost totally inactivated by simple freezing or by freeze-drying. These early preparations, however, afforded materials which enabled us to develop an assay procedure and to examine some of the properties of antigenic fractions. In the course of these examinations, newer methods of antigen preparation were devised, based on the isolation of subcellular fractions from sucrose solutions. Brent *et al.*[2], Manson *et al.*[3] and Davies[4] have recently reported the preparation of transplant antigens from sources and by methods that differ from those described below.

Mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, with either *A/Jax*, *C3H*, *C57BL6*, or *Balb/c* used as the donor strains, and male *C57BL6* or male *A/Jax* as the recipient strain. Antigens were prepared from the tissue of either all male or mixed male–female populations. All animals were allowed to reach 20 g in weight before use.

As the source of antigenic materials, we used 30–50 spleens or pooled sets of lymph nodes (cervical, axillary, infrascapular, mesenteric, and inguinal) or 70–80 thymus glands. Organs were collected and rinsed in cold buffered sodium chloride solution (0·13 M sodium chloride, 0·01 M

phosphate, *p*H 7·2–7·4) followed by rinsing with solution *A* (0·25 M sucrose, 0·03 M ethylenediamine tetraacetic acid adjusted to *p*H 7·2–7·4 with potassium hydroxide, sp.g. about 1·03). 5–8 spleens were sliced longitudinally on a 4–5-in. square of 40-mesh stainless steel screen and were pressed through it with the end of a flexible spatula blade. Lymph nodes and thymus glands were cut coarsely with a small scissors and fragmented directly in a homogenizer. Spleens were kept wet with solution *A* and eventually washed into the plunger-type homogenizer previously described[5], with short intense bursts of the same solution *A* delivered from a polyethylene wash bottle. A final volume of 3–4 ml. per spleen or set of lymph nodes has proved convenient but not critical. The cellular material was then comminuted in the homogenizer with a 'loose' plunger (10–12 complete cycles), and the resulting homogenate was placed in 250 ml. polypropylene bottles and sedimented twice for 15 min at about 1,550*g* (max.) in order to remove nuclear debris, whole cells, etc. The sediments were combined and reserved for a 'chemical cell-count' by DNA estimation, since solution *A* causes irreversible aggregation of cells and makes a direct count in a hæmocytometer impossible.

The cloudy supernatant suspension was skimmed as free of floating fat as possible and sedimented for 1 h at 30,000 r.p.m. in the No. 30 rotor of a model L Spinco preparative ultracentrifuge (105,000*g* max.). The supernate, I, was decanted from the sediment, II, and the sedimented pellets were gently rinsed with 0·25 M sucrose.

Sediment II was further fractionated by centrifugation at successively greater speeds in solution *B*, 0·25 M sucrose, 0·002 M ethylenediamine tetraacetic acid (adjusted to *p*H 7·5–7·6 with potassium hydroxide). Volumes of 0·75–0·9 ml. per spleen or set of lymph nodes have proved convenient.

The suspension in solution *B* was sedimented in the following sequence of rotational speeds in the indicated head or rotor of the Spinco preparative ultracentrifuge (model *L*), the sedimented materials being collected at each speed: (*a*) 5,000 r.p.m. for 60 min in head *SW* 25 (4,000*g* max.); (*b*) 8,000 r.p.m. for 60 min in the same head (8,000*g* max.); (*c*) 20,000 r.p.m. for 120 min in rotor number 30 (60,000*g* max.), and (*d*) 40,000 r.p.m. for 60 min in rotor number 40 (140,000*g* max.). By means of this scheme the following fractions were isolated in solution *B*: (*a*) a dark gelatinous pellet, III; (*b*) a gelatinous pellet, somewhat turbid in appearance, IV; (*c*) a large gelatinous pellet, shaded yellow, V; (*d*) another gelatinous pellet, VI. For the sedimentations at 5,000 and 8,000 r.p.m., a Spinco *SW* 25 (swing) head was required in order to recover the poorly packed layers. At the higher speeds Spinco No. 30 or 40 rotors (angle head) were satisfactory.

All sediments were resuspended in 0·002 M ethylenediamine tetraacetic acid (*p*H 7·2–7·4) and were centrifuged for about 5 min at 250*g* to sediment adventitious material. Aliquots were then removed for biological assay, chemical assay, and freeze-drying. The aliquots for bioassay were brought to their final volumes with buffered 0·13 M sodium chloride, so that the desired dose was contained in a volume of 2 ml. The results of a typical bioassay[5] are outlined in Table 1. (In the scoring system in Tables 1 and 2, the integers 0–6 correspond to 0, 10, 25, 50, 75, 90 and 100 per cent survival of epithelium. Zero, therefore, represents maximal measurable antigenicity.)

Table 1. Fractionation of Lymph Node Cells and a Comparison of their Activities. Experiment 63-17 (*Balb/c* to *C57BL6*)

Fraction No.	Score (Averages of scores of 6 or 7 animals)	Dose per animal mg (dry weight)	Recovered total dry weight (mg per donor animal)
III	0·6	2·2	2·3
IV	0·1	0·8	0·7
V	1·1	1·0	1·0
VI	2·8	0·3	0·15

As can be seen in Table 1, all fractions show considerable activity, but our results have shown the consistent pattern of appearance of the greatest activity in fractions IV and V. These correspond to fractions containing membranes of the cytoplasm and cell surface. The use of ethylenediamine tetraacetic acid probably causes the complete disassociation of microsomal RNA–protein–lipoprotein complexes, so that the morphological features of the active fractions would be obscured by this treatment.

Preliminary efforts at sucrose gradient sub-fractionation of sediment II have afforded several active sub-fractions. One fraction was recovered between sp.g. 1·17 and 1·18, and a second between sp.g. 1·125 and 1·150 (appropriate layered sucrose concentrations, 0·002 M in ethylenediamine tetraacetic acid brought to *p*H 7·3–7·5 with potassium hydroxide). Separation was accomplished in an *SW* 25 head of a Spinco model *L*, 25,000 r.p.m. for 120 min. These observations are now being extended.

All the fractions described here can be freeze-dried in 0·002 M ethylenediamine tetraacetic acid solution (*p*H 7·2–7·3) with retention of a high degree of antigenic activity when re-assayed as long as 25 weeks after preparation. Activity is also well preserved after storage overnight at 3° C in the same solution. Typical data are shown in Table 2. Freeze-drying was carried out in an apparatus of our own design, under pressure of 0·03–0·05 mm mercury. Drying was completed in 2–3 h, and all samples were collected under pre-purified (water-pumped) nitrogen and stored in the freezing compartment of an ordinary refrigerator (−5° to −12° C).

Table 2. Comparison of Freshly Prepared Antigen with Freeze-Dried Material

	Age of Antigen	Dose in dry wt. (μg)*	Recipient strain	Score (Averages of groups of 6 or 7 animals)
Exps. 62-13 (*C57BL6* Spleen) Fracts. IV and V (0·57 mg recovered per donor animal)*	Fresh	200	*A/Jax*	0·3
	Freeze-dried (8 wk.)	350	*A/Jax*	0·9
	Freeze-dried (25 wk.)	200	*A/Jax*	0·2
Exps. 63-3. (*C57BL6* lymph node and spleen) Fract. V (0·70 mg recovered per donor animal)*	Fresh (not tested)			
	Freeze-dried (3 days)	1,080	*A/Jax*	0·1
	Freeze-dried (22 days)	490	*C3H*	0·9, 1·0
	Freeze-dried (23 days)	110	*C3H*	1·0
		110	*A/Jax*	1·0
		110	*Balb/c*	0·3

* Not corrected for inclusion of EDTA.

Although we have prepared antigenic fractions from pre-frozen tissue, the activities of these fractions have not been as high, on a per organ basis, as material prepared from fresh organs.

Analyses, carried out in attempts to define even broadly the classes of materials present in the various sub-fractions, have indicated only that the active material is essentially lipoprotein. When applied to this material, procedures which are known to denature lipoproteins cause the loss of antigenic activity (deoxycholate, 'Triton *X*-100', organic solvents, etc.). The difficulties presented by the contamination of micro-samples with relatively large amounts of absorbed ethylenediamine tetraacetic acid and sucrose have been solved in part by dialysis. In general, the antigenic materials have shown the following ranges of composition: lipid, 30–60 per cent (lipid sterol as cholesterol, 40–70 per cent of lipid); protein, 20–40 per cent; DNA, less than 0·1 per cent (when detected by colorimetric determination with indole[6,7]); RNA less than 5 per cent (as determined by ultra-violet absorbance at 260 mμ of the appropriate Schmidt–Thannhauser fraction[7]).

While 0·25 M sucrose is a customarily used concentration[8], we found that it was necessary to use an ethylenediamine tetraacetic acid concentration of 0·03 M in order to suppress the tendency of DNA to disperse in solutions

of low ionic strength. This has not been completely successful since the amounts of DNA appearing in the supernatant of sediment I have been found to be about 5 per cent of the total DNA recovered in the typical preparation. Similar observations have been reported, for example, in the fractionation of ascites tumour cells[9]. Although DNA-histone is found to be less than 0·1 per cent in our most active preparations, we cannot yet rule out the nucleus as the source of some antigenic material.

The material described by Davies[4] which is isolated from ascites tumour fluid is a lipoprotein that is active in the hæmagglutination inhibition system of Gorer. More recently, Manson *et al.*[3] have reported on a material isolated from a strain-specific mouse spleen cell and lymphosarcoma, grown in cell culture, which also appears to be a lipoprotein. It is reported to be active in a transplant system, but differences in the assay system that they have used and our assay system preclude a comparison of the two preparations.

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'Refuin': a Non-cytotoxic Carcinostatic Compound proliferated by a Thermophilic Actinomycete

'Refuin' is a high-molecular fraction of a fermentation beer produced by a thermophilic actinomycete. The fraction is recovered by precipitation with ammonium sulphate and by fractional ice crystallization. Although the fermentation temperature is 50° C, the active principle is heat-labile, losing all activity after 6 h at room temperature. Electrophoretic analysis reveals a ninhydrin staining positively charged band.

The morphology and general physiology of the *Thermoactinomyces* sp. producing 'Refuin' was determined by methods previously described[1].

'Refuin' was evaluated for antibiotic activity, *KB*-cell line tissue culture toxicity, and for carcinocidal or carcinostatic activity on four mouse tumour systems: sarcoma 180, carcinoma 755, leukæmia 1210, and Ehrlich ascites.

Activity against Gram-negative organisms was not noted. The Gram-positive activity was highly specific. Although *B. subtilis* was not inhibited and *S. aureus* 209 *P* was only slightly inhibited, *Mycobacterum smegmatis*, *Torulopsis albida* and *S. aureus* respiratory deficient mutant culture *AT CC UV* 2–13680 were strongly inhibited.

The anti-tumour activity in the sarcoma 180, adenocarcinoma 755, and leukæmia 1210 was conducted according to the National Institutes of Health protocols 1 : 301–302 of June 1962. A daily intraperitoneal injection of 50 mg/K of 'Refuin' causes a 75 per cent inhibition of the sarcoma 180 tumour and a 60 per cent inhibition of the adenocarcinoma 755 tumour. No leukæmia 1210 activity was observed.

The Ehrlich ascites assay results are summarized in Table 1. 10^6 ascites cells were inoculated intraperitoneally and immediately followed by a subcutaneous injection of 50 mg/K of 'Refuin'. Although the subcutaneous route is not reported to be effective for administration of other anti-tumour agents, 'Refuin' showed significant activity when administered either subcutaneously or intraperitoneally. Although the intraperitoneal route gives better results, the subcutaneous route permits more critical evaluation of our system. Of special interest was the effect of 'Refuin' in converting the fluid ascitic cells to a small solid tumour in 45 per cent of the treated mice. Upon continued administration of 'Refuin', necrosis and ultimate disappearance of this tumour resulted in many of these mice.

Table 1. Activity of 'Refuin' on Ehrlich Ascites Mouse Tumour System when Administered Subcutaneously

	Treated group	Control group
Number of mice	100	47
Number of mice showing conversion of ascites to solid tumours	45	0
Average survival of those mice showing progressive development of ascites	19·9 days	9·8 days
Number of mice showing total disappearance of ascites and tumour	18	0

Mortalities or excessive loss of weight due to administration of 'Refuin' were not noted. A LD_{50} on unimplanted mice was not obtained since the maximum dose that could be conveniently administered intravenously (700 mg/K) proved non-lethal.

In the *KB* cell line tissue culture system 'Refuin' caused negligible inhibition. This would suggest that the oncostatic activity of 'Refuin' is dependent on the host defences. This lack of tissue culture activity, the lack of systemic toxicity, and its very limited antibiotic spectrum differentiates 'Refuin' from the other anti-tumour agents known at present.

All drugs other than hormones and steroid that are of value in cancer therapy have shown considerable toxicity. As stated by Moore[2] toxicity is a prerequisite for anticancer activity. Indeed, since the drugs presently used for the palliation of neoplastic disease exert their greatest effect on rapidly dividing cells, they also induce toxic symptoms in bone marrow, gastrointestinal mucosa, skin, liver, and lymph nodes. The clinical evaluation of 'Refuin' now in progress has confirmed the lack of systemic toxicity of this drug and gives promise of confirmatory clinical evidence of anti-tumour activity.

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IMMUNOLOGY

Preparation of an Antiserum Specific to a Spontaneous Mouse Leukæmia after the Induction of Artificial Immunological Tolerance to Normal Mouse Tissue

The feasibility of preparing antiserum to the transplantable Ehrlich ascites tumour after the induction of immunological tolerance to normal mouse tissue antigens in the neonatal rabbit has been reported previously from this laboratory[1,2]. Such tolerant anti-Ehrlich ascites tumour sera, when tested *in vivo*, were toxic for the tumour and relatively innocuous for the murine host.

Non-tolerant anti-Ehrlich ascites tumour sera displayed far greater murine toxicity, and no greater, or decreased, protection against the transplanted tumour. Antisera to normal tissues from animals previously made tolerant to the same tissues have no increased anti-tumour effect, and non-tolerant anti-normal tissue sera have not only no increased anti-tumour activity, but are highly murine toxic. Other authors, using *in vitro* tests, have reported similar findings on other tumours[3,4].

Since the Ehrlich ascites tumour is not spontaneous in the strain of mice used (*C3H*), the specificity reported cannot unequivocally be attributed to antibodies directed against antigenicities unique to the malignancy, but may represent antibodies to antigenicities of the original strain in which the tumour arose which are not represented in *C3H* mice. In order to see if protective specificity to a spontaneous tumour could be demonstrated after the induction of artificial immunological tolerance toward normal tissue antigens, experiments were begun using isogenic *AKR/Jax* mice and the lymphatic leukæmia which arises spontaneously in this strain. The preliminary experiment using this system is reported here.

By a method similar to that used with the Ehrlich ascites tumour system[1,2], immunological tolerance toward a homogenate (consisting mostly of whole cells) of normal *AKR/Jax* mouse spleens, thymuses, livers, lungs, and peripheral and mesenteric lymph nodes was induced in neonatal Dutch rabbits. The first injection, containing 50 mg of tissue (wet weight), was given within 12 h after birth. Ten more daily injections of the same amount of tissue were given followed by another ten injections each containing 100 mg (wet weight) of tissue. During the 10th post-natal week, these rabbits were divided into two groups. One group received 1·25 g (wet weight) of a similar tissue homogenate from leukæmic mice, given in four injections every other day (tolerant anti-leukæmia serum). The second group received the same amount of tissue according to the same injection schedule from normal mice (tolerant anti-normal tissue serum). Litter-mates uninjected at birth received like injections of one of the homogenates from either leukæmic or normal mice (non-tolerant anti-leukæmia serum, and non-tolerant anti-normal tissue serum). All rabbits were bled for antiserum 14 days after the last immunizing injection.

The spleens, and peripheral and mesenteric lymph nodes from leukæmic *AKR/Jax* mice were obtained and minced and the single cells were collected in 0·9 per cent sodium chloride; 7×10^7 cells were injected subcutaneously into normal *AKR/Jax* mice. A total of 1·2 c.c. of one of the antisera or normal rabbit serum was injected by the intraperitoneal route into these animals: 0·4 c.c. within 2 h after tumour administration, and 0·4 c.c. on each of the two subsequent days. The survival of mice in each of the groups is given in Table 1.

Table 1. Effects of 1·2 c.c. of tolerant and non-tolerant anti-*AKR* leukæmia and tolerant and non-tolerant anti-*AKR* normal tissue sera on the survival of *AKR* mice inoculated with 7×10^7 leukæmic cells

Antiserum given	No. of mice per group	Mean survival (days)	Last day of survival of any group member
Tolerant anti-leukæmia	6	22·2	27
Non-tolerant anti-leukæmia	6	16·7	25
Tolerant anti-normal tissue	3	16·7	19
Non-tolerant anti-normal tissue	4	17·8	19
Normal rabbit serum	4	15·5	18

It can be seen that the tolerant anti-leukæmia serum conferred a survival advantage on mice so treated that was not seen in any other group. The mean survival for this group was 22·2 days, and the last surviving mice were also in this group. Tolerant and non-tolerant anti-normal tissue serum survival levels did not appreciably differ from those seen for normal rabbit serum. The mean survival of mice receiving non-tolerant anti-leukæmia serum was of the same order, although one mouse did survive almost as long as some members of the tolerant anti-leukæmia group.

It should be noted that mice receiving the non-tolerant anti-leukæmic or anti-normal *AKR* sera did not regularly display the immediate murine toxicity seen when comparable amounts of non-tolerant anti-Ehrlich ascites tumour serum or non-tolerant anti-*C3H* normal tissue serum were administered to *C3H* mice in previous experiments with this system[1,2]. Only one mouse given non-tolerant anti-leukæmia serum succumbed from the immediate effects of the antiserum. This may be due to toxic characteristics of antisera against the *AKR* normal tissue or leukæmia, different from those against the *C3H*-Ehrlich ascites tumour system. However, the more probable explanation is that the anti-*AKR* sera were generally weaker, resulting from the circumstances that the rabbits were immunized with four rather than the usual six injections. This situation occurred because of a lack of available injection antigen.

It thus appears that while tolerance to normal tissues is induced in neonatal rabbits by the injection of normal antigens of an isogenic strain of mice, this does not prevent the formation of antibodies, on subsequent immunization with a leukæmia arising spontaneously in this strain. It is probable that some or all of these antibodies are specific for antigens found uniquely in the malignant state.

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Persistence of Antigen in Nucleoprotein Fractions of Mouse Spleen Cells during Antibody Formation

The fate and distribution of antigenic determinants in immunized animals is largely unknown[1]. There has been much speculation and experimentation concerning persistence of antigen during the period of antibody formation, as well as possible relationship of persisting antigen to specific immunological tolerance[2,3]. Recent work has indicated that neonatal injections of soluble antigens derived from *Shigella* micro-organisms may result in specific immune unresponsiveness in young mice[4,5]. It was postulated that 'excess' antigen, analogous to pneumococcal polysaccharide, may persist for extended periods of time in the mouse tissues, and thus induce an unresponsiveness similar to 'immunological paralysis'[6].

In an attempt to learn more about the normal fate and biological activity of *Shigella* antigens injected into mice, an investigation was designed to test for possible persistence of antigen in mouse spleens at various periods following immunization. For this purpose *NIH* white mice, 6–12 weeks of age, were injected intravenously with 0·1–0·5 ml. soluble antigenic material (20 μg N) derived from *Shigella paradysenteriae* by partial trypsin digestion of a 10 per cent suspension of alcohol-killed *Shigella*[4,7]. Serum samples were obtained by retro-orbital puncture from each mouse at various times following injection to ascertain agglutinin response[4]. Groups of such immunized mice were killed at 1 day, 1, 2 and 3 weeks following injection and spleen cell suspensions prepared in Hanks's balanced salts solution. Cell-free homogenates were prepared using 'Teflon'-tipped tissue homogenizers at 4° C. Nuclei and mitochondria containing fractions were obtained by differential centrifugation, while ribonucleoprotein (RNP) and deoxyribonucleoprotein (DNP) rich fractions were obtained by salt extraction procedures[8,9].

No detectable *Shigella* antigen or anti-*Shigella* antibody could be observed in any of the fractions, except in those

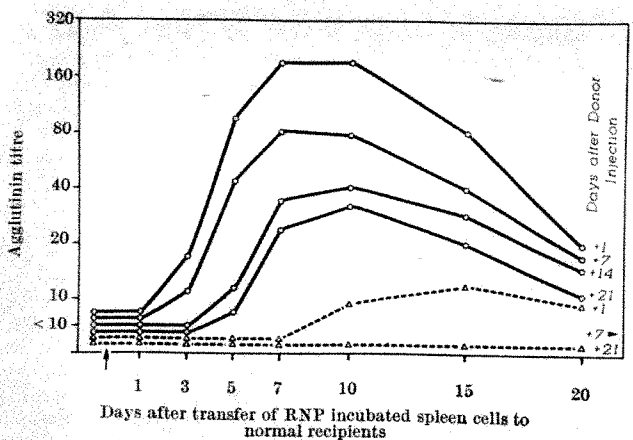


Fig. 1. Agglutinin formation in normal, non-immunized recipient mice receiving normal mouse spleen cells which had been incubated with spleen ribonucleoprotein fractions (RNP) derived from *Shigella* immunized donor mice. Each curve represents mean titre of at least six recipients. 0·4–0·9 mg N of immune spleen RNP incubated for 1 h with 2–9 × 10^7 normal spleen cells, either untreated (0) or heated for 30 min at 56° C (△) prior to incubation. RNP prepared by salt extraction method (ref. 9) 1, 7, 14 and 21 days after intravenous injection of donor mice with 0·5 ml. soluble *Shigella* antigen (20 μg N)

obtained one day following injection, by means of complement fixation, tanned cell hæmagglutination, precipitin, and agglutination tests. Injection of these fractions into non-immune recipient mice failed to elicit a marked anti-*Shigella* response. In order to test further for possible presence of *Shigella* antigenic material in the sub-cellular spleen fractions 1·0-ml. samples (0·4–0·9 mg N) were incubated for 1 h with 1·0 ml. sterile Hanks's solution containing 30 per cent normal rabbit serum and 2–9 × 10^7 normal spleen cells obtained from another group of non-immunized *NIH* mice, 6 to 10 weeks of age. Following incubation, each cell suspension mixture was carefully washed three times with sterile Hanks's solution by serial centrifugation and injected intravenously into a fresh group of non-immunized *NIH* recipient mice, 6–10 weeks of age. One mouse was used as a recipient for each incubation mixture. At least six spleen cell suspensions were incubated for each original spleen cell fraction. Controls consisted of: (1) similar incubation mixtures containing cell suspensions previously heated at 56° C for 1 h prior to incubation; (2) cell suspensions incubated with saline instead of spleen cell fractions; (3) cell suspensions incubated with cell fractions obtained from control, non-immunized donor mice.

Serum samples were obtained from each recipient mouse at 1, 3, 5, 7, 10, 15 and 20 days following transfer and tested for anti-*Shigella* agglutinins. Nearly all the mice receiving normal, unheated spleen cell suspensions incubated with the immune spleen sub-cellular fractions responded with readily detectable agglutinin formation within 3 to 5 days following cell transfer (Table 1). Very few recipients receiving control-heated spleen cells incubated with donor spleen cell fractions responded with any agglutinins. Those that did had lower titres and a marked delay in the appearance of agglutinins. No recipient receiving normal spleen cell suspensions incubated with saline only, or incubated with fractions from normal control donors, revealed any agglutinin formation above the threshold level of measurement. Other experiments failed to reveal a similar agglutinogenic effect with sub-cellular fractions from liver or lung tissues from similar immunized donor mice.

Table 1. AGGLUTININ FORMATION IN NON-IMMUNIZED MICE RECEIVING NORMAL SPLEEN CELL SUSPENSIONS INCUBATED *in vitro* WITH SPLEEN CELL FRACTIONS FROM *Shigella* IMMUNIZED DONOR MICE*

Immune spleen cell fraction†	Day following *Shigella* injection‡ 1	7	14	21
RNP	196 (11/14)§	68 (10/12)	49 (7/11)	47 (8/10)
DNP	48 (8/15)	31 (6/10)	21 (3/8)	8 (0/10)
Mitochondria	75 (7/11)	32 (4/8)	30 (3/7)	15 (1/6)
Nuclei	69 (8/10)	48 (4/9)	10 (1/8)	8 (0/8)
Whole homogenate	132 (10/11)	62 (7/11)	40 (2/6)	28 (3/8)

* 2–9 × 10^7 normal, washed spleen cells incubated with 0·4–0·9 mg immune spleen cell fractions.
† Prepared from immune whole cell suspensions by differential centrifugation and salt extraction procedures (refs. 8 and 9). Negative results obtained with similar fractions from non-immunized donors.
‡ Original donor mice injected intravenously with 0·5 ml. soluble *Shigella* antigen (20 μg N).
§ Mean peak agglutinin titres of recipient mice. No. of recipients with peak titres 1 : 20 or greater per number receiving incubated spleen cells.

The rapid appearance of agglutinins in normal mice receiving the fresh normal spleen cells incubated with sub-cellular fractions from immunized donors suggested that immunogenic quantities of *Shigella* antigens were present in the original fractions and could stimulate an agglutinin response by the incubated spleen cell suspensions. The major immunogenic activity of the donor spleen cells appeared to be associated with ribonucleoprotein-rich fractions (Table 1 and Fig. 1), although all the fractions from immune donors incubated with normal spleen cells stimulated some degree of agglutinin response. RNP fractions obtained from donors 3 weeks after immunization, when agglutinin responses were diminishing, still revealed agglutinogenic activity (Fig. 1), while the other fractions, including homogenates, nuclei, mitochondria and DNP-rich material, had either low or no immunogenic activity at the second or third week following immunization.

The results presented are in agreement with suggestions by others that immunogenic activity of antigenic determinants may persist for some time in tissues of immunized animals[10,11]. However, the possibility of the transfer of *de novo* antibody-forming capacity by means of immune spleen cell nucleoproteins or sub-cellular fractions, as suggested previously[12,13], has not been eliminated by these experiments.

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RADIOBIOLOGY

A Rapid Method for Estimation of Iodine-131 in Milk

Cosslett and Watts[1] have described a method for the removal of iodine-131 from milk by means of an anion exchange resin prepared in the chloride form. They reported 96–98 per cent retention of radioactive iodine by the resin and assumed that the iodine residual in the milk was present as a non-ionic species. Lengemann and Swanson[2], Garner[3] and Morgan[4] have all found non-ionic iodine (that is, protein-bound) in cow's milk, whereas Glascock[5], Wright, Christian and Andrews[6] believed that all physiologically occurring iodine-131 was completely ionized.

Morgan[4] concluded that resin treatment of cow's milk was of value only in removing ionic iodine. In order to clarify this point, the following experiment was performed. A 5-l. pooled sample of milk, representing a collection from about 300 cows, was subjected to ultrafiltration. At

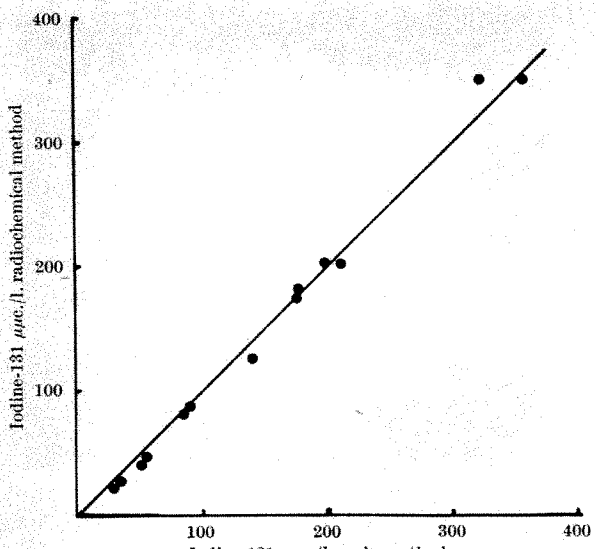


Fig. 1. Comparison of methods

this time the iodine-131 concentration in milk was about 100 μμc./l. as a result of fall-out from weapon testing. Radiochemical analysis of the ultrafiltrate and residue indicated that 60 per cent of the iodine-131 was present in the ultrafiltrate. Samples of the original milk were then passed through an anion exchange resin column. Duplicate analysis showed 100 per cent retention of iodine-131 by the resin. From this we concluded that radioactive iodine complexed with protein was quantitatively removable by resin treatment.

Details of our experimental technique for the estimation of iodine-131 in milk are:

50 ml. of wet 'Deacidite *FF*', 3–5 per cent cross-linking (*SRA* 63) 14–52 mesh in the chloride form, are transferred to a column of dimensions 25 cm × 1·5 cm. One litre of fresh milk is then passed through the column at a flow-rate of 5 ml./min. On completion the resin is blown out of the column into a clean beaker using compressed air, washed twice by decantation with 100 ml. demineralized water and transferred to a polystyrene container (6·5 cm diam. × 2 cm high) which fits on top of a sodium iodide crystal. We have shown that this washing procedure causes no loss of activity. Indeed, the resin can be acetone-washed and air-dried without any measurable loss. The iodine-131 content is determined in a γ-spectrometer using the 0·364 MeV photopeak. With a 3 in. × 3 in. sodium iodide (Tl activated) crystal, the detection limit is 10 μμc. with a precision of 15 per cent if counting is for 50 min.

Because of our success in removing iodine-131, we compared the reproducibility of the method to our standard radiochemical procedure[7] after spiking milk with iodine-131 in the ionic form. Details are given in Fig. 1.

Our technique agrees well with that described by Boni[8], and we have found the method reliable over a period of several months.

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Splitting of Uracil Dimer by High-energy Electrons

Thymine or uracil in frozen aqueous solutions are readily converted into their dimers by ultra-violet irradiation[1–3]. Using ion exchange chromatography on 'Dowex 1', the dimers of both pyrimidines-2-^{14}C were prepared in chromatographic homogeneous forms. Recently, it was established by us that thymine dimer is easily split into thymine monomer by irradiation with high-energy electrons[4].

During the experiments recorded here an aqueous solution of uracil dimer (120 μg/ml.) was irradiated with 100 kr. electrons of 34 MeV at room temperature. After exposure to β-rays 30 per cent of the radioactivity of the uracil labelled with carbon-14 were shown to be situated in monomer uracil by the method of paper chromatography. By the same dose of 200 kV X-rays the splitting effect could not be found at this high quantity, while the damages of the monomer molecules rose significantly. Similar results have recently been published for X-irradiation of thymine dimer[5].

The easy splitting of thymine dimer may be of some biological importance in connexion with the restoration of damaged cells. Dimerization of pyrimidines in the nucleic acid chain is also believed by some authors to cause lethal or mutagenic effects by ultra-violet irradiation[1,6]. Therefore, several experiments were carried out on the reactivation of ultra-violet irradiated bacteria, and our preliminary results indicate a rise in survival of ultra-violet irradiated bacteria after irradiation with low doses of electrons.

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BIOLOGY

Infertility in Mice caused by Nutritional Stress before Mating

Mice from which all food was withheld for 48 h from the end of the third to the end of the fifth day after finding copulation plugs did not litter[1]. The fasting appeared to depress hypophyseal gonadotrophic function, causing degeneration of the deciduomata and secondary embryonic death. Although embryonic death is an important cause of infertility, failure of fertilization and death of ova before nidation are also commonly involved in the pathogenesis of mammalian infertility. This communication reports the results of attempts to influence the viability of the ovum by imposing nutritional stress before ovulation. As it was essential not to inhibit œstrus and mating a shorter period of starvation was given.

In a series of 5 experiments, 8–12-week-old virgin mice of the Sydney White strain were caged in groups of 2, 3 or 4. 166 were fasted for 24 h 3–8 days after caging. Male mice were introduced, one per cage from 3 days before to 3 days after the fasting period. 61 comparable mice were not fasted and were kept as controls and were mated at a similar time after caging the females. Water was always

freely available. Mice were examined daily after introduction of the bucks for evidence of mating. This was found in 102 starved and 49 control mice within 5 days of the end of the fasting period and in the case of control mice 6 days after introduction of the males either by the finding of copulation plugs or spermatozoa in vaginal smears. Treated mice were grouped according to the time 0–5 days which elapsed between the end of fasting and finding spermatozoa or copulation plugs. The mated mice were allowed to complete the expected gestation period and the numbers of mice littering were noted. The results are given in Table 1.

Table 1. A Comparison of Fertility (Littering Rates) of Mice Fasted for 24 h at Six Different Starvation-Mating Intervals and of Non-Starved Mice

Class No.	End of starvation-mating interval (days)	No. mated	No. littering	Percentage littering
1	0	12	4	33
2	1	21	4	19
3	2	21	3	14
4	3	13	7	54
5	4	14	11	79
6	5	21	15	71
		102	44	43
7	Control—no starvation	49	28	57

Comparison of the sum of classes numbered 1, 2 and 3 with the sum of 4, 5 and 6 and with 7 showed highly significant differences ($\chi^2 = 9{\cdot}71$, $P = < 0{\cdot}01$ and $\chi^2 = 6{\cdot}68$, $P = < 0{\cdot}01$).

These results indicate that fasting for 24 h significantly reduced the littering rates of the females mated one and two days after the end of the fast.

I thank Miss A. E. Dowell for assistance and Mrs B. Clarke for statistical analysis.

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The Bill-snap Reflex : a Feeding Mechanism in the American Wood Stork

In the course of field observations of the American wood stork (*Mycteria americana*) in Florida, the birds were seen to capture fish habitually from water so turbid or choked with vegetation that the use of visual cues was improbable. In such feeding behaviour, a stork typically inserts its partially open bill into the water, moves the bill slowly from side to side, and walks forward. Submerged vegetation is sometimes stirred with one foot and the wing on the same side is flashed open, presumably to startle concealed fish into the open bill. When a fish makes contact with the bill, the mandibles are quickly closed with considerable force. These feeding techniques have been previously described[1–3].

Using two captured wood storks, we measured the time required for closure of the bill after stimulation of the lower mandible. One of the birds was then temporarily deprived of frontal vision, and its ability to capture fish by means of the bill-snap reflex was examined.

Measurements of the reflex speed were accomplished by using a piezoelectric crystal to which a stiff wire had been attached. The electrical output of the crystal was led to one channel of an electronic switch the output of which triggered the sweep of an oscilloscope. The second channel of the electronic switch contained a 500 c.p.s. timing signal obtained from a General Radio model 1304*B* oscillator calibrated against line frequency. Single sweeps of the oscilloscope beam were photographed, and measurements were made from the photographs of the time elapsing between stimulation of the lower mandible and closure of the bill. The unrestrained birds were blindfolded during the measurements and had been deprived of food for two or three days prior to the experimental session.

Examination of the oscilloscope photographs revealed that nine trials were usable from one bird and 16 trials from the other. Many trials were discarded because the birds snapped the sensing device repeatedly, causing multiple sweeps of the oscilloscope beam to appear on the photograph. In a few trials there were no responses. Only those photographs showing a single sweep and a clear indication of the elapsed time were used. A typical oscilloscope trace is shown in Fig. 1, and results for the two storks are given in Table 1. Additional measurements of the speed of the bill-snap reflex, made on two other days with the same birds, gave similar values.

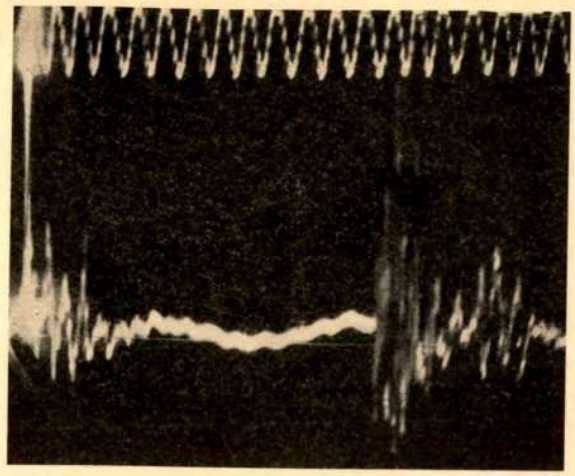


Fig. 1. Typical oscilloscopic record of bill-snap reflex. Upper trace: timing signal, 500 c.p.s.; lower trace, output of piezoelectric cartridge. The initial stimulus triggers the sweep of the oscilloscope; bill closure is indicated by the onset of the signal at the right of the lower trace

Table 1. Bill-snap Reflex Measurements on Two Wood Storks, *H* and *E*

Statistic	*H*	*E*
Mean response time	24·7 ms	25·3 ms
Standard error	0·92	1·07
Range	20–27 ms	19–32 ms
No. of trials	9	16

In triggering the bill-snap reflex, the lower mandible was found to be more sensitive to stimulation than the upper mandible. The portions of the tomia which generally come into initial contact with the fish are very horny, and, although no histological examination was made, we think it unlikely that any tactile receptors are present there. It seems more likely that the bill closure is a myotatic reflex, occasioned by a stretching of the jaw musculature when the bill is depressed[4].

Restriction of frontal vision in one bird was effected by gluing segments of blackened ping-pong balls to the denuded cranium in such a way that the bird was deprived of at least the central 75° of visual field. The blinders were attached while the stork was anæsthetized with ether; 3 h elapsed between recovery from anæsthesia and the experimental observations. The birds were allowed to feed together in a round wading pool, approximately 2 m in diameter and 25 cm deep, to which they were accustomed. Thirty to forty live fish, 10–15 cm long, were placed in the pool. Feeding behaviour of the two birds was observed and motion pictures were taken.

Visual restriction had little effect on the general feeding behaviour of the stork and no effect on its ability to capture fish. The partially blinded bird captured fish as well as its normal companion, and clearly utilized the reflex closure of the mandibles in response to stimulation by moving fish. The bird with normal vision showed some visual orientation to the prey; but actual capture was usually triggered by contact between bill and fish.

As Roeder[5] has suggested, 'strike times' of predators may have evolved to equal or better 'startle times' of potential prey. No startle times for typical wood stork prey (that is, fish or other lower vertebrates) were found in the literature. One of the quickest vertebrate startle responses appears to the eye blink after a strong auditory

stimulus in human beings, which has a latency of about 40 m.sec[6].

This feeding mechanism permits wood storks to secure food unavailable to species which locate their prey by vision alone. It also enables them to feed at night. Furthermore, this feeding technique is highly effective under the conditions of high fish density which prevail during the wood storks' breeding season in Florida[7].

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Gigantism-inducing Factors in *Chlorella pyrenoidosa*

In a previous communication[1] we showed that when *Chlorella pyrenoidosa* is injected intraperitoneally into diabetic rats, the alga grows to twice its normal size. The causes of this phenomenon and its mechanism are the subject of this communication.

Since *Chlorella* is a facultative chemo-organotrophic alga which, according to Fogg[2], utilizes hexoses for preference, plasma glucose may well be the factor which causes gigantism. This, on being metabolized by the alga, might explain the disappearance of glycosuria in diabetic rats, as shown by us[3]. On the other hand, Lorenzen[4] maintains that, in certain conditions, temperature at 37° C causes an inhibition of division and an increase in the size of cells in *Chlorella*, and so the temperature inside the rat might also be the cause of gigantism.

50 ml. of a suitable culture medium for *Chlorella*[5] or 50 ml. of distilled water was placed in 250-ml. matrasses, and in both cases Pfanstiehl Lab. 16 mM glucose was added. Each matrass was sown with 10 mg (dry wt.) of the alga. The cultures were subjected to various conditions in order to examine the effects produced in them. Some matrasses were exposed to daylight, others to darkness; the temperatures ranged from 23° C to 37° C. Some of the matrasses exposed to daylight were subjected to a mixture of 95 per cent air + 5 per cent carbon dioxide. Others were not subjected to this atmosphere. The experiments, which lasted 96 h, were duly controlled without glucose. Every 24 h, the transformations undergone by the cells were observed through the microscope, the diameter of 50 adult cells in each matrass measured, and the average assessed.

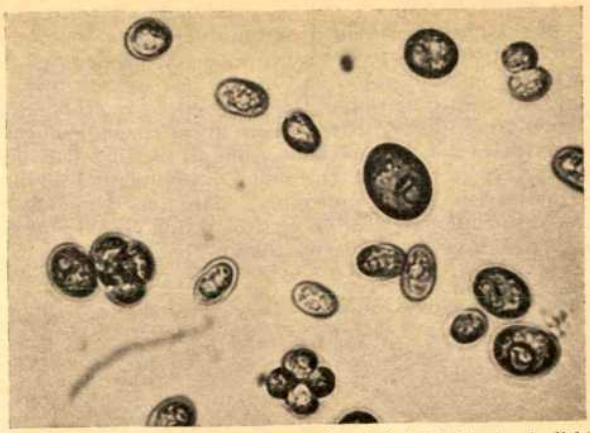


Fig. 2. Normal *Chlorella pyrenoidosa* cultivated at 23° C during daylight (× *c.* 800)

The adult cells of *Chlorella* increased in size in the presence of glucose and accumulated reserves, although their organization showed no change. The processes of division, sporulation, and growth of the culture were considerably attenuated. At the end of 72 h, changes appeared in the microscopic structure of the mother cells of the autospores, which were by now considerably larger in size than when checked (Fig. 1*a* and *b*). Autospores began to form, and at the end of this process each mother cell became a giant sporangium with a large number of autospores (Fig. 2), whereas the test tubes generally contained 4 autospores (Fig. 3). Light, temperature, and culture medium also exerted influences (Fig. 1*a* and *b* and Table 1). Subjection to the aforementioned atmosphere had no effect on the process of gigantism.

The effect of glucose having been proved, we investigated, under the same conditions and at the same concentration, whether the pentoses, hexoses, and disaccharides shown in Table 1 had a similar action on the glucose. Only fructose and mannose had the same effects as glucose and gave rise to equal findings. The other sugars showed no such action.

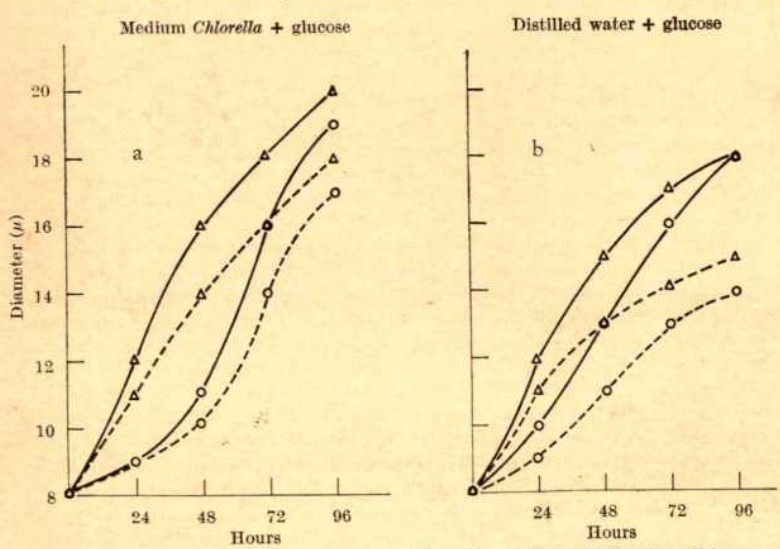


Fig. 1. Variation in diameter of *Chlorella* cells cultivated under different conditions. Daylight, —○—, at 23° C. —△—, at 37° C; darkness, – – ○ – –, at 23° C; – – △ – –, at 37° C

Sugars which stimulated gigantism caused a delay or an inhibition of cell division and sporulation; so that, according to Soeder[6] (since the sugars would increase the total concentration of the medium), cells larger than the normal ones would be originated and would contain a large quantity of reserve substances, which, later on, would cause giant sporangia with numerous autospores to appear. The fact that other sugars (ribose, xylose, galactose, sucrose, maltose, and lactose), which also increase the total concentration of the medium, do not promote gigantism is due to their not being metabolizable.

96 h after the beginning of the experiment the giant sporangia were detached from practically all the cells by centrifuging at 500 r.p.m. for 5 min; they were repeatedly washed with sterilized water, and sown in *Chlorella* mineral medium, but without sugar. A considerably more marked growth was observed than when sowing normal cells, for each sporangium released a large number of autospores. Previous treatment of the

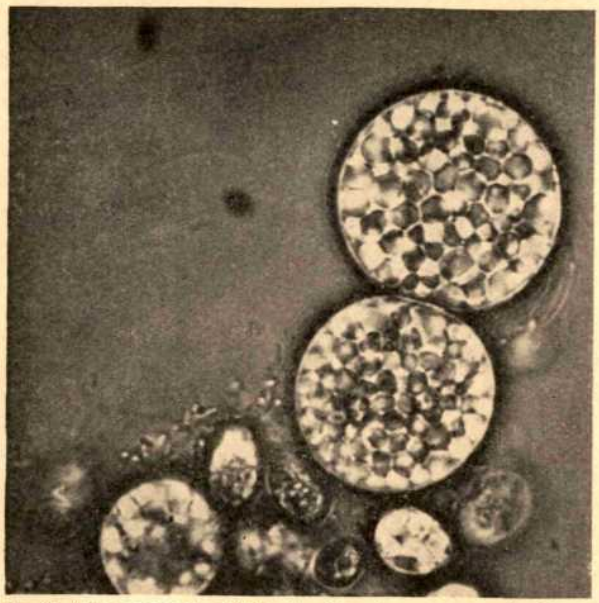


Fig. 3. Giant sporangia of *Chlorella* with autospores grown in glucose medium for 96 h in daylight at 23° C (× 1,200)

inoculum with gigantism-inducing sugars may be a good means of accelerating the growth of the culture in the examples presented here.

Table 1. Size (μ) of *Chlorella* Grown in Different Conditions for 96 h

Sugar added	Culture medium	Illumination	Temperature 23° C	Temperature 37° C
Glucose	Med. Chl.*	Daylight	19	20
		Darkness	17	18
Fructose				
Mannose	Distilled water	Daylight	17	18
		Darkness	14	16
Ribose		Daylight	8	8
Xylose	Med. Chl.			
Galactose		Darkness	8	8
Sucrose				
Maltose				
Lactose	Distilled water	Daylight	8	8
Control†		Darkness	8	8

* Culture medium for *Chlorella*.
† No sugar added.
No. of experiments, four with different sugars.

In cultures in a mineral medium in which the inocula used were giant sporangia obtained through treatment with a sugar or isolated from the abdominal cavity of diabetic rats, we found, particularly at the beginning, a high percentage of adult forms with an average diameter of 12μ, of normal organization and appearance, which gave rise to sporangia having 8–16 autospores. We have not been able to count the chromosomes of *Chlorella*, but the fact that they slowly return to the normal size of the adult cell from which we started (8μ diam.) and the high percentage of giant forms lead us to believe that the phenomenon thus observed is not a polyploidism, but a trophic change caused by the sugars which are metabolizable to the alga (glucose, fructose, and mannose), from which state the cells regressed on encountering the sugar-less mineral medium, whereas they again became gigantic when returned to a medium containing an inducing sugar.

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Influence of Bacillus Calmette-Guérin Infection on the Intensity of Homograft Reaction in Rats

In order to investigate the non-specific stimulation of the reticulo-endothelial system and consequences of this stimulation on the intensity of specific immune reactions it was decided to find out whether bacillus Calmette-Guérin (B.C.G.) infection would influence the rate and intensity of homograft reaction. Results have already shown[1] that the first set homograft rejection was markedly accelerated in B.C.G. infected rats. A similar effect was described by Balner, Old and Clarke[2]. They observed an accelerated skin graft rejection in B.C.G. infected female *C57BL*/6 mice grafted with isologous male skin.

Adult, 2-month-old, random-bred *Y* rats of both sexes weighing about 150 g were used. By exchanging a skin graft between random chosen animals it was shown that the median skin graft survival-time was in the first set 12·5 ± 0·06 days; the second set it was 8·0 ± 0·08 days. This relatively well-expressed difference between the first and second set homograft rejection was very suitable to determine the acceleration of the first set homograft rejection in B.C.G. infected rats. All experiments were done by using tail graft technique described by Bailey and Usama[3], the surface of the graft being about 1·0 cm × 0·5 cm. Rats were injected intracutaneously into tight region with 0·5 ml. of standard fresh prepared B.C.G. vaccine (Institute for Sera and Vaccines, Beograd).

Two hundred and twenty B.C.G. infected rats were divided in eight groups, each consisting of 25–30 animals and grafted with homologous skin on day 5, 8, 10, 15, 20, 25, 30 or 40 after infection. Each rat received a second skin graft 30 days following the first, both originating from the same donor. The control group contained 54 rats which were grafted only.

Table 1. Skin Homograft Survival Time in Rats infected with B.C.G. Vaccine on the Day Zero

First skin homograft Day of grafting	Median survival time	Second skin homograft Day of grafting	Median survival time
5	9·2 ± 0·05	35	8·1 ± 0·05
8	9·1 ± 0·05	38	8·1 ± 0·05
10	9·7 ± 0·05	40	8·2 ± 0·09
15	9·0 ± 0·09	45	7·8 ± 0·05
20	8·2 ± 0·06	50	8·0 ± 0·04
25	8·7 ± 0·04	55	8·1 ± 0·06
30	8·9 ± 0·04	60	8·0 ± 0·04
40	11·1 ± 0·04	70	8·0 ± 0·05
0 (control non-infected)	12·5 ± 0·06	30	8·0 ± 0·08

The results are shown in Table 1. As can be seen, B.C.G. infection exerted a marked acceleration of the first graft rejection. This effect was mainly expressed in the group grafted 20 days after B.C.G. infection. The homograft survival-time in that group came up to the values obtained in the second set reaction. The stimulation of the reticulo-endothelial system exerted by B.C.G. vaccine did not last for a longer period since 40 days following injection it revealed a tendency to disappear. It is obvious that no marked acceleration of the second graft rejection could be expected since the proximate second graft was placed 35 days after the injection, that is, at the time when the reticulo-endothelial system stimulation seemed, as already mentioned, to disappear. However, as no difference in the second skin graft survival-time was obtained when compared with controls, it was concluded that the second homograft does not depend on the intensity of the first set reaction. It is known that the difference between the first- and second-set homograft rejection is expressed more strongly in weaker antigenic differences in comparison with the strong ones. In the present experiments the differences between the first- and second-set homograft reaction was reduced by B.C.G. injection, thus simulating the strong antigenic differences. Zaalberg[5] obtained in *C57BL* female mice the first set

male skin graft rejection in a range of 12–91 (mean 45) days, having been in the second set in a range of 14–26 days. These last values correspond almost completely to the male first set skin graft rejection time obtained in B.C.G. infected *C57BL* female mice in the experiment performed by Balner *et al.*

From these results it can be deduced that the course and intensity of an immune reaction might be markedly dependent not only on antigenic differences but also on the functional status of the reticulo-endothelial system.

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Fixation of Atmospheric Nitrogen by Root Nodules of *Comptonia peregrina*

Comptonia peregrina (L.) Coult. is a common shrub in the eastern part of North America. It is an important component in the shrub layer of the Pinetum banksianæ[1]. *Comptonia peregrina* and its variety *C. p. asplenifolia* (L.) Fern. have root nodules[2]. The structure of these has already been described[3].

In view of the similarity in structure between the nodules of *Comptonia* and those of *Myrica*, it was probable that the nodules of the former, like those of the latter[4], can fix atmospheric nitrogen. To prove this, the ability of *Comptonia* nodules to fix nitrogen-15 was investigated.

The test material used consisted of: (*a*) Parts of roots with nodules and with adhering soil particles, taken 2 h before the beginning of the experiment at the Botanical Garden, Munich. The root parts were placed in a glass-holder containing at the bottom a small layer of water (about 5 mm deep); (*b*) Parts of roots as in (*a*), but embedded in moist soil; (*c*) Parts of roots with nodules, collected 24 h before the experiment from the Botanical Garden, Darmstadt. During the experiment they were embedded in moist soil; (*d*) Moist soil, sampled in the rhizosphere of *Comptonia*, at prevailing soil moisture conditions; (*e*) As in (*d*), but after full water-saturation of the soil.

The material was transferred into a 'circulation system'[5], evacuated three times at 30 mm mercury and filled intermittently with argon with the purpose of expelling air. A gas mixture containing about 30 vol. per cent nitrogen and 20 vol. per cent oxygen in argon (with a nitrogen-15 content of 16·23 atomic per cent ± 0·068) was then introduced in the system. After six days the experiment was stopped. The nodules (with the adhering soil particles) were detached from the roots and the roots and nodules separately dried at 75° C in a vacuum oven. The soil, without roots and nodules, was dried at 105° C. Then the material was ground, the total nitrogen sampled by the Kjeldahl method, and the nitrogen-15 content determined with the aid of a mass-spectrograph (Atlas, CH_3/IS IV)[6]. The limit of the isotope-method used lies within an increase in nitrogen content of 0·01 per cent of the original amount.

Table 1 shows that fresh nodules (material *a* and *b*) fixed a remarkable amount of atmospheric nitrogen compared with the moderate increase in nitrogen content of the nodule-bearing roots. The soil showed very little or no increase in nitrogen content. The gain in nitrogen by the roots could be explained by the fact that they derived their additional nitrogen from the nodules. Nitrogen fixation by the soil-embedded root nodules (material *b*) was far more effective than that of material exposed to moist air (*a*).

Table 1

Material		Nitrogen content (% dry weight)	Nitrogen increase (% of original N)
a	Nodules	1·897	0·151
a	Roots	1·623	0·015
b	Nodules	2·597	2·224
b	Roots	1·702	0·578
b	Soil	0·354	0·013
c	Nodules	2·098	0·000
c	Roots	1·475	0·000
c	Soil	0·384	0·011
d	Soil	0·348	0·020
e	Soil	0·362	0·000

A greater elapse of time between collection and experimentation (material *c*) leads to a complete loss of the ability to fix nitrogen. It is possible also that the active nodules (material *a* and *b*) fix nitrogen only during the first phase of the 6-day experimental period.

These results confirm that root nodules of *Comptonia* can fix atmospheric nitrogen. A quantitative evaluation of the fixation capacity of intact plants cannot be derived from the results of the present experiment. Results previously obtained by Stewart[7] with *Alnus* would suggest that this capacity would be greater than that of isolated root parts.

In view of our results it is quite probable that *Comptonia peregrina* plays an important part in the nitrogen status of soils in the natural area of the species in North America.

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Infection of Celery Seedlings by Viable Spores of *Septoria* spp.

Blight of celery caused by *Septoria* spp. was first definitely recognized in England in 1906 and afterwards was reported to spread rapidly over the country[1]. As early as 1910 it was known that the pycnidia of the fungus on the 'seed' contained spores which produced the disease when they were suspended in water and sprayed on to plants.

Chittenden[1], writing in 1914–15, gives reasons for suspecting that the seed was "the principal if not the only source of infection", and he claims to have been able to demonstrate infection of seedlings when infected seed was "sown in the ordinary way". Since then it has been commonly assumed that spores from pycnidia on the seed

infect young seedlings, but so far as I am aware there has never been a published description of a reliable method to show this. Mukkath and Wood[2] recently have suggested that celery seedlings may become infected by mycelium carried within the seed and that viable spores on the surface may be less important than previously assumed. On the other hand, Hewett[3] has found viable spores in an appreciable proportion of infected seed samples and considers these to be an "important potential source of disease outbreaks".

About 1914, 90 per cent of the commercial seed samples examined were found to be infected with *Septoria* spp. In 1957–58 Marshall[4] found 78·8 per cent infection, and among the present season's seed, that is, 1962–63, I have found only one sample out of 30 examined to be free of *Septoria*. Last year 17 out of 50 infected seed samples examined at Imperial College were found to have viable spores. The majority of these samples were less than one year old when tested.

The purpose of this communication is to describe a laboratory method for demonstrating that infected seed gives rise to infected seedlings and that infection can result from clean seed contaminated with viable *Septoria* spores. Here it may be noted that the same seed grown under ordinary greenhouse conditions never produced infected seedlings.

The method is as follows. A closely fitting glass cylinder, 7 cm diam. and 7 cm tall, is placed inside a tall 600 ml. glass beaker. The top of the ring is covered with muslin held in place by a rubber band, and the mouth of the beaker closed with a Petri dish lid after adding water up to the level of the muslin. The beaker is autoclaved at 15 lb. pressure for 20 min. When cool about 300 seeds (0·15 g) are placed evenly on the muslin and the beaker kept in the laboratory in the dark for one week. Thereafter it is placed on a laboratory bench 12 in. below 80-W fluorescent tubes switched on during 8 a.m.–8 p.m. daily. There is no reason for supposing that this method of illumination is critical. It is used mainly to ensure good growth of seedlings under laboratory conditions where the temperature is reasonably constant, about 20° C. Five weeks after sowing the seedlings are examined for infection by means of a stereoscopic microscope, magnification 20 times.

Three seed samples which were shown to have viable spores by the cultural method used in this laboratory[5] have been examined and the results set out in Table 1.

Table 1. Infected Seedlings obtained from Naturally Infected Seed

Seed sample	% pycnidial infection	% spore germination	No. of seeds sown (approx.)	No. of seedlings obtained	No. of infected seedlings
a	7	37	3,600	853	21
b	9	31	3,600	875	10
c	25	15	1,800	463	4
Control	7	0	3,600	822	0

With the same method no infected seedlings have been found if the seed was first treated with formalin or hot-water.

In another test clean seed which had also been treated with hot water was used. It was contaminated by steeping for half-an-hour in a thick suspension of *Septoria* spores (about 10 million per ml.) obtained from pure culture, and then allowed to dry over-night before placing in the beakers. The results are shown in Table 2.

It is clear, therefore, that seedlings can become infected by viable *Septoria* spores carried superficially on the surface of the seed, and in the absence of any other source of viable fungus. It has been shown that seed with pycnidia containing viable spores can also give infected seedlings and here too, presumably, it is the viable spores that are the source of infection. The possibility that the fungus on such seed can give infected seedlings in other ways is being investigated.

Table 2. Infected Seedlings obtained from Clean Seed contaminated with Viable *Septoria* Spores

Seed sample	No. of seed sown (approx.)	No. of seedlings obtained	No. of infected seedlings	Germination of spores washed from seed (%)
a	1,800	694	56	75
b	1,800	501	28	61
Control	900	382	0	0

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Pathogenicity of *Streptomyces scabies* on Potato Tubers cultured *in vitro*

In potato breeding trials conducted here extensive investigations are required to determine the relative resistance of selected seedlings to the common scab disease caused by *Streptomyces scabies* (Thaxt.) Waksman and Henrici. The tissue-culture technique[1,2] for establishing a ready source of aseptic tubers potentially offers a rapid and convenient method for indexing their resistance and for studies on the host–parasite interactions. To evaluate this technique for studies on the common scab disease, tubers were produced *in vitro* using etiolated stolons[1] and aerial stem nodes of greenhouse-grown plants[3]. White's amended medium, containing 10 per cent sucrose for etiolated sprouts and 8 per cent sucrose for stem nodes, was used.

Tissue cultures were prepared from the following varieties: Green Mountain and Katahdin (scab-susceptible); Huron, Russet Burbank, and Cherokee (moderately resistant); Hindenburg, Richter's Jubel, Arnica and Ackersegen (highly resistant). Fifty tuber cultures of the Green Mountain variety and 20 tuber cultures of the remaining varieties were inoculated with a mycelial-spore suspension, in sterile distilled water, from a pathogenic strain of *S. scabies* (*L*272) which had been growing for 7 days on Czapek's agar. Because of the smooth surface of cultured tubers the inoculum tended to run off. Care was taken that all tubers had a drop of inoculum adhering to the surface. Cultures were maintained in the dark at 25° C.

In contrast to previous findings[2] no scab developed on tubers from any of the varieties. Occasionally a small lesion was noted on a few tubers from the susceptible Green Mountain. Similar results were obtained when this work was repeated using a second pathogenic strain of *S. scabies* (*L*28) as the inoculum.

These results suggested that either environmental conditions were unfavourable for disease development or the tuber surface in some way prevented invasion by the organism. The susceptible Green Mountain variety and the resistant Richter's Jubel variety were used in subsequent studies.

To test the effect of an altered environment the essentially chemically inert 'Vermiculite' was used as follows. 'Vermiculite' (fine) and water (100 : 60 (v/v)) were poured into a Petri dish, covered and sterilized at 121° C for 20 min. Enlarging tubers of 16-day-old cultures of Green Mountain and Richter's Jubel varieties were transferred, aseptically, to the sterile 'Vermiculite', inoculated, and covered lightly with the moist medium. The dish was covered and cultures were incubated at 25° C. Under these conditions 45 per cent of the susceptible Green Mountain developed scab within 10 days. Small lesions

were seen on a number of tubers within 3 days. With the exception of isolated instances no scab developed on the resistant Richter's Jubel variety. Where scab occurred the lesions were very superficial.

To determine the effect of an altered tuber surface on scab development, enlarging Green Mountain and Richter's Jubel tuber cultures were removed from the vials and soaked in sterile water for 5 h and in three concentrations of methanol for varying periods. Half of the tubers in the treatments were placed back in the vials and the remainder in moist 'Vermiculite'. Care was taken to prevent injury to tubers when making transfers. Cultures were inoculated (*L*272) and incubated as before. The water treatment enhanced scab development on both varieties. Green Mountain tubers in 'Vermiculite' showed some lenticel infection within 48 h and considerable scab in 4 days. 70 per cent were severely scabbed after 10 days. An occasional isolated lesion occurred on tubers placed back in the vials, but these took longer to develop and remained relatively superficial. 62 per cent of Richter's Jubel tubers in 'Vermiculite' developed scab within 10 days of treatment. Scab took longer to develop on this variety and the lesions were very superficial. There was no scab on Richter's Jubel tubers replaced in the vials.

When tubers were soaked for periods of up to 1 h in 10 and 25 per cent methanol and placed in the culturing media as described, 40 per cent of the Green Mountain developed scab in the 'Vermiculite' medium, whereas no scab developed on tubers replaced in the vials. There was no scab on the Richter's Jubel variety in either case. However, when tubers were soaked in 40 per cent methanol for 5 min severe scab developed on 80 per cent of the Green Mountain variety in 'Vermiculite', but very little on tubers replaced in the vials. With the Richter's Jubel variety 35 per cent of the tubers in 'Vermiculite' developed superficial scab. There was no scab on tubers in the vials.

In some instances the 40 per cent methanol treatment caused reddish-brown areas to develop on the tuber surface. The rose end was not usually affected. The Richter's Jubel variety showed less discoloration and when present occurred mainly at the lenticels. The discoloured areas did not appear to be more susceptible to infection.

These investigations suggest that the tissue culture technique fails to produce a set of circumstances that will permit the parasitic invasion of the scab organism. On the other hand, the placing of the tuber-bearing culture into a Petri dish of moist 'Vermiculite', and inoculating, does allow effective invasion. Additionally, it demonstrates degrees of resistances in that the invasion of the Green Mountain was accomplished while the Richter's Jubel remained clean. The washing of the cultured tubers, prior to their exposure to the pathogen in 'Vermiculite', apparently attenuates the resistance, permitting a greater invasion of Green Mountain and also the development of some symptoms in the Richter's Jubel. The results also suggest, after invasion, a differential vigour of the pathogens' establishment in that scab was less severe in the resistant Richter's Jubel.

The effect of the washing is not understood although it suggests the elimination or dilution of soluble native products or the alteration either of osmotic values or relative permeabilities. The 'Vermiculite' action may be strictly mechanical, providing a more uniform spread of the inoculum and a moister tuber surface. With refinement the technique may become an important adjunct to the present pathological assessment of breeding stock.

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MICROBIOLOGY

Events associated with Development of Lipid Plaques on Plasma Agar

In a previous paper[1] we have reported that many strains of *Staphylococcus aureus* elaborate a diffusible extracellular lipolytic substance that produces visible lipid plaques on plasma agar, often in large amounts.

Careful examination of plasma agar preparations in which this substance is acting show that its effects occur in a stepwise fashion and that plaque formation is actually a later manifestation of this phenomenon. This communication describes these sequential changes leading to the formation of such lipid plaques.

Ten per cent plasma plates were prepared by adding 2·5 ml. plasma from 24 different normal human ACD bloods to 22·5 ml. trypticase soy agar at 48° C. Each plate was inoculated centrally with 1 loop of broth suspension from a slant of *S. aureus* Guy, incubated at 37° C for 5 days, and thereafter left at room temperature. To obtain agar segments containing the diffusible extracellular lipolytic agent, plain trypticase soy agar plates were inoculated centrally with *S. aureus* Guy, incubated at 37° C for 5 days and at room temperature for 2–5 weeks. Active sterile agar segments near the growth were cut out and placed on plasma plates. The plates were examined macroscopically and microscopically daily for 5 days and periodically thereafter.

After 1–2 days' incubation at 37° C, a finely dispersed precipitate appeared in an area 0·5 cm wide surrounding the growth of *S. aureus*. The diameter of the precipitate-containing area increased at the rate of 0·2–0·3 cm a day. Of the 24 plasmas tested, marked lipid production was consistently observed in 1–2 days in 7 plasmas; moderate production of lipid occurred during 3–6 days with 11 plasmas, and slight or no lipid production was seen in 6 plasmas.

In discussing the microscopic observations in this work, we have divided the plasma agar into three zones parallel to the plates: the bottom face of the agar (*a*); the central area (*b*); and the surface of the agar (*c*).

After 1 day at 37° C, the bottom face of the agar (*a*) was crowded with numerous minute particles in an area extending out 0·3 cm beyond the periphery of growth. At this time there were no changes in zones (*b*) or (*c*). By the second day, on the bottom face (*a*), elongated particles (Fig. 1) had developed, apparently from agglomeration of the smaller particles observed on the previous day; and round, dense bodies appeared throughout in the middle zone (*b*). By the third day, numerous closely packed clumps of round particles (Fig. 2) were seen on the bottom face (*a*), and, in zone (*b*), additional dense bodies (Fig. 3) had developed. In zone (*c*) lipid plaques (Fig. 4) had appeared about 0·5 cm away from the *S. aureus* growth overlying the area containing sub-surface particles.

After 4–5 days' incubation, the clumps in zone (*a*) became large, irregular and filamentous; the dense bodies in zone (*b*) increased in number and size, and more plaque material was produced on the surface of the agar. In plates made with plasmas which yielded the largest amount of plaque material, the changes occurred so rapidly and concurrently (often 24 h or less) that the sequence of changes described above was not seen. Similar changes were observed when active sterile agar segments were used instead of living *S. aureus*.

Raised spherical, refractile globules were seen in and near the surface of lipid plaques (× *c.* 80). These increased in size and number when plates were placed at 4° C for 1–2 h (Fig. 5). Such globules were always associated with lipid plaques and had a definite configurative relationship to them, developing at points from which the plaques appeared to originate.

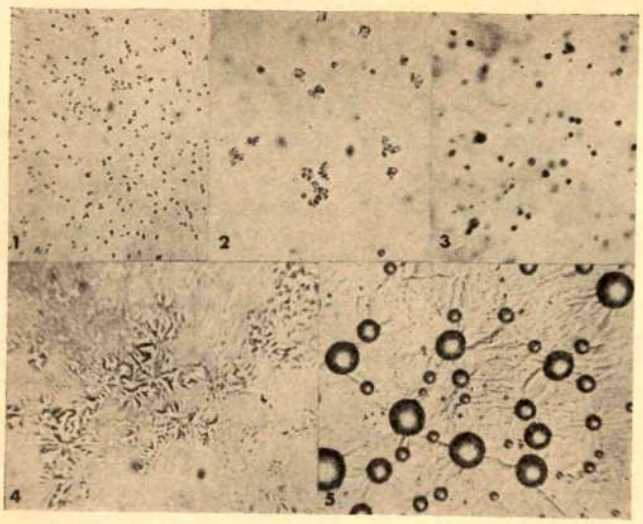


Fig. 1. All plates (× *c.* 80) were previously inoculated centrally with *S. aureus* Guy.

(1) Incubation, 37° C. 2 days. Elongated particles at bottom face of agar. Zone *a*. (2) Incubation, 37° C. 3 days. Clumps of round particles at bottom face of agar. Zone *a*. (3) Incubation, 37° C. 3 days. Dense bodies in middle zone *b*. (4) Incubation, 37° C. 3 days. Delicate lipid plaques on surface zone *c*. (5) Incubation, 37° C. 5 days. Lipid globules in and near surface plaques

Using micromanipulation techniques, it was possible to enter some of the larger globules with a very fine capillary and withdraw what proved to be liquid contents. When cooled below 4° C, this material solidified, remelting readily with slight warming. It was immiscible with water, but dissolved readily in various organic solvents. The very minute amounts of material that could be collected in this manner were subjected to several microchemical tests using reagents such as rhodamine *B*, α-*cyclo*dextrin, bromthymol blue, 2,7-dichlorofluorescein and sudan black *B*[2,3] and to chromatographic procedures described previously[1]. The results of these tests together with the observed physical properties indicate that the liquid content of these globules is lipid in nature, and that it consists primarily, if not entirely, of octadecenoic acid. Efforts are now being made to collect enough of this material for more exhaustive analysis.

The observations reported here show that the initial events associated with the eventual production of surface lipid plaques occur on a microscopic scale in the depths of the medium. The first sign of activity is the formation of minute particles on the bottom face of the agar. There follows a sequence of events as we have described resulting eventually in the visible surface plaques. There is a direct relationship between the number of sub-surface particles and the amount of lipid produced on the surface. Further, the sub-surface particles invariably spread out within the agar in advance of and just below the outward moving surface lipid. Thus in screening *S. aureus* strains for the ability to liberate free lipid from media, evidence of such activity can be obtained within 24 h simply by microscopic examination of the depths of the agar whereas surface lipid may not be visible for several days.

It has been shown that certain strains of *S. aureus* elaborate extracellular material that liberates octadecenoic acid and other lipid materials from plasma[1]. The presently available information indicates that the particles visible within the agar and the surface lipid material are the water-insoluble cleavage products produced by this lipolytic substance. It is presumed that lipolytic enzymes are involved; the elaboration of such enzymes by strains of *S. aureus* has been recorded[4] although little is known of their nature or action. Studies to investigate further the nature of this lipolytic substance, or substances, and of the products formed are now being initiated.

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PSYCHOLOGY

Development of Alcohol Preference in *BALB/c* Mice

McClearn and Rodgers[1] have reported differences in alcohol preference of various strains of mice tested on a two-bottle choice between water and a 10 per cent ethanol solution. The *BALB/c* strain is one of several identified as having low alcohol preference. Adult mice of this strain were found to avoid the ingestion of ethanol solutions in concentrations as low as 2·5 per cent[2].

During subsequent investigations, it was found that young *BALB/c* mice voluntarily ingested a considerable amount of 10 per cent ethanol solution. The discrepancy between this observation and the earlier ones might be due to developmental changes in preference for alcohol; the *BALB/c* mice examined initially were approximately 1 year old, while the age of those displaying some preference for alcohol varied from 6 to 9 weeks. The work recorded here was therefore undertaken in order systematically to investigate the possible developmental changes in alcohol preference of this strain.

The *BALB/c* mice were obtained from the Cancer Research Genetics Laboratory of the University of California, Berkeley. Males were used for the first investigation, and males and females for the second. The animals were caged individually and were provided with tap water and a 10 per cent ethanol solution in 25-ml. graduated cylinders. The position of the cylinders was changed every 3 days so as to avoid the establishment of possible position habits. Daily readings were made of the liquid consumption, and a daily preference ratio was computed as the volume consumed from the ethanol solution divided by the total consumed from both cylinders. A mean of the daily preference ratios for a two-week period was calculated for each animal. Food ('Diablo Labration') was available *ad lib.*

The first observations were made on independent samples of mice at various ages of 3–20 weeks. It should be noted that the animals were production stock, and the ages were known only within ±3·5 days. The results of the first study are shown in Fig. 1. The number

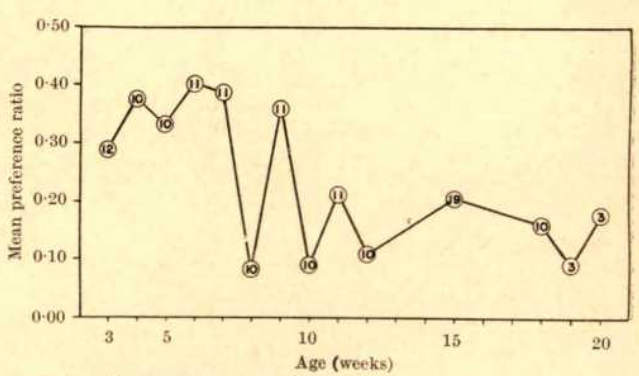


Fig. 1. Alcohol preference of *BALB/c* mice as a function of age. (Age shown as at beginning of 2-week test period)

indicated at each data point represents the number of animals tested at that particular age. The results clearly indicate that the younger animals show higher mean preference for alcohol than do the older animals. Indeed, there is a strong suggestion of an abrupt threshold. It is quite likely that the rather discrepant results at the 8th and 9th weeks may be explained by the inexact information concerning age. If this is so, it would indicate that the threshold occurs some time during the 9th week of life.

The second investigation involved 8 female and 16 male *BALB/c* mice. Alcohol preference was measured on the same animals twice, once at 4 weeks and again at 16 weeks of age. Ages were determined exactly for animals of this experiment. The testing and scoring procedures were exactly the same as in the first study. During the interval between the first and second test, the animals were housed in groups of 3 or 4, and had only water available as their drinking fluid. Food was given *ad lib.* The results of the second investigation are shown in Fig. 2.

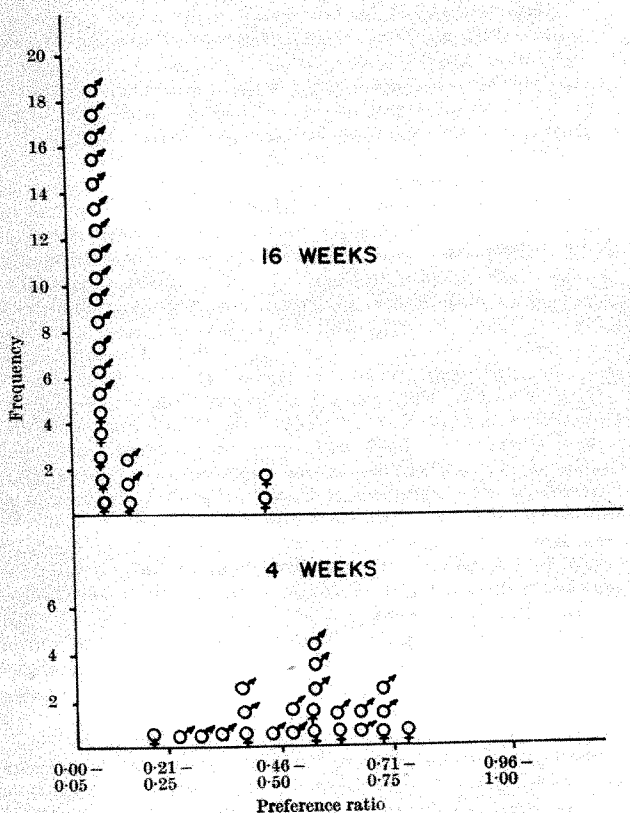


Fig. 2. Alcohol preference of a group of *BALB/c* mice measured at 4 and again at 16 weeks of age. (Age shown as of beginning of 2-week test period)

Since inspection revealed no sex differences, the data for both sexes were analysed together. The mean preference ratio was 0·50 at 4 weeks of age and 0·12 at 16 weeks of age. The difference was found to be significant ($P < 0.005$) by the Wilcoxon test[3]. The low mean alcohol preference at 16 weeks of age is in accord with the results obtained by McClearn and Rodgers[1]. These results indicate that exposure to ethanol during the high acceptance period does not result in an 'addiction' which persists into the adult, non-preferring phase. It remains to be determined if continued exposure to ethanol throughout the intervening period would result in a similar decline in preference.

The higher voluntary intake of ethanol solution by young *BALB/c* mice does not appear to be due to a simple failure of discriminative capacity. Inspection of the daily records indicates that preference for ethanol solution developed only gradually over the 2-week period, and there was very little intake of ethanol solution in the first few days. Thus, the mean preference ratio of the second week of testing was considerably higher than that of the first week.

These two investigations, one cross-sectional and the other longitudinal, agree in demonstrating a striking change with age in the alcohol preference of *BALB/c* mice. The available evidence indicates that the change from alcohol-accepting to alcohol-avoiding is an abrupt one. Investigations of correlated changes in anatomical, physiological, or biochemical processes might provide useful suggestions as to the nature of the mechanism producing the strain difference in alcohol preference of adult mice.

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Maintenance of High Accuracy without Augmented Feedback

We would like to direct attention to results obtained from a keyboard training experiment.

Using a 10-key keyboard, subjects (18 postmen aged 30–40 years) were taught a logical association between pairs of keys and letters of the alphabet, except *Q*. The nature of the association is irrelevant here; but one might note that instruction required four 45-min sessions spread over two days, to achieve error-free response to letters called out by the instructor. This initial instruction was given to classes of six men, and in subsequent practice there were three men in a class.

After the first two days of alphabet learning, subjects copied town names from typed lists, receiving four 5-min runs morning and afternoon for 8 days. Speed and accuracy were equally stressed, but whereas the amount copied during each run was obvious to the subjects, they were given no information at all concerning errors, the output from the keyboard being in the form of punched tape. The speed and accuracy achieved during practice (all runs and subjects meaned daily) are shown in Fig. 1.

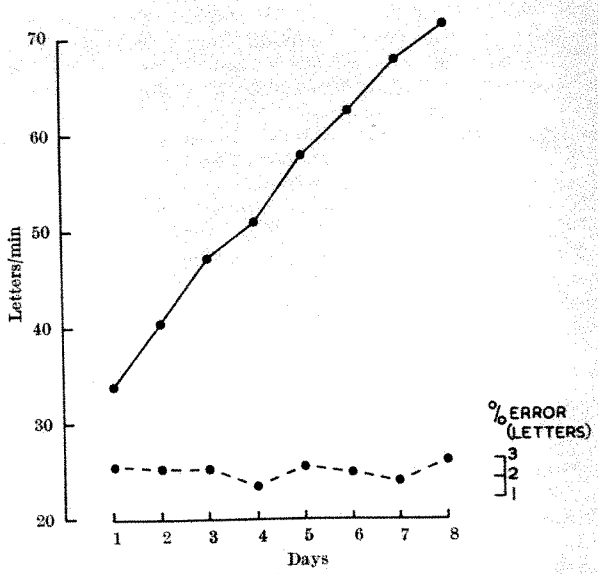


Fig. 1. Speed and accuracy of keying

It should be noted that the code used, while logical, was more complex than that usually utilized in laboratory tasks, and the task as such was a very close approximation to a real-life situation. Because of this, the results obtained are of particular interest. It was shown that subjects were able to improve steadily in speed without loss of accuracy, although they were not given immediate augmented feedback[1]. This finding lends support to a hypothesis which states that augmented feedback can be withdrawn as soon as a vocabulary has been learned to the point when, given sufficient time, subjects can produce the items without errors. From this moment onwards subjects are held to be able to provide sufficient intrinsic feedback to keep performance at a high level of accuracy. The hypothesis, therefore, provides a perfectly simple criterion for the point during training at which augmented feedback can be withdrawn. Since this point may well be reached much earlier than is generally assumed, the design of training schedules and devices may not have to be as complex as some current writers appear to think[2].

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VIROLOGY

Experimental Transovarial Transmission of Kyasanur Forest Disease Virus in *Haemaphysalis spinigera*

Transovarial passage in ticks has been investigated for several viruses of the tick-borne encephalitis complex. Russian workers have reported that Russian spring–summer encephalitis virus can pass transovarially in *Ixodes persulcatus*, *I. ricinus*, *Dermacentor silvarum* and *Haemaphysalis concinna*[1]. Benda[2], working with *Ixodes ricinus* and the European strain of tick-borne encephalitis in Czechoslovakia, demonstrated transovarial transmission in only 6 per cent of ticks, while Smorodintsev[3] in the U.S.S.R. reported that it occurred in 5–25 per cent of ticks. In an investigation confirming transovarial transmission of tick-borne encephalitis virus in *I. ricinus*, Rehacek[4] found that it occurred in 2 per cent of ticks infected by feeding and in 20 per cent of ticks infected by inoculation after feeding. Laboratory studies with a third member of the complex, Kyasanur forest disease virus, and its vector *Haemaphysalis spinigera* are reported here.

In a preliminary experiment, 8,713 larvæ, the progenies of 79 laboratory-infected *H. spinigera*, were processed in 92 pools for virus and all were negative. These females had moulted from larvæ and nymphs which had fed on 3–5-day-old chicks infected with Kyasanur forest disease. In each case infection in the tick was confirmed by isolation of virus in mice after deposition of eggs.

To determine whether the virus could pass transovarially if given the opportunity to reach the ovaries of the ticks without going through the alimentary canal, 35 unfed adult female *H. spinigera* were inoculated in the membranous portion of the joint between the coxa and the trochanter of the third leg with a fine needle drawn from 2-mm capillary tubing. The inoculum consisted of approximately 1,500 adult mouse LD_{50} of strain *G* 11338 of the virus which had undergone three mouse brain passages and a further five intra-muscular passages of infected blood in 1-day-old white leghorn chicks. It is presumed that the virus entered the hæmocœle directly and that there was no injury to any internal organ.

One week after inoculation, the ticks were released on a month-old calf. Twenty-nine of the 35 ticks engorged and laid normal fertile eggs. When tested after oviposition, these ticks were all positive for virus, and virus was also found in their excreta.

The larvæ from each tick were then divided into two groups of 500–1,000 each, and the first group, in pools of 200, was inoculated intracerebrally into adult mice. Virus was recovered from larvæ of 17 of the 29 ticks.

The second group of larvæ was released on 1-day-old white leghorn chicks to examine their ability to transmit the virus by bite. The 29 chicks used were bled twice, on the third day and again on the fifth day after release of the larvæ, and the blood was inoculated intracerebrally into adult mice. Virus was found in the blood of 16 chicks. The larvæ fed on these chicks were held for 10–15 days at 25° C and 90–95 per cent relative humidity to moult into nymphs. The nymphs of 26 groups were processed in pools of 50–100 each, and virus was isolated from 11 groups.

In this experiment the proportion of infected females capable of transmitting virus transovarially to larvæ was considerably greater than that reported by Rehacek[4]. Nevertheless, although larvæ were processed in fairly large pools, the proportion of pools negative for virus seemed to indicate that the number of infected larvæ produced per female was very small.

Since transovarial transmission had occurred in ticks infected by inoculation into the body cavity but not in ticks infected by feeding, it became necessary to investigate whether in the latter group virus was absent from the body cavity and the hæmolymph. A total of 140 female *H. spinigera*, previously infected in the larval and nymphal stages by feeding on infected chicks, was divided into seven groups of 20 each. The legs of the ticks were carefully detached either with fine scissors or with forceps; all instruments had been sterilized and the same instrument was not used for more than one tick. The detached legs and the 'de-legged' bodies of each group were inoculated separately into adult mice, and virus was recovered from all pools, both of legs and of bodies. This result would seem to indicate that virus is present in the hæmocœle of ticks infected by feeding.

Further work is necessary before these results can be used for interpreting field data. In particular, the experiments with progenies of ticks infected by feeding must be repeated under a greater variety of conditions. The potentiality of transovarial transmission of Kyasanur forest disease virus in *H. spinigera* has, however, been established.

We thank Dr. T. Ramachandra Rao, director, Virus Research Centre, for his critical and helpful advice throughout the investigation.

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Synergistic Effect of 2-(α-Hydroxybenzyl)-benzimidazole and Guanidine on Picornavirus Reproduction

Both 2-(α-hydroxybenzyl)-benzimidazole (HBB) and guanidine are specific and potent inhibitors of picornavirus multiplication[1]. However, in virus-infected cell cultures maintained for a prolonged period in the presence of either compound, drug-resistant mutants regularly emerge[2–4]. Recently it has been shown that mutants of poliovirus resistant to either HBB or guanidine exhibit only a small degree of reciprocal cross-resistance[5,6].

Combined treatment with HBB and guanidine was, therefore, expected to have a greater suppressive effect on virus reproduction than either compound alone. This proved to be the case in experiments with 'Coxsackie B3' and 'ECHO 5' viruses[5,6].

The large effect of combined treatment suggested, however, that it was not solely due to inhibition of mutants exhibiting limited cross-resistance, but that, in addition, HBB and guanidine acted synergistically.

The following experiment was carried out to test this possibility. 'Coxsackie A9' virus was grown in primary monolayer cultures of rhesus monkey kidney cells in a single infectious cycle[7]. HBB and guanidine hydrochloride, either alone or in combination, were added to the cultures at the end of the viral adsorption period, that is, 1 h after virus inoculation. The cultures were collected 7 h later and virus yields were determined by the plaque technique[7].

Table 1 shows the result of a typical experiment. HBB, 19 μM, or guanidine, 219 μM, had no effect on virus reproduction when given alone. 2·25-fold higher concentrations of HBB or guanidine reduced the virus yield 50–300 times. Yet still higher concentrations of either compound were required to suppress virus reproduction completely. On the other hand, 19 μM HBB and 98 μM guanidine, when given in combination, inhibited virus growth completely. The complete inhibition cannot be explained on the basis of inhibition of mutants resistant to one compound but sensitive to the other, since neither compound was used at inhibitory concentration.

Table 1. Synergistic Effect of HBB and Guanidine on the Multiplication of 'Coxsackie' A9 Virus

Compound	Concentration μM	Virus yield: Plaque-forming units/ml.	Virus yield: Treated, per cent of untreated control
None	—	$3·0 \times 10^8$	100
HBB	19	$3·0 \times 10^8$	100
	43	$6·5 \times 10^6$	2·2
	98	$1·6 \times 10^5$	0·05
Guanidine	219	$3·5 \times 10^8$	117
	493	$1·1 \times 10^8$	0·4
	1,110	$1·4 \times 10^5$	0·05
HBB + Guanidine	19		
	98	$2·0 \times 10^5$	0·07

Two other combinations (29 μM HBB + 219 μM guanidine; 43 μM HBB + 98 μM guanidine) were also found to inhibit virus reproduction completely, and even 9 μM HBB + 98 μM guanidine had some effect (72 per cent inhibition).

The mechanism of the synergistic effect of HBB and guanidine on virus multiplication is not known at present. Both compounds inhibit the production of virus-induced RNA polymerase[8], and of viral RNA and coat protein[7,9–12]. It may, therefore, be hypothesized that the compounds affect different aspects of the virus-specific reaction sequence which leads to the synthesis of viral RNA.

From a practical point of view, combined use of both compounds holds promise for the treatment of virus diseases.

This investigation was supported by a grant from the U.S. National Foundation.

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ANTHROPOLOGY

Selection Intensity in Brazilian Caingang Indians

It is becoming increasingly clear that if we wish to understand the biological evolution of man we must examine in detail the selective pressures and breeding structures of human populations which are in different stages of cultural development. Crow[1] suggested a relatively simple method for measuring selection intensity in man. This communication describes the results obtained with Crow's method collected from among the Caingang Indians living in the State of Rio Grande do Sul, Brazil.

These Indians have already been subjected to a series of investigations which aim at understanding the evolutionary forces which shaped their present genetic constitution[2,3]. Details about the location of these groups and their demographic structure were described in a previous paper[2].

Table 1. Selection Intensity in Caingang Indian Populations of Rio Grande do Sul, Brazil

Population	N_1	$\bar{x}$	Vf	N_2	pd	Im	If	I
Ligeiro	24	4·5	16·4	262	0·439	0·783	0·810	2·2
Cacique Doble	15	5·2	10·5	156	0·327	0·486	0·389	1·1
Guarita	46	7·4	14·4	768	0·410	0·695	0·263	1·1
Nonoai	49	6·0	14·4	681	0·417	0·715	0·400	1·4

N_1, No. of women studied whose age was 40 or more; $\bar{x}$, average No. of live births per woman; Vf, variance in progeny number due to fertility; N_2, total No. of live births (women of all ages); pd, premature deaths (died before the reproductive age); Im, mortality component; If, fertility component; I, index of total selection intensity.

The results are shown in Table 1. The average number of live births per woman aged forty years or more varied from 4·5 to 7·4. The corresponding variance was 10·5–16·4. The mortality before the reproductive age ranged from 32·7 to 43·9 per cent. From these results the mortality and fertility components of the index of total selection intensity, Im and If respectively, can be calculated. Im varied from 0·486 to 0·783; If from 0·263 to 0·810. The extreme index values obtained were 1·1 and 2·2. Spuhler[4] has recently reviewed the information concerning these variables in ten tribal populations. The index of total selection intensity varied in these groups from 0·6 to 3·7. The values presented here, therefore, are in the lower half of the range observed. This shows that the Caingang are not subjected to too stringent selective pressures, despite the heavy mortality before the age of reproduction observed among them. In five of the ten populations reviewed by Spuhler the mortality component was higher than the fertility one, while in the five others the opposite occurred. Among the Caingang three of the populations presented higher Im and only one higher If values. Mortality is still very important in determining the gene frequencies in these Indian groups. Total selection intensity is about the same in Cacique Doble, Guarita and Nonoai, but, due to fertility differentials, shows a two-fold increase in Ligeiro.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

TUTORIAL ASSISTANT IN PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (August 6).

SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER (mathematician, with a first- or second-class honours degree in mathematics or applied mathematics with experience in the application of numerical analysis and digital computers, and preferably some knowledge of numerical methods), to lead a section of the team providing a mathematical service to the Laboratory—The Director, National Engineering Laboratory, D.S.I.R., East Kilbride, Glasgow, quoting Ref. E/AQ/030 (August 7).

LECTURERS or ASSISTANT LECTURERS (3) IN PHYSICS at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 8).

DIRECTOR (with a university degree with specialization in zoology, preferably in mammalology, and administrative experience, particularly in a museum or kindred institution) OF KAFFRARIAN MUSEUM—The Director, Kaffrarian Museum, King William's Town, C.P., South Africa (August 10).

POST-DOCTORAL FELLOW (chemist or physicist) IN SPECTROSCOPY IN THE DEPARTMENT OF CHEMISTRY, to work on the application of lasers to Raman Spectroscopy in collaboration with Dr. D. A. Long—The Registrar, University College of Swansea, Singleton Park, Swansea (August 10).

TECHNICIAN (with City and Guilds Certificate, mechanical and constructional aptitude, conversant with laboratory and field techniques and with the routine running of a research biological laboratory, preferably in a marine setting, and able to supervise a small technical staff) in the Dove Marine Laboratory (University of Durham)—The Director, Dove Marine Laboratory, Cullercoats (August 10).

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER (with a degree, Dip. Tech., or H.N.C. in electronic engineering or equivalent, and a good knowledge of electronic circuitry including pulse and high frequency techniques) IN THE BASIC PHYSICS DIVISION, to work on the development and maintenance of equipment for fundamental research in microwave and radio-frequency spectroscopy—The Director, National Physical Laboratory, D.S.I.R., Teddington, Middlesex (August 12).

ASSISTANT EXPERIMENTAL OFFICER (with a degree in a natural science or an appropriate Dip.Tech. or H.N.C., and preferably training or interest in statistics or applied physics), to join a team working in the field of building design and town planning—The Director, Building Research Station, D.S.I.R., Garston, Watford, Hertfordshire (August 12).

LECTURER (preferably with an interest in control systems, analogue computers, electrical power or electrical machinery) IN ELECTRICAL ENGINEERING—The Registrar, The University, Sheffield (August 12).

SENIOR LECTURER or LECTURER (physical chemist, suitably qualified to teach to degree level in physical chemistry for the B.Pharm. honours degree and research experience in physical chemistry applied to pharmaceutical or related topics) IN PHARMACEUTICAL CHEMISTRY (Physical Aspects)—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (August 12).

ASSISTANT EXPERIMENTAL OFFICER (graduate), to assist in aphid investigations at the Entomological Field Station, Cambridge—The Director, A.R.C. Unit of Insect Physiology, Department of Zoology, Downing Street, Cambridge (August 13).

ASSISTANT PROFESSOR IN THE DEPARTMENT OF ZOOLOGY, to participate in the cell biology programme and to teach proto-zoology, cytology, or a related subject at the senior undergraduate level—Dr. C. V. Finnegan, Department of Zoology, The University of British Columbia, Vancouver 8, B.C., Canada (August 15).

ASSISTANT LECTURER (preferably with postgraduate research experience in one of the following fields: polypeptides, nucleotides or polysaccharides) IN ORGANIC CHEMISTRY—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (August 16).

LECTURER or ASSISTANT LECTURER (with a good honours degree, and preferably a special interest in plant physiology or biochemistry) IN BOTANY—The Registrar, King's College (University of London), Strand, London, W.C.2 (August 16).

POST-DOCTORAL RESEARCH FELLOW IN CHEMISTRY at Wollongong University College, The University of New South Wales, for work on structural problems relating to natural products, chiefly alkaloids, likely to possess pharmacological activity—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (August 16).

TUTORIAL STUDENT (graduate, or about to graduate) IN THE DEPARTMENT OF BIOPHYSICS, to read for a higher degree and give a limited amount of assistance by demonstrating—The Registrar, King's College (University of London), Strand, London, W.C.2 (August 16).

EXPERIMENTAL OFFICER IN THE DEPARTMENT OF CHEMISTRY in the Faculty of Science, to be concerned with preparative work involving the synthesis of inorganic molecules containing isotopically labelled atoms—The Registrar, The University, Manchester 13 (August 17).

LECTURER IN STATISTICS; and an ASSISTANT IN MATHEMATICS in the Faculty of Science, Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (August 17).

LECTURER (with a good honours degree in science or technology, preferably some industrial experience, and interested in research work) IN PAPER SCIENCE—The Registrar, The Manchester College of Science and Technology, Sackville Street, Manchester 1 (August 17).

POST-DOCTORAL RESEARCH FELLOW IN THE DEPARTMENT OF NATURAL PHILOSOPHY, St. Salvator's College, for work on high resolution electron spin resonance at X-band and Q-band frequencies, and/or for work on the measurement of spin-lattice relaxation times by the use of microwave techniques—The Secretary, The University, St. Andrews, Scotland (August 17).

LECTURER (dental, medical or science graduate with bacteriological experience) IN DENTAL BACTERIOLOGY in the Turner Dental School—The Registrar, The University, Manchester (August 24).

SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER (with a first- or second-class honours degree in physics or engineering or equivalent, and preferably some familiarity with computer logical design), to work in collaboration with other physicists studying gamma-ray dosimetry and measurement of neutrons and of radioactivity—The Director, National Physical Laboratory, D.S.I.R., Teddington, Middlesex, quoting Ref. 121.YV (August 24).

SENIOR RESEARCH OFFICER (Soil Chemist) (with a Ph.D. degree or post-graduate research experience of equivalent duration and standard, preferably in soil chemistry, supported by satisfactory evidence of research ability) IN THE DIVISION OF LAND RESEARCH AND REGIONAL SURVEY, Commonwealth Science and Industrial Research Organization, Canberra, to undertake chemical investigations on New Guinea soils with particular reference to the clay fraction—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 618/135 (August 24).

SENIOR LECTURER (preferably qualified to lead and develop research in applied geophysics or the physics of thunderstorms) IN THE DEPARTMENT OF PHYSICS, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 25).

LECTURER IN PATHOLOGY at the Western Infirmary—The Secretary of University Court, The University, Glasgow (August 26).

SENIOR BIOCHEMIST (post-doctoral, with experience of peptide structural analysis), for research on brain peptides; and a RESEARCH BIOCHEMIST or HISTOCHEMIST, to work on problems of neurological metabolism in tissue cultures in the Department of Pathology—The Registrar, The University, Leeds 2 (August 26).

ASSISTANT LECTURERS (2) (with a medical or scientific qualification) IN BACTERIOLOGY—The Registrar, The University, Manchester 13 (August 31).

LEADER (with an established research reputation in one of the relevant scientific disciplines (biochemistry, physiology, microbiology, physics or chemistry), the ability to lead a research group and guide younger workers, and preferably experience in meat research) IN MEAT RESEARCH in the Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, Cannon Hill, Brisbane, Australia, to advise the Chief of the Division on the development of new basic and applied projects and the expansion of existing activities—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 305/71 (August 31).

SENIOR BIOCHEMIST IN THE DEPARTMENT OF CHEMICAL PATHOLOGY, United Liverpool Hospitals, to assist with the Department's routine work and research programme, particularly in relation to the metabolic ward now reaching completion at the Liverpool Royal Infirmary—The Secretary, United Liverpool Hospitals, 80 Rodney Street, Liverpool 1 (August 31).

SENIOR LECTURER or LECTURER (graduate in physics, chemistry, biochemistry or engineering, with experience of research in food processing) IN FOOD SCIENCE IN THE DEPARTMENT OF AGRICULTURAL SCIENCES at the School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham (August 31).

RESEARCH STUDENT IN GEOGRAPHY—Secretary of the Graduate School, London School of Economics and Political Science (University of London), Houghton Street, Aldwych, London, W.C.2 (September 6).

CHAIR OF MATHEMATICS; and a CHAIR OF PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR. 05G (September 7).

POST-DOCTORAL RESEARCH FELLOW IN INORGANIC CHEMISTRY, for the study of magnetic properties of transition metal complexes in association with work carried out by Prof. Ballhausen in Copenhagen—The Registrar, The University, Manchester 13 (September 7).

LECTURER (graduate in physics or agricultural science) IN SOIL PHYSICS IN THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, Waite Agricultural Research Institute, University of Adelaide—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 9).

READER IN PURE MATHEMATICS; a SENIOR LECTURER IN MATHEMATICAL PHYSICS; a LECTURER IN ORGANIC CHEMISTRY; and a LECTURER IN ELECTRICAL ENGINEERING at the University of Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 9).

ASSOCIATE PROFESSORS/SENIOR LECTURERS IN MICROBIOLOGY AND PARASITOLOGY; and SENIOR LECTURERS/LECTURERS IN PATHOLOGY (2) BACTERIOLOGY; VIROLOGY; MYCOLOGY; and PARASITOLOGY at Massey University College of Manawatu, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Massey University College of Manawatu, Palmerston North, New Zealand (New Zealand and London, September 15).

CHAIRS IN BIOLOGICAL SCIENCE (2) at Massey University College of Manawatu, Palmerston North, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

PROFESSOR OF THEORETICAL PHYSICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 15).

SENIOR LECTURER and a LECTURER IN PHYSICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 15).

ASSISTANT CONSERVATOR OF FORESTS (over 30, a university degree in forestry, and preferably at least ten years experience as a Forest Officer in South-East Asia, preferably Malaya) IN THE FOREST DEPARTMENT, North Borneo—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting T/RC.324/107/01.

BIOCHEMIST (Senior or Basic grade) at the Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick—The Group Secretary, West Bromwich and District Hospital Management Committee, Edward Street, West Bromwich.

CHEMIST (male, with an honours degree in chemistry or equivalent qualification, and not less than two years postgraduate experience or training) IN THE DEPARTMENT OF AGRICULTURE, Tanganyika, for research work on coffee, including soil and plant analysis connected with nutritional problems and soil survey work—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting T/RC.213/145/05.

DEMONSTRATOR IN ZOOLOGY—The Registrar, University College of Wales, Aberystwyth.

ELECTRONICS PHYSICIST or ENGINEER (preferably university graduate, but candidate with equivalent qualifications and suitable experience will be considered) IN THE DEPARTMENT OF CHEMISTRY, to take charge of a workshop concerned with the development and construction of new electronic equipment for research, and for the maintenance and repair of electronic instruments and equipment—The Bursar (B.49), The University, Sheffield 10.

EXPERIENCED CYTOLOGY TECHNICIAN (qualified in vaginal cytology), for routine investigations on obstetric and gynæcological patients—The Administrator and Secretary, University College Hospital, Gower Street, London, W.C.1.

GRADUATE BIOCHEMIST or CHEMIST, to work on aspects of the biosynthesis of phospholipids in the nervous system with Dr. G. B. Ansell—The Head of the Department of Experimental Neuropharmacology, The Medical School, The University, Birmingham 15.

LABORATORY ASSISTANTS (2) (preferably G.C.E. "A" level, H.N.C. or equivalent), to join a research team investigating the control of breathing in man—Dr. I. P. Priban, c/o Superintendent, Autonomics Division, National Physical Laboratory, Teddington, Middlesex.

MICROBIOLOGIST (graduate in any of the biological sciences considered) IN THE BACTERIOLOGY LABORATORY—The Administrator and Secretary, University College Hospital, Gower Street, London, W.C.1.

PHYSICIST and a PHYSICAL CHEMIST (of Ph.D. standing, preferably with research experience in a related field) to join a team engaged in basic, long-term research into the photoconducting behaviour of organic materials—The Director, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Midlothian.

POSTDOCTORAL FELLOW IN PHYSICAL CHEMISTRY, to work on a fundamental study of thermal diffusion in binary liquid mixtures under the direction of Dr. H. J. V. Tyrrell—The Registrar, The University, Sheffield 10.

RESEARCH ASSISTANT (preferably with a Ph.D. and an interest in the electrophysiology of nerve fibres), to work on a programme of study of nervous tissues *in vitro*—Prof. C. E. Lumsden, Department of Pathology, The University, Leeds 2.

RESEARCH ASSOCIATE (honours graduate, preferably with experience of cytology), to work on human chromosomal aberrations in the Medical Genetics Laboratory of the Institute of Child Health—Prof. Douglas Hubble, Institute of Child Health, Francis Road, Birmingham 16.

RESEARCH FELLOW (with an honours degree in chemistry, biochemistry or agricultural chemistry or in a closely allied field), for work in the field of protein nutrition—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 63/11.

RESEARCH OFFICER, HEAD OF THE ANIMAL HUSBANDRY DIVISION (under 50 years of age, with a good honours degree, preferably in sciences which include animal physiology and biochemistry, and experience in initiating research programmes in the field of animal husbandry and management) in the East African Agricultural and Forestry Research Organization, Muguga, Kenya, to be responsible to the Director for the initiation and carrying out of research programmes on important problems of animal management in East Africa—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. C1/R.7217/383/01.

SCIENTIFIC OFFICER (male or female, with an honours degree in science or biology) IN THE LABORATORIES OF THE BLOOD TRANSFUSION SERVICE—The Director, Regional Blood Transfusion Service, Roby Street, Manchester 1.

RESEARCH SCIENTIST (with a degree in a scientific subject and an interest in the physical properties of powders), for fundamental investigations into flour milling processes; and an ORGANIC CHEMIST, to join a small team working on chemical modifications of starch—The Director of Research, Cereals Research Station, Research Association of British Flour Millers, Old London Road, St. Albans, Herts.

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SENIOR TECHNOLOGIST (with sound training and experience in electron microscopy and ultra-microtomy, and preferably with a science degree or diploma) IN THE MEDICAL RESEARCH UNIT—Prof. C. E. Lumsden, Department of Pathology, School of Medicine, The University, Leeds 1.

TECHNICIAN (expert in preparing thin and polished sections and competent to take charge of other technical duties) IN THE DEPARTMENT OF GEOLOGY—The Chairman, Geology Department, University of Ottawa, Ottawa, Ontario, Canada.

TECHNICIAN, for clinical research in the Respiratory Function Laboratory—The Administrator and Secretary, University College Hospital, Gower Street, London, W.C.1.

TISSUE CULTURE TECHNICIAN, for work in the Radiobiology Unit—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

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Ghana. Report of the Forestry Division—Ministry of Agriculture for the calendar year 1960. Pp. 48. (Accra : Government Printing Department Publications Branch, 1963.) 6*s*. 6*d*. [146

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World Health Organization. Public Health Papers. No. 17 : Paying for Health Services—a Study of the Costs and Sources of Finance in Six Countries. By Brian Abel-Smith. Pp. 86. 3 Sw. francs ; 5*s*. ; 1 dollar. No. 18 : Medicine and Public Health in the Arctic and Antarctic : Selected Papers from a Conference. By K. Lange Anderson and 9 other authors. Pp. 169. 6 Sw. francs ; 10*s*. ; 2 dollars. Technical Report Series. No. 253 : Conference on Medicine and Public Health in the Arctic and Antarctic, Geneva, 28 August–1 September 1962—Report. Pp. 29. No. 254 : Public Health Responsibilities in Radiation Protection—Fourth Report of the Expert Committee on Radiation. Pp. 23. 1 Sw. franc ; 1*s*. 9*d*. ; 0.30 dollars. No. 255 : CCTA/WHO African Conference on Ancylostomiasis, Brazzaville, 22–29 August 1961—Report. Pp. 30. 1 Sw. franc ; 1*s*. 9*d*. ; 0.30 dollars. No. 256 : The Care of Well Children in Day-Care Centres and Institutions—Report of a Joint UN/WHO Expert Committee convened with the Participation of FAO, ILO and UNICEF, Geneva, 23 October–1 November 1962. Pp. 34. 2 Sw. francs ; 3*s*. 6*d*. ; 0.60 dollars. Distribution of and Trade in Pharmaceutical Preparations : a Survey of Existing Legislation. (Originally published in the *International Digest of Health Legislation*, 1962, 13, 381–466). Pp. 88. 3 Sw. francs ; 5*s*. ; 1 dollar. World Directory of Veterinary Schools. (Published under the auspices of the Food and Agriculture Organization of the United Nations and the World Health Organization.) Pp. 216. 16 Sw. francs ; 28*s*. 8*d*. ; 5.25 dollars. (Geneva : World Health Organization : London : H.M. Stationery Office, 1963.) [146

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United States Department of Agriculture. Leaflet No. 520 : Controlling the Sugarcane Beetle on Corn in the Southern States. Pp. 6. (Washington, D.C. : Government Printing Office, 1963.) 5 cents. [216

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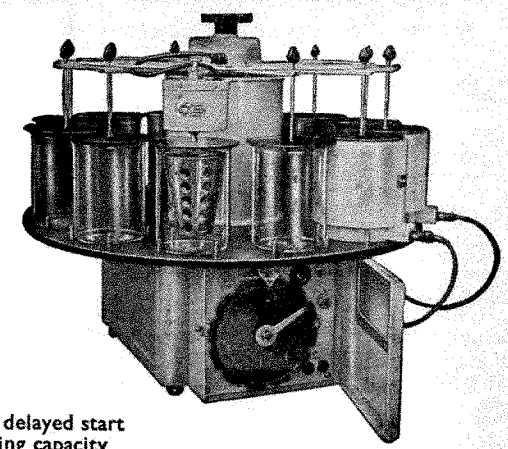
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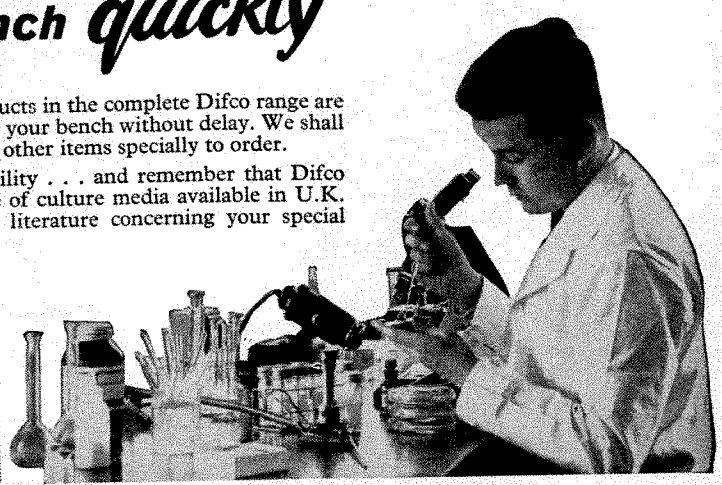
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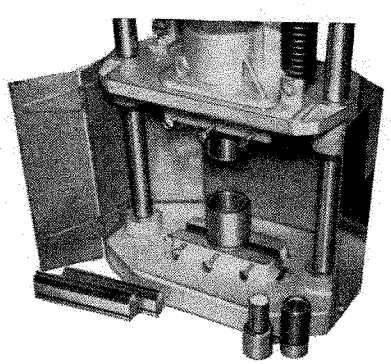
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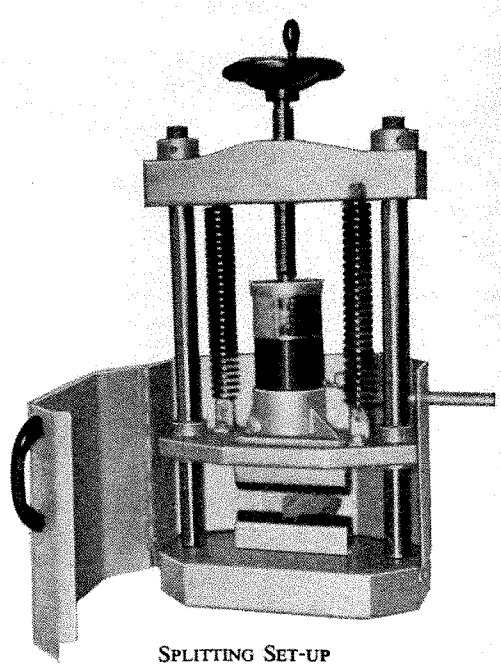
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MANAGEMENT PERFORMANCE

BRITAIN'S failure to maintain a challenging place in the race for productivity has, in turn, been ascribed to lack of early enough awareness by the Government of what was involved, lack of competence by industrial management, and lack of appreciation by the nation at large that the balance of trade has so changed that we can no longer live on our 'invisible' earnings. The setting up of the National Economic Development Council at the beginning of 1962 was itself admission that the Government was at last taking national economic planning seriously, while recent moves to set up a senior institution concerned with management education is evidence that there is now considerable support for the belief that the quality of management can be considerably improved by some basic education and training.

The amount of management training being carried out in institutions of all kinds is still far too small, however, and this and other projected developments will have little effect on management performance for some time. Many of the older managers will be little affected by any of the proposals for improving management education, and they form the bulk of present-day management. What can be done to help them to improve their own performances ?

The majority of these men are individuals who left school at the 'elementary' or grammar school stage and whose further education has been confined to what they could pick up in evening classes and on the job. Despite much criticism, these managers have served industry well and many of them possess considerable business shrewdness and practical awareness of what management involves and requires. Over the past decade plenty of advice has been showered on them, much of it of that 'gimmicky' kind which temporarily fills the pages of industrial journals and then quickly disappears. The need for better communications, 'brain-storming', 'posting' of problems, joint consultation, management by exception, management by objectives—all have merit but all have suffered through too much advocacy and too grandiose claims. Courses and conferences of all kinds have been arranged for them, and, while many of the more specious have not justified the claims of their organizers, others have at least been useful in persuading some managers to take a more empirical look at their own processes of management.

In this ephemeral world of panaceas three firm trends have emerged. The first is that enough experiment with management education has been carried out to justify all the recent moves to extend the quality and quantity of management courses on a secure and permanent basis. The second is that the invasion of mathematical concepts into industrial practice and procedure has only just begun. The use of operational research, network analysis, critical path planning and value analyses to improve stock and quality control, production scheduling, plant performance, distribution and warehousing and financial control have already reached the stage when production managers, engineers, marketing sales and financial management realize that their jobs can no longer be adequately carried out without substantial statistical support.

The third trend is of more recent origin and provides not only a continuing basis for the control of management performance but could also be the short term answer to the means of improving the country's productivity each year by the 4 per cent target which the National Economic Development Council regards as essential for healthy growth. Much management education and training has held little meaning for existing managers because they have failed to see the relevance to their particular jobs. They have failed to find yardsticks to measure their own performance and that of their colleagues, and the training provided has often appeared academic and remote. Even the annual appraisal of their performance during the year has seemed to consist of no more than a rigid mixture of generalization and exhortation.

Over the past few years some of our more enterprising organizations—not all big and not all private enterprise—have begun to develop training and development programmes for management based on their individual performances during an observed period. This is usually carried out as part of the annual appraisal procedure but bears no relation to the superficial way in which this is frequently performed in many institutions. Effective appraisals of management performance are carried out in considerable depth and in such a way that subjectivity is eliminated to the greatest possible extent. Although much of the early thinking about the control of management performance was initiated in the United States*, the detailed application to produce measurable results would appear to have been more successfully carried out in Britain. In a recent American work, which has attracted considerable attention†, the author gives useful advice about how systems and procedures in a business may be overhauled. His sections on the actual means of evaluating executive performance in the revision of these systems and procedures is superficial and sketchy and implies that, in common with other American authors, Leonard has failed to grasp the nettle. Drucker, on the other hand, has made a significant contribution to means of improving over-all business effectiveness and it would be useful if he would now turn his attention to improving individual management performance‡.

The true assessment of management performance and the building of an individual development programme cannot be begun unless the job is first clearly defined. In co-operation with the manager and his immediate chief, an experienced investigator needs to draw up a detailed job specification indicating the manager's duties and responsibilities, the tasks he undertakes to carry out those duties and responsibilities, the standards he is expected to reach to perform the tasks satisfactorily, and the controls which can be used to measure standards achieved against set tasks. Ideally, all standards should be capable of quantitative estimation but this is unlikely ever to be completely achieved. In practice, however, the fact that standards have been painstakingly set means that they are discussed with much greater objectivity during appraisal reviews.

This systematic approach to management performance has already produced some interesting results in those companies where it is being used. The company quickly

finds weaknesses in administration and procedures which it is able to eliminate; it receives suggestions from men whose views have not previously been sought about ways of improving its administration and procedures. Individuals taking part in appraisal procedure are encouraged when persistent difficulties are removed, while there is an all-round improvement in morale and teamwork when all-round improved performance begins to influence productivity. In the short term this seldom fails to occur and, over a longer period, can produce more than the National Economic Development Council target of 4 per cent without any increased capital expenditure. This is particularly so where appraisal of performance standards is followed by education and training programmes designed specifically to meet the needs of individual managers. In practice again, the needs of individuals have been followed by much more education and training on the plant than on external courses, and this in itself has led to a greater interest in means of improving productivity. While this systematic approach to management performance is still at an early stage, it offers one of the most fruitful ways of harnessing the practical knowledge and experience of existing managers which has yet been devised. Unfortunately, it requires skilled job analysts and men experienced in promoting in-plant education and training facilities and these are almost as rare as industrial mathematicians.

The main difficulty in introducing such a systematic approach to improved management performance is that it demands great skill in the handling of human relationships. Where the job analyst is a man employed by a company he should be drawn from the higher ranks. Sometimes it is more useful to use an experienced and independent 'outsider' who could profitably be a social research worker or a management consultant. Rosemary Stewart has directed attention to the valuable work which social research workers in industry can do but, surprisingly, she seems unaware of all that enterprising and critical managements are doing to improve their own performances§. Their efforts would well repay study by those who work in academic institutions. Industry is increasingly learning to make use of techniques being developed in academic institutions—selection processes, job analysis and the training of skilled operators, executive job grading for salary determination and so on—and many of the practical applications could well be used in the originating centres. The systematic approach to means of improving managerial performance which industry has adopted could profitably be used in other institutions.

* See various publications of the American Management Association and the Society for the Advancement of Management.

† *The Management Audit: An Appraisal of Management Methods and Performance*. By William P. Leonard. Pp. 238. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1962.) 120*s*.

‡ *Harvard Business Review* (May–June 1963).

§ *The Reality of Management* (Heinemann, 1963).

THE MARK OF THE MASTER

Chromosome Marker

By K. R. Lewis and B. John. Pp. x+489. (London: J. and A. Churchill, Ltd., 1963.) 65*s*.

ALTHOUGH it is a quarter of a century since the publication of the last edition of Darlington's *Recent Advances in Cytology*, I still find it difficult to read a new book on chromosomes with a mind unbiased by comparison with the *Advances*. *Chromosome Marker* is no exception, and in fact the authors almost invite the comparison as they pay just and handsome dedication to their master. It is inevitable therefore that, despite the title which only became meaningful after reading much of the book, I had hoped for the much-needed synthesis of the past twenty-five years work on chromosomes. This is still to be written. *Chromosome Marker*, for example, does not make the near-synthesis that is now possible of the seemingly conflicting evidence on chromosome structure derived from tritium labelling, enzyme digestion, X-ray breakage and electron microscopy. Nor does it bring together the data on the vexed question of the mechanism of crossing-over. This was not the authors' intention.

The scope of the book, which is divided into four sections, includes a description of the normal behaviour and form of chromosomes in division, chromosome duplication and movement, and the mutational changes which occur in chromosomes. Full and useful accounts are given of the tritium-labelling experiments on duplication, on the action of the centromere in chromosome movement, and the morphological changes in some dipteron chromosomes which are related to cellular activity. Two sections of the book are devoted to types of visible chromosome markers of all kinds and their use in cytological and genetical experiments. A full and useful summary of Riley's work on the genetic control of chromosome pairing in wheat is given. All this is of considerable interest, and is the most valuable part of the book. It should be particularly valuable in the expanding field of human cytology where much of the plant and animal cytology is now being applied.

The final section of the book on the "Chromosomes in Evolution" invites comparison with the Darlington classic *Evolution of Genetic Systems*, and it is here that there is some disappointment. This may be because this aspect of genetics is one without a productive methodology. It has to rely largely on ideas and deduction, and therefore a limited advance is inevitable.

The book includes many quotations from the classics; these occupy a prominent place at the beginning of the chapters. It also has a short classification of the plant and animal kingdoms but it does not include a glossary of terms. Most of the scientific terms are explained when they are first used in the text. It is possible to find references in the index to Twain, Mark; Conan Doyle, Sir Arthur; and Carroll, Lewis; but terms such as 'desynapsis' or 'sympatric', used in the text, are not in the index. There is some unnecessary repetition in the book; for example, Chapter 2 begins, "All the characteristics of an organism are the result of interaction between the genotype and the environment. This is the primary postulate of genetics". The next but one chapter begins, "The phenotype is a product of interaction between the genotype and the environment. This is the basic premise of Genetics". Despite these and other small irritations, the book is one that should be read carefully by all who are interested in chromosomes and heredity. D. Lewis

COMPARATIVE PHILOSOPHY

Introduction to Comparative Philosophy

By P. T. Raju. Pp. xii+364. (Lincoln: University of Nebraska Press, 1962.) $7.50

STUDENTS in universities and readers in general have long been accustomed to books bearing such titles as "Comparative Anatomy" or "Comparative Religion", but "Comparative Philosophy" is novel. For this reason alone, *Introduction to Comparative Philosophy* is welcome. Moreover, in other ways, too, its appearance is significant. In a world wherein events make themselves felt everywhere with increasing speed, there is an obvious need for a wider understanding both between peoples as such and between individuals of different outlook and of varying

backgrounds. To achieve any measure of success in such an enterprise is not easy: nevertheless, Prof. Raju has made a notable contribution and what he has to say is deserving of very careful study. It should be observed at once that a strong point of the present treatment is the clear résumé provided at the end of every main section, setting out the essentials of each of the major philosophies —Western, Chinese and Indian. These summaries are again analysed and compared, thus achieving a particularly lucid methodology.

After a general introduction, the main theme is tackled as follows. First, Western philosophy and the struggle for the liberation of the outward; secondly, Chinese philosophy and human mindfulness; and thirdly, Indian philosophy and the explication of inwardness. A chapter on comparisons and reflexions, and a couple of appendixes complete the whole. These headings are themselves of value, as showing the way in which the author's mind works. He is a distinguished Indian scholar, and perhaps a little more introspective than most of us; yet he manages to keep his feet well on the ground and writes (for the most part) with the impartiality to be expected from a seeker after truth. The chronological table at the end is not without omissions which some authorities would regard as unfortunate, but as an example of its range, the Western column begins with Moses and ends with A. J. Ayer.

Prof. Raju is careful to point out that he is deliberately including Jewish philosophy as part of the Western tradition. This is sensible and makes easier the discussion of mediaeval thought (especially scholastic), and the development of a conscious thesis, more particularly by the great figures at the University of Paris, and the repercussions of their labours on the nascent seats of learning at Oxford and Cambridge, not to mention the influence of the friars.

These pages mirror the thoughts of a philosopher rather than those of a scientist or of a mathematician. That is sound enough, but it still leaves the feeling that a comparison of universal knowledge down the ages—and that is the subject under discussion—is only possible if some attention is given to the logicians, and the disciplines which are covered by them. It is true that China was defective here, but she was not without science and technology, which in their turn orientated her characteristic humanism. Needham's monumental volumes receive no mention at all, which is almost as much as to say that the "good life" of Confucius—and China's marked pragmatism—was untouched by her ability to invent and to apply. This in no way detracts from the importance of what the author has to relate, but it does mean that the reader must take his own steps to acquaint himself with all the facts before he can justly measure what has happened.

Indian philosophy is essentially concerned with salvation, and its emphasis is inward. There is no privileged intuition, and no heroes as such, and perhaps, consequently, no dogma. All this is well put, but the fact remains that had India produced a Plato or an Aristotle at the crucial moment in historical time, much might have been gained, and little lost.

Prof. Raju's erudition is enviable, and its scope remarkable. With an engaging modesty he realizes the limitations of language, and the possible loss of fine-structure due to translations. Nevertheless, his rendering of some parts of the Western tradition indicates something of a blind spot when faced with the relation between revelation and reason, or, if one so prefers, the recognition of philosophy as the hand-maiden of theology. Supposedly he has not absorbed the works of Gilson and Lessing (they are not included in the index), particularly the remark to the effect that the great truths of religion were not rational at the time of their revelation, but that some of them in any event were revealed that they might become so. In other words, St. Augustine and St. Thomas Aquinas did far more than merely re-edit Plato and Aristotle: at their hands something very important was added. It is entirely correct for Prof. Raju to maintain that he is not writing about religion, except incidentally. Yet he passionately wants philosophy to be a way of life for mankind (and the West has thwarted that aim to some degree by making it so much of a departmental study in most of its universities). If that is so, then the West may perhaps curb some at least of its outward tendency—as it is already doing—in an attempt not to frustrate reason, but to direct it where it may be most fruitful, strengthening the traditional humanism of China, and upholding the deeply felt naturalism of India.

The whole fabric of this book demonstrates the author's charity and his capacity to comprehend. Ratiocination may weary and faith become weak and errant, but *credo ut intelligam* still points the way for many people, and they, among others, could find their life illuminated by these admirable studies in comparative philosophy.

F. I. G. Rawlins

FLYSCH DEPOSITS

Sedimentology of some Flysch Deposits

A Graphic Approach to Facies Interpretation. By Dr. A. H. Bouma. Pp. xii+168. (Amsterdam: Elsevier Publishing Company, 1962.) 64*s*.

THE term 'flysch' was proposed by B. Studer in 1827 for a group of early Tertiary geological formations in the Pre-Alpine zone of Switzerland. It was quickly recognized that these formations had distinctive sedimentary characteristics, and the name came to be applied to similar rock-series of many different ages in many parts of the world. All these had in common an association with the later stages of major orogenic cycles; but no attempt at precise definition was made. Great impetus was given to the study of these rocks by the recognition, by Ph. Kuenen and his collaborators in the early 1940's, that marine turbidity currents were largely responsible for the formation of flysch; this theory at once provided an explanation for many of their sedimentary features, and a reason for their orogenic association. Since that time a voluminous literature has appeared in the journals of the world; the present volume is, however, the first 'hard-cover' text to appear in English. Since such a book might be expected to have a wider circulation among the 'non-specialists' than individual papers, and therefore to carry more authority, it must be pointed out that this volume represents no more than a thoroughly competent study of the kind carried out by many authors in many parts of the world, but which by accident of publication do not reach a wider public.

The major part comprises a description in detail of the Eocene–Oligocene flysch of the Peira-Cava area, 20 km north of Nice. No better introduction to the flysch could be obtained than from the magnificent, readily accessible sections here, and Dr. Bouma's text describes them lucidly. From their study he develops the hypothesis that each sandstone bed contains some elements of a sequence of rock types (named T_{a-e}, or sometimes, confusingly, T_1) each deposited by a different part of the turbidity current flow. He points out significantly that the base of this sequence may be missing (in regions distant from the source) or the top; but never the middle. Using the well-established methods of palæo-current studies he builds up a convincing picture of the palæogeography of the Alpes Maritimes. He then compares his rocks with a variety of others in Europe, and concludes that some are flysch and some are not, without giving a very clear indication of his criteria.

The first chapter is devoted to a description of a method for the production of 'graphic logs' for these sedimentary sequences. This is carefully worked out, and certainly

will facilitate accumulation of data. Dr. Bouma fails to convince that it is essential—the whole of the present volume can be perfectly comprehended without recourse to this system.

The glossary, as the text, sometimes gives the impression of being definitive where no general agreement among specialists exists—this particularly applies to the nomenclature of sole-markings. To some extent this is compensated by a full bibliography; read with an awareness of the complexities of this controversial subject, Dr. Bouma's contribution is very valuable indeed.

J. E. Prentice

PERPETUUM MOBILE

Tectonics

By Prof. Jean Goguel. English translation from the French Edition of 1952 by Hans E. Thalmann. (Series of Geology Texts.) Pp. viii+384. (San Francisco and London: W. H. Freeman and Company, 1962.) 70*s*.

THIS is a translation, with minor additions, of *Traité de Tectonique* which first appeared in 1952. We quickly realize just how much the geophysical sciences have advanced our knowledge of the Earth's crust during the last ten years. Since 1952, numerous publications have given the results of seismic exploration, gravity and magnetic surveys and heat-flow measurements at sea. We have had geophysical investigations of continental margins, deep-sea trenches and mid-oceanic rifts. A wealth of results in palæomagnetism has influenced our thinking about continental drift. Numerous advances in theoretical seismology have enabled a better probing of the crust and upper mantle. The use of electronic computing techniques has aided seismological investigations and the interpretation of geophysical surveys of granites, sedimentary basins and rift valleys. All these provide material for a re-appraisal of the tectonic history of the Earth. The most striking example is that the basic difference of the structure of oceanic and continental crust was not firmly established when this book was written. This must now surely be the starting point in structural geology, and it is sad that most university geology departments still teach the subject without recognizing the sea floor covers more than two-thirds the Earth's surface and whole *mémoires* have appeared on the results of oceanic exploration.

A translation of this kind is therefore necessarily outdated. However, the book contains much useful information and gives a profound coverage of classical thoughts and observations on the tectonics of the continental areas. There are excellent accounts of the observational powers of the geologist in investigations of erosion and deformation, morphology, stratigraphy, rock deformations and folding and faulting. There is a useful account of elementary geological mapping, including the use of bore-hole information and underground workings. There is a brief review of geophysical exploration and the geometric presentation of results and structural maps. All this is followed by a systematic description of basic structural forms and by various interpretations. Throughout, numerous examples provide adequate illustrations.

Prof. Goguel gives an impression of an ever-mobile Earth's crust. It seems that at almost any given time in geological history, field evidence can be produced from somewhere on the Earth's surface to reveal tectonic movements. Another phenomenon which is well brought out is the behaviour of the crust and mantle to stresses of different time constants. It is important to realize that the materials of the crust and mantle behave as solids to stresses for times of the order of 0·5 sec and as fluids to stresses for times of the order of 10^4 years. There is field evidence for this phenomenon and it is strange how little it has been considered in theories of crustal movements and continental drift.

Finally, what is the fundamental cause of tectonic deformations? The Earth could be cooling, and the crust reflects stresses due to shrinkage. The Earth could be warming up and the sialic crust dissipated due to expansion. The Earth could be alternately warming and cooling and the various tectonic deformations through geological time could be a consequence of both expansion and contraction. Lastly, the surface structures and deformations may be due to convection currents in the mantle. In recent years, this last-mentioned hypothesis has been fashionable and Jean Goguel gives it much attention. From recent work in palæomagnetism and exploration of the sea floor, especially the discovery of faults with horizontal displacements of the order of 1,000 km, mantle convection must be a strong possibility.

The book is well produced and carefully thought out. It also serves as a good introduction to continental geological literature. The translation is fair, a curiosity being that the translator seems to have given up translating metres to feet and inches after p. 14; a reminder that it is high time geology joined the other sciences in using the metric system.

R. W. Girdler

AUTOMATIC CONTROL

Automatic Control

An Introduction to the Theory of Feed-back and Feed-back Control Systems. By A. E. De Barr. (The Institute of Physics and the Physical Society Monographs for Students.) Pp. vii+118. (London: Chapman and Hall, Ltd.; New York: Reinhold Publishing Corporation, 1962. Published on behalf of the Institute of Physics and the Physical Society.) 7*s*. 6*d*. net.

Automatic Control Handbook

Advisory Editor, G. A. T. Burdett. Pp. xviii+864. (London: George Newnes, Ltd., 1962.) 90*s*.

Anatomy of Automation

By George H. Amber and Paul S. Amber. Pp. x+245. (Englewood Cliffs, New Jersey and London: Prentice-Hall Inc., 1962.) 60*s*.

THREE recently published volumes in the field of automatic control may broadly be described as dealing respectively with conventional linear theory, components and circuit techniques, and the philosophy of automation in industry.

Automatic Control is an inexpensive paper-backed volume in the Monographs for Students series in which is found a concise presentation of the basic linear theory of feed-back control systems, and a short chapter on non-linear systems. The author dispenses with the introduction to Laplace Transforms found in most standard text-books on this subject, and derives transfer functions and characteristic equations using only the differential operator. This approach leads to a demonstration of the dependence of the natural modes of the system on the roots of the characteristic equation that is not wholly satisfactory, but the saving in space is considerable and enables the concepts of circuit theory to be covered in the first chapter. Also introduced in this chapter of twenty-nine pages are Bode and Nyquist plots, segmented gain characteristics, and the result of the gain-slope theorem. In this last-mentioned connexion an error appears on p. 13 where minimum phase systems are described as having merely poles, rather than both poles and zeros, without positive real parts.

Closed-loop systems are introduced in the second chapter, which includes the frequency-response locus and Hall Chart, and in the third chapter a simple physical system is introduced together with an analysis of damping

methods and of velocity lag. In this chapter some small errors appeared in the diagrams where the impedances connected to the operational amplifiers should be directly connected, not through error detectors.

The stability problem is discussed in Chapter 4 and an unfortunate error occurs early on, where it is stated that poles must be positive for transients to decrease with time, although this statement is corrected in the text which follows. The Nyquist criterion and phase-gain method of stability analysis are described, and also the Hurwitz criterion, although not the somewhat simpler one of Routh. Damping and compensation are also discussed. The brief discussion of non-linear systems in the following chapter includes a derivation of the parabolic response of an on-off system, but no mention of phase-plane or other analytical methods.

In the final chapter, typical examples of simple controllers taken from the mechanical, electrical, hydraulic and pneumatic fields are described. Thus the author reinforces the point made at the outset and maintained throughout the book, that linear theory is applicable in all these fields and that the student of control must be aware of this and appreciate the merits of each. Despite its conciseness, the presentation, apart from the errors mentioned, is exceedingly clear, and this small volume is ideal for students and should be very valuable in teaching the subject.

In a very different category of price, size and purpose is the volume entitled *Automatic Control Handbook*. Using a minimum of mathematics but with many photographs and diagrams, this book describes the principles of operation and the application of many of the components and devices of automatic control. Among the twenty-one chapters contributed by different experts are those devoted to motors, relays, magslips and selsyns, magnetic and rotating amplifiers, voltage and temperature control, materials, transducers, photoelectrics and nucleonics, analogue computers, mechanical handling, lubrication, hydraulic and pneumatic control, machine-tool control, gyroscopes and television. Digital systems receive little attention, and this is perhaps the most significant omission in an otherwise comprehensive list. The nature of this work is such that the words "feed-back", "transfer function" and "non-linearity" seldom appear and, as is to be expected in a composite edition such as this, there is no thread of theory relating the various chapters. This is particularly noticeable on reaching the chapter devoted to analogue computers which outlines methods of solving problems which can only be formulated with the aid of basic feed-back theory. Thus, while not attempting to study the closed-loop problem, this volume will none the less be valuable to the designer as an aid to the selection of methods or components, which for problems in open-loop control is often all that is required. A number of advertisements are included, as well as a comprehensive index.

Anatomy of Automation is described as a "broad survey of production and automation trends to aid business executives and production men". This book is mainly concerned with definitions and classification, and in the first chapter the authors define a "scale of automation" containing ten groups and given the symbol A, which they compare with the symbol F used to represent the Fahrenheit scale of temperature. A chapter recognizing the office, military, domestic and industrial fields of automation is followed by an examination of various production operations with the view of classification.

The remaining chapters are devoted to describing the levels of automation which have been found advantageous in various situations, and thus to aiding the industrialist in making the necessary decisions in this direction. The book is completely non-mathematical, to a large extent non-technical, and is well supplied with illustrations. An index and bibliography are provided.

E. M. Deeley

NUTRITION OF PIGS AND POULTRY

Nutrition of Pigs and Poultry

Proceedings of the University of Nottingham Eighth Easter School in Agricultural Science, 1961. Edited by Dr. J. T. Morgan and Dr. D. Lewis. Pp. ix + 366. (London: Butterworth and Co. (Publishers), Ltd., 1962.) 70*s*.

THOSE who have attended Easter Schools of the University of Nottingham are in no doubt of the quality of the papers presented and of the liveliness of the subsequent discussions. Not all authorities on their special subjects are equally expert in the art of oral presentation, and few listeners are capable of assimilating all the views and facts presented at the time. The subsequent publication of the *Proceedings* with discreet editing of both papers and discussion is therefore always to be welcomed; it provides a stimulating 'refresher" for those who attended, and to the wider range of readers who could not be present, a comprehensive review of up-to-date information in the numerous fields embodied in the subject of the School. Indeed, it goes further, for in the presentation of the present position of knowledge of a particular topic, it helps to emphasize the gaps in knowledge and the directions in which new research is urgently required. The organizers of these Schools are sufficiently far-sighted, to the extent that their resources will permit, to invite speakers from the United States and from various European countries to make specifis contributions. Language difficulties are largely eliminated by skilful editing, and the net result is that these *Proceedings* represent an authoritative work of great value. Workers in the field of nutrition of pigs and poultry are very conscious of the value of research from other countries, and the present volume is enhanced by the contributions from outside the United Kingdom.

It is an ambitious project to cover the nutrition of pigs and poultry in a single volume. The *Proceedings* cover four main aspects: (1) general physiology and principles of nutrition; (2) environmental physiology; (3) nutrition of poultry and (4) nutrition of pigs, and in terms of page space these subjects are dealt with in the ratios of 1 : 1 : 1 : 2. Section 1 serves as an introduction and is mainly a re-examination of fairly well established knowledge. Section 2 brings home the fact that the scientific study and evaluation of the environmental physiology of both pigs and poultry is a comparatively new field, demanding far more intensive investigation than so far has been given to it. Although these studies are still largely academic the practical importance of this work will mount steadily over the next few years. It is now becoming recognized that much of the older nutritional work with these species completely disregarded the all-pervading influence of environmental factors, which assume an ever-increasing importance in the practical translation of laboratory findings to the field of economic animal husbandry.

Section 3 is concerned with poultry nutrition, and it is well accepted that the nutrient requirements of poultry generally are better understood than those of any other economically important class of livestock. The main trend to-day is to work out a more complete understanding of the optimum relationships between energy, proteins, minerals and vitamins and to translate these into terms of practical dietary formulation. Section 4 covers the nutrition of the pig in all aspects from the nutrition of the fœtus, the pregnant and lactating sow, and the piglet through the normal phases of growth and fattening. In this major section of the *Proceedings* most contributors were able happily to marry fundamental scientific concepts to practical implementations and thus give sustained interest to the mixed audience of fundamental and applied scientists, advisers and commercial interests. In consequence these published *Proceedings* will no doubt have a strong appeal to a reading public of very diverse outlooks connected with animal nutrition.

One cannot pay too high a tribute to the skill of the editors, who have managed to weld some twenty separate contributions and associated discussions into a coherent and masterly volume that should serve as a valuable reference work for several years to come. As they state in the preface " . . . the objectives of precision in nutrition can only be built upon a more complete knowledge of the basic biochemistry and physiology" of pigs and poultry. Although these objectives have not yet been reached and our basic biochemical and physiological knowledge is far from complete, this volume admirably indicates how far we have advanced, and as such it is to be commended to all with interests in the nutrition of pigs and poultry.

A. Eden

DOMESTICATION AND BEHAVIOUR OF ANIMALS

The Behaviour of Domestic Animals

Edited by Prof. E. S. E. Hafez. Pp. xiv + 619 + 21 plates. (London : Baillière, Tindall and Cox, Ltd., 1962.) 75*s*. net.

THE origin and adaptiveness of the races of domestic animals is a topic of wide interest, for it is a field in which the zoologist, the comparative anatomist, the ethologist, the physiologist, the farmer, the animal trainer, the psychologist, the anthropologist and the archæologist all have a stake. The production, therefore of a massive book on the subject raises high hopes and this book is indeed a considerable achievement. It is divided into four parts: (1) behaviour and domestication, (2) fundamentals of behaviour, (3) behaviour of mammals, (4) behaviour of birds; together with an epilogue entitled "Comparative Behaviour." Such a large book by so many authors is, however, bound to be very uneven and there is plenty to criticize in individual contributions; moreover, the editors have not succeeded in making any real unity out of a diverse series of topics covered by a diverse collection of authors.

Of the two chapters in Part 1, Scott's introductory essay is too vague and too limited to help the book on very much. E. B. Hale's chapter, on the other hand, entitled "Domestication and the Evolution of Behaviour," contains a good deal of interest; but even he misses out some of the most important points—due, I think, to being insufficiently at home with the modern ethological approach. Thus there is no satisfactory discussion as to the evidence bearing on the nature and evolution of innate or instinctive behaviour from a study of the characteristics of this behaviour in domesticated species. Again, it is well known that, among domestic animals, improperly directed sexual behaviour is particularly common—so common as to be almost a characteristic of domestication. In this connexion it has been argued that those animals most likely to have been domesticated are those which could learn most readily to transfer their responses from individuals of their own species to individuals of the human species. That is to say, species which could be readily imprinted, and so psychologically modified, would be those which would be most likely to respond successfully to attempts at domestication. But a contrary thesis has also been maintained: namely, that under domestication the probability or possibility of imprinting on to biologically inappropriate objects is so much greater than in the wild that there would tend to be selection against the ability to be imprinted during domestication; for if it were not so, imprinting would almost certainly interfere with normal reproduction. Here is an issue of fundamental importance and interest which seems to be almost entirely ignored.

Part 2 commences with J. L. Fuller's chapter on the genetics of behaviour. It is scarcely the author's fault that this is not so interesting as the title might lead one to expect; behavioural genetics is in fact an extremely difficult subject and—in the higher animals at least—the inheritance seems always to be polygenic. It will be a long time yet before there is enough material to write a satisfactory summary of this subject. Some of the chapters show too exclusive a dependence on the American literature and, in particular, a neglect of the European continental contribution. This is specially evident in what should otherwise have been a valuable chapter, that on physiological mechanisms and behaviour patterns. On the other hand, the chapter on behavioural pharmacology is both unusual and extremely useful. Denenberg in Chapter 6 contributes a valuable discussion on the effects of early experience.

Coming to Part 3, we find chapters written much more from the point of view of the student of animal husbandry. They are interesting, valuable and competent in their way; but they tend too often to be a collection of isolated facts unrelated to the ethological principles underlying the subject. Thus it is almost unbelievable that anyone should set out to write a chapter on the behaviour of dogs and make no mention of the classic work of Schenkel on wolves, or even of the excellent though popular account of the Cryslers on the same species, or of Murie on the coyote. Some of Lorenz's work in this field is unjustly neglected, and throughout the whole book the contributions of Hediger on the behaviour of animals in zoos and circuses seems to have been entirely forgotten. Of the mammalian chapters, those on the horse and the cat are perhaps the most thorough and widely based. Part 4, which deals with bird behaviour, contains valuable and original work by Hale and Schein on turkeys, and Collias on ducks. To summarize, the book is in many respects an achievement to be welcomed; but large though it is, its faults are chiefly those of omission and sometimes of too restricted an outlook.

W. H. Thorpe

THE REALM OF FISHES

A History of Fishes

By J. R. Norman. Second edition revised by P. H. Greenwood. Pp. xxxi + 398. (London : Ernest Benn, Ltd., 1963.) 21*s*. paperback.

THIS is an extremely interesting and informed book, a revision of a work first published in 1931. Comparison with the original edition reveals that much of the text remains unchanged, as does the general plan of the book; the chapter headings and many of the text figures are the same. Thus, at first glance, the new edition is closely similar to the old, and this gives some idea of the reason for the success of the book (which went through no less than four impressions after the Second World War). Norman's book was a remarkably original one in its emphasis on the study of fishes as living animals, not as museum specimens, and his approach being essentially modern, few alterations have been necessary to the basic plan of the book.

There have, of course, been many advances in our knowledge of fish in the past thirty years, particularly in so far as their physiology and behaviour are concerned, and in the study of fossil forms. Many new fishes have been discovered, some, like the cœlocanth, of first importance. On closer inspection, it is evident that the new edition has been very carefully revised to include the results of recent work, and although a few pages shorter than the old, it contains a great deal more information. A number of new text figures have been drawn by the artist who illustrated the first edition, most of these are of fossil forms such as *Jamoytius*, but other new figures are of *Latimeria*, of different forms of the jaws and their articulations, and so on. Although Dr. Greenwood apologizes in his preface for the possible appearance of a dogmatic tone due to the demands of compression, in

fact it seems that great care has been taken to give both sides of controversial problems. It is, for example, pleasant to find clearly stated the alternative hypotheses required if that of continental drift be not accepted, or again, to see a discussion of Dr. Tucker's views on the breeding of eels after the classical account is given.

In general, all the interpolations and additions in this revision are judicious and valuable, and few topics which have arisen since Norman's day, or have increased in importance since he wrote, are not to be found in this new edition. It is a pity that space could not be found for a discussion of the effects of the land-locked sea lampreys of the Great Lakes on the commercial fisheries there, perhaps the most striking example to be found of the direct competition of men and fishes for the same food source (particularly as the statement about the value of these fisheries on p. 335 is scarcely true to-day); but in less than 400 pages there must be some omissions.

Very few errors or ambiguities were detected by me but three may be mentioned. It is difficult to understand the distinctions drawn in this edition between true and virtual parasitism, both terms being applied to the special case of the dwarf males of certain angler fishes (pp. 211; 244) which live attached to the females.

One error in the first edition which has not been remedied is that the female ray of Fig. 101 (Fig. 107 of the first edition) is in fact an immature male, and this is unfortunate, since the figure purports to show the difference between the male and the female. Finally, it is something of a surprise to find that Dr. Greenwood has placed the lancelet, *Branchiostoma*, among the Hemichordates (p. 258).

These are minor defects, and Dr. Greenwood is to be congratulated on a very successful revision of one of the best popular books on fish. It would be difficult to find so much information so well presented in any other book of comparable size and price, and this new edition well deserves the success of its predecessor. Q. BONE

CLASSIFICATION OF NEMATODES

Soil and Freshwater Nematodes

By Dr. T. Goodey. Second edition, revised and rewritten by Dr. J. B. Goodey. Pp. xvi + 544. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1963.) 100*s*. net.

NEMATOLOGISTS have looked forward to a new edition of this outstanding work on the classification of the soil- and fresh-water inhabiting and plant-parasitic nematode genera for quite some time already. The first edition has been out of print for many years now. Moreover, large sections of the book were becoming more and more out of date. To incorporate the large amount of new information which has become available since the first edition was published this new edition has been entirely rewritten, and expanded from about 400 to more than 600 pages. The number of illustrations has increased from 190 to 298 and a larger format has been chosen. Several illustrations have benefited from the better-quality paper than that used for the first edition. However, the reviser has justifiably maintained the original plan of the book.

Contrary to other more or less similar works (for example, Meyl's *Freilebende Nematoden* in *Die Tierwelt Mitteleuropas* 1, 5*a*, 1960) which are compilations of extremely abbreviated descriptions of species, the new edition of *Soil and Freshwater Nematodes*, like the first, concentrates on the definition of genera and their classification in higher categories. The definition of each genus is followed by an illustrated description of the type species or of another characteristic species if this serves the purpose better: to make the genus definition more than an abstraction and therefore much easier to use.

It is this judicious combination of definitions of higher systematic categories with full descriptions and illustrations of a limited number of species which makes the book so usable also to non-taxonomists. The species belonging to a genus are listed alphabetically with author, date and synonyms after the description of the type or representative species and the section on each genus closes with a paragraph on the bionomics of its members. An improvement compared to the first edition is the insertion of tabular keys at the beginning of each systematic category. They are not the usual dichotomous keys but tables which list in columns the members of the category with, under appropriate headings (cuticle, stoma, œsophagus, etc.), their taxonomically important features. The tables are intended to enable the reader to determine the order, family and genus to which specimens actually belong, but they also make a comparison of the members of the category possible, thereby providing a quick insight in the concepts the classification has been based on.

In adapting the classification to the present state of knowledge the reviser has not only made a choice from the various concepts found in the literature but has also expressed his own, hitherto unpublished, views. He has reduced the status of the nematodes from that of a phylum to that of a class with the name Nematodea and abandoned the division into Phasmidia (Secernentes) and Aphasmidia (Adenophori). On the other hand, several lower categories have been raised in rank and a division of the genera under consideration in ten orders instead of the four of the first edition has been adopted, resulting in a higher degree of uniformity within most of them.

The orders Tylenchida and Rhabditida were maintained virtually unchanged apart from placing *Teratocephalus* and *Euteratocephalus* (formerly in Rhabditida) in a new order *Teratocephalida*. Within these orders considerable changes in classification were made. The Chromadorida and the Enoplida of the first edition are now divided into four and two orders respectively : Araeolaimida, Desmoscolecida, Monhysterida, Chromadorida, Enoplida and Dorylaimida.

One could scarcely expect systematists not to disagree with the classification of the original author and of the reviser on several points. For example, I question the inconsistency in the evaluation of the structure of the œsophagus in the Tylenchoidea, allowing it to prevail over all other characters in one case (Neotylenchidae) and giving it only secondary importance in others. I must admit, however, that it will not be easy to find entirely satisfactory alternatives in this and other cases unless new material and a better knowledge of nematode anatomy, for example of the topography of the œsophageal glands in Tylenchoidea, give us a better insight in systematic relationships.

Neither queries nor differences of opinion, however, suggest that the new edition of *Soil and Freshwater Nematodes* is not an excellent work and of great importance to all those who study nematodes as taxonomists, plant pathologists or while engaged in faunistic studies.

J. W. SEINHORST

INDIAN WATER PLANTS

Aquatic Angiosperms

A Systematic Account of Common Indian Aquatic Angiosperms. By K. Subramanyam. (Botanical Monograph No. 3). Pp. viii + 190 (illustrations). (New Delhi: Council of Scientific and Industrial Research, 1962.) Rs. 20.00; 40*s*.; 6.00 dollars.

THIS book is the third in a series of botanical monographs published by the Council of Scientific and Industrial Research, New Delhi, with the aim of collecting together the scattered results of researches published in

India and elsewhere. It is hoped that these monographs will eventually provide a source of material to be used in the preparation of Indian botanical text-books.

The present volume deals systematically with the numerous common aquatic flowering plants of India, and in addition to descriptive matter provides keys for their identification at family, generic and species levels. The characteristics of the Indian climate are such that many plants which grow during the monsoon season could well be classed as aquatic plants, since the soil in which they grow is often immersed in some depth of water; however, in the interests of brevity the author has excluded both monsoon and mangrove plants from his treatise.

Although they contain much interesting information, on the whole the descriptions of the various species are somewhat dull; however, the book will no doubt be used principally as a work of reference. The text is sprinkled with numerous botanical terms, some of which are not in common usage, and the provision of a glossary might have been helpful. In providing morphological, anatomical and especially embryological information about the species described, where this is available, the author has drawn largely from Indian literature. The pharmaceutical and other economic uses of the plants are also given, where appropriate, and this information is interesting and sometimes surprising; aquatic plants are utilized in numerous ways, both as medicaments and in the manufacture of articles such as sun hats. A useful appendix supplies the chromosome numbers of many of the plants described.

This well-produced book is illustrated with many clear line-drawings and a few photographs; the latter are of rather indifferent quality and add little to the value of the text. The usefulness of the line drawings would probably have been increased by the provision of scales of magnification, which are supplied in only some instances. With a few exceptions, the text seems to be largely free from errors.

As a systematic work, the present volume appears to fulfil its purpose admirably; it will provide a very useful addition to Indian botanical literature, and to the literature of aquatic plants in general. No description of a Flora which can claim several representatives of such fascinating families as the Podostemaceae could fail to interest the morphologist as well as the systematist; it is to be hoped that this book will stimulate the further investigation of aquatic angiosperms in all their aspects.

Elizabeth G. Cutter

THE ROYAL ARMY VETERINARY CORPS

The History of The Royal Army Veterinary Corps 1919–1961. By Brigadier J. Clabby. Pp. 244+56 photographs. (London: J. and A. Allen and Co., 1963.) 50*s*.

THE history of the British Army Veterinary Services in the First World War (*History of the Great War: Veterinary Services*, by Blenkinsop and Rainey, London; H.M.S.O., 1925) is now succeeded by a history bringing the story up to 1961.

It is of interest to compare the outlook and methods in the two Wars, one before the mechanized age had got into its stride and the other after the general public had ceased to regard animals as of importance for military purposes except for food. Again, it is of interest to consider the recently issued history of the U.S. Army veterinary services (*Nature*, 198, 822; 1963.) It could be said that the British histories follow a rather typical, amateur-sounding trend, very enterprising and perhaps rather personal, and with some hankering after the old days of the horse and even the camel. On the other hand, the American account is very impersonal, efficient, bustling and very well planned. The achievements of both countries in military veterinary service in the First World War were outstandingly good.

By 1939 cavalry had disappeared as such and the artillery services and most of the former pack transport had been mechanized; army veterinary officers had seen the red light and found civilian careers on a large scale. Actually horses and mules found a place in operations to a much greater extent than could be anticipated and were of much importance in certain spheres in Italy, Africa and Asia. Considerable numbers were captured from the Italians in Africa and taken over in the ensuing operations.

Bizarre new problems had to be met. Mules were dropped from aeroplanes by parachute. In Wingate's Chindit force mules were a serious handicap to his troops when penetrating enemy country because of their braying, which warned the enemy and was a danger to morale. At relatively short notice mules were 'devoiced' and dispatched as replacements. This involved a surgical operation on the vocal cords and a special scheme for the procedure on a large scale had to be devised hurriedly and without previous experience of what surgical interference was required to achieve the desired results.

Most of the book is devoted to detailed accounts of the work done and systems followed in the five widely separated theatres of war.

This is an arresting book which will be of interest to all concerned with riding, draft and pack animals in particular and with all aspects of animal life. In war, animals have, unfortunately, increased dangers from many sources, and cruelty and even pain not related to cruelty. The army veterinary services are very active in measures to prevent unnecessary suffering. A hazard to the animal that has always accompanied war is when persons unaccustomed to animals are drafted to animal units; in the Second World War this was a very common occurrence because, by then, so few persons had experience with horses. Many loved the job and did it well, but others were probably disappointed in a non-mechanical duty and not very efficient. Mules reported to be vicious were found on occasion to be underworked and overfed: in any event for a tyro to be placed in charge of an army mule could be described as an experience.

A surprise was that the Japanese did not understand surra in horses; it is not serious in cattle, but they are carriers of the trypanosome concerned, and they thought that it could be ignored in the horses. From their known interest in Burma before the War it would not have been expected that their observers had slipped up in such a fashion.

War dogs really came into their own in the Second World War. It is a serious failure that their value was not recognized in earlier days. There was plenty of evidence of the reliability, after the right training and in the right hands, of suitable dogs for certain very intricate work. Survival of a medieval outlook may have tempered the judgment of war lords. As infantry patrol aids, guards, messengers in certain types of country, for finding casualties and even for finding buried *caches* of arms and for mine detection they have value. An illustration shows a dog with a rifle that it had found in a concealed position.

In modern war there is important linkage with civil services, air raid and bomb precautions, Animals of all kinds require service, as hazards to the human population, as well as for veterinary attention. With the importance of war gases, missile damage, fall-out, etc. the animal side requires special attention and the army veterinary services are trained accordingly.

The book is full of interesting illustrations and is well written and produced.

W. A. Pool

European Brewery Convention

Report of the Barley Committee: Trials, 1961. Pp. 136. (London: Institute of Brewing, 1963.)

THIS twelfth report of the series compares the agricultural and malting performances of various new barley varieties in fifteen European countries. In addition to details of sowing, ripeness, yields, quality characteristics such as nitrogen-content, grain size and dormancy, the volume contains a wealth of tabulated data on the lay-out of the individual trials, their location, soil analysis, fertilizer treatment, field observations such as those relating to disease resistance, meteorological data and statistical evaluation of yields. Moreover, samples of the barleys were converted into malts by small-scale methods and, here also, the newcomer to this field will perhaps be surprised both by the variation in the results and by the multitude of malt properties which have to be brought into view to provide an adequate comparison.

It will be clear that this is a specialized publication of particular importance to those concerned to see that with this quantitatively and economically outstanding crop the varieties grown shall best meet the rather different needs of the farmer and the maltster. It has, however, a wider lesson to teach by revealing how complex a problem is posed by this seemingly simple objective. Thus not only are the barleys subject to almost unavoidable variations in methods of cultivation and uncontrollable differences of weather, the malts also vary widely. The latter circumstance is in part due to the fact that different regions often prefer malts of quite different character, but is also the result of malting plants apparently giving different qualities of product even when much care is taken to standardize the procedure employed. These considerations are additional to more familiar ones such as the adequate botanical description, identification and stability of the new varieties. The present volume, therefore, both summarizes clearly the results of a phenomenal number of hours of endeavour and provides a valuable guide to those who may be concerned with similar evaluations of other crops on a wide geographical basis. A. H. Cook

Advances in Astronautical Propulsion

Proceedings of a Seminar held in Milan by Istituto Lombardo Accademia di Scienze e Lettere in collaboration with the Advisory Group for Aeronautical Research and Development of Nato, 8–12 September 1960. Edited by Corrado Casci. (International Series of Monographs in Aeronautics and Astronautics. Division 9: Symposia, Vol. 11.) Pp. xxvii+366. (London and New York: Pergamon Press; Milan: Editrice Politecnica Tamburini, 1962.) 100*s*.

THE papers which compose this volume are concerned with most aspects of astronautical propulsion and a few other closely related subjects. The book begins with a useful introduction to the various methods of electrical propulsion, by the late Theodore von Kármán. Then comes a fine survey of the mechanics of space propulsion by L. Crocco (in Italian), covering the dynamics of high- and low-thrust systems and the properties of transfer orbits. There follow four contributions on conventional rocket propulsion, including a passionate plea for solid fuels from J. Buchanan, and a pleasing discourse on liquid-fuel rockets by A. O. Tischler. Nuclear rockets form the subject of two papers, a practical survey of fission rockets by R. W. Bussard, and a forward-looking review by E. Sänger, covering such topics as photonic fission rockets and intermittent water plasma fusion rockets. Other papers deal with magnetohydrodynamics and power generation in space. The dynamical and heating problems of atmospheric re-entry are admirably discussed in three papers by A. Ferri, L. Broglio and P. A. Libby. Finally, H. L. Dryden outlines some of the future United States plans and also "dips into the future far as human eye can see".

The papers (though not all well written) together constitute an excellent review of the subject. But the book is marred by printing errors so numerous and glaring that they often distract the reader's attention and sometimes obscure the meaning. D. G. King-Hele

Thermodynamic Properties of Helium to 50,000° K

By Wilbert J. Lick and Howard W. Emmons. Pp. 122 (including charts). (Cambridge, Mass: Harvard University Press; London: Oxford University Press, 1962.) 24*s*.; 2.95 dollars.

THE authors give an excellent and precise account of the methods used in the preparation of these tables, although the preceding introduction is a discouragingly vague attempt to justify their existence. In contrast to many other similar compilations, it is indeed welcome to find an extremely graphic physical discussion of the type of atomic model and particle interactions assumed in the analysis. The partition functions for electronic excitation and ionization are based on Unsölds treatment, and thus allow for a density-dependent cut-off of levels and reduction in ionization potential due to particle interactions. The justification and validity of this approach is discussed clearly and at length. There are 108 pages of thermodynamic data—including sound speed—for equilibrium mixtures from 8,000° to 49,800° K and from 10^{-4} to 10^{3} atm., listed in steps of 200° K for each ten-fold pressure increment. The chemical species considered are He, He^{+}, He^{++}, and e; the molar constituents of these are similarly tabulated. The data is also conveniently presented in the form of two splendidly large and clear Mollier charts. This will be welcome to those concerned with the use of helium in high-enthalpy electrically driven shock tubes, although an order extension in pressure would be useful in this respect. The extreme range covered will be more than adequate for those interested in its use in plasma-arc and space propulsion facilities.

Ian R. Hurle

Plant Hairs

By Prof. J. C. Th. Uphof. Die Verbreitung der Haartypen in den Natürlichen Verwandtschaftsgruppen. Von Dr. Karl Hummel und Karin Staesche. (Handbuch der Pflanzenanatomie, Band IV, Teil 5). Pp. xi+292+5 plates. (Berlin-Nikolassee: Gebrüder Borntraeger, 1962.) 96 D.M.

THE long article by Uphof looks like a general essay on hairs and emergences, but is really a pure compilation. The author, on the evidence of his own bibliography, has not been personally active in this field of research, and does not deal with the plants themselves, but only with the literature concerning them. Furthermore, his scale of values is antiquarian rather than botanical. Nothing can disguise the intrinsic sterility of such a treatment. The botany expounded here is one in which iodine is the principal histological stain, and in which the electron microscopy of the cotton fibre is firmly subordinated to the "faint reticulation" which appeared on staining with Congo red in 1925.

The shorter contribution by Hummel and Staesche is a statement of the distribution of the principal hair-types (unicellular, uniseriate, stellate, etc.) among the angiosperms. The only conceivable objection to it is that the material might just as well have been tabulated in a fraction of the space.

At this price the purchaser is entitled to a professional standard of book-production. He does not get it. The English text reads like a bad translation, misprints abound, and Hummel repeats both his own statements and Uphof's to an extent which borders on the farcical. In all, this is a serviceable classified guide to a scattered literature, giving enough detail to obviate in many cases all need for reference to the originals. From every other point of view, it represents a lost opportunity.

K. J. Dormer

STUDIES OF RADIOACTIVE FALL-OUT FOLLOWING 1961 U.S.S.R. TESTS

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ON September 1, 1961, it was announced that the testing of nuclear weapons in the atmosphere had been resumed by the U.S.S.R.[1]. Thus, the voluntary moratorium which commenced in Autumn 1958 and which had been officially maintained by the United Kingdom, the U.S. and the U.S.S.R. was ended abruptly. Afterwards, between September 1961 and November 1961, the U.S.S.R. conducted about fifty such atmospheric tests. Most of these were detonated in the Arctic regions. Following a series of underground tests in late 1961 and early 1962 the U.S. resumed atmospheric testing in April 1962, most of which occurred in the Pacific on Christmas Island. As a result of the time delay between the respective resumptions of atmospheric testing by the U.S.S.R. and the U.S., it is clear that the dominant source of nuclear debris at Westwood, New Jersey, during late 1961 and early 1962 was the U.S.S.R. tests.

In this article we discuss several aspects of the U.S.S.R. radioactive debris, including the overall deposition, the rate of fall-out and the comparison of 1962 and 1959 situations. Of special importance is the total yield of the U.S.S.R. series at 120 megatons and the relatively small fission yield of 25 megatons[2]. Also the fate of the nuclear debris from the two high-yield weapons 25 and 55–60 megatons is particularly worthy of discussion.

Fall-out Prior To Renewal Tests

In a detailed investigation of the recent tests it is instructive to examine the deposition of fall-out prior to September 1961. When testing was suspended in 1958 the rainwater sampling programme of our laboratories at Westwood was curtailed after the short-lived nuclides from the earlier tests decayed. Through the early part of 1959 many short-lived isotopes such as barium-140 and strontium-89 were measurable with ease and their behaviour during this period was extremely useful in ascertaining the age and origin of the debris[3]. As time progressed, however, and their concentrations decreased, the importance of monitoring some longer-lived nuclides increased. Thus during 1960 and 1961 attention was given to the determination of cerium-144, ruthenium-106, rhodium-102, etc. Cerium-144 and rhodium-102 were considered most valuable in determining the age and source of the debris in precipitation at these times. Throughout the period the variability of the deposition of strontium-90 was examined as a reference nuclide and because of its importance in radiation hazards.

In Fig. 1 the monthly concentrations of strontium-90 in precipitation at Westwood, New Jersey, are plotted as a function of time. The strontium-90 concentrations show pronounced peaks in spring in both 1960 and 1961 as they did in the previous years. It is interesting to note that the peak concentrations are attained in different months—April, May and June—a phenomenon which is governed by at least four factors: (*a*) schedule of testing; (*b*) magnitude and types of tests; (*c*) frequency and types of precipitation; (*d*) mechanisms of release of nuclear debris from the stratosphere for high-yield bursts.

One of the most important conclusions to be reached from these data is that, in spite of the moratorium on atmospheric testing, the spring maximum in 1961 was almost as high as that in 1960. Table 1 will reveal this fact a little more clearly.

Table 1. Deposition of Strontium-90 at Westwood, New Jersey (January–June 1959–62)

Year	Precipitation (in.)	Sr-90 (mc./mi.²)	Sr-90 Average concentrations (mc./mi.²/in.)	(μμc./l.)
1959	18·45	20·28	1·10	16·7
1960	18·35	3·36	0·18	2·78
1961	22·61	3·96	0·18	2·66
1962	22·88	19·57	0·85	13·0

The average concentrations of strontium-90 for the first six-month periods in 1960 and 1961 were almost equal, but the actual deposition in 1961 was slightly higher than that in 1960 chiefly due to increased precipitation. Such data tend to indicate a similarity in the magnitude at the source region of the stratosphere in 1960 and 1961 and hence some method of replenishment of debris lost in 1960. Replacement of the material deposited in 1960 must have occurred therefore in the latter part of the year. Some confirmation of this suggestion can be seen in the rhodium-102 results which were reported elsewhere[4] and in the cerium-144 results.

Our cerium-144 results are presented in Fig. 2. Here the average monthly cerium-144/strontium-90 ratios for two types of samples are presented. One series of results

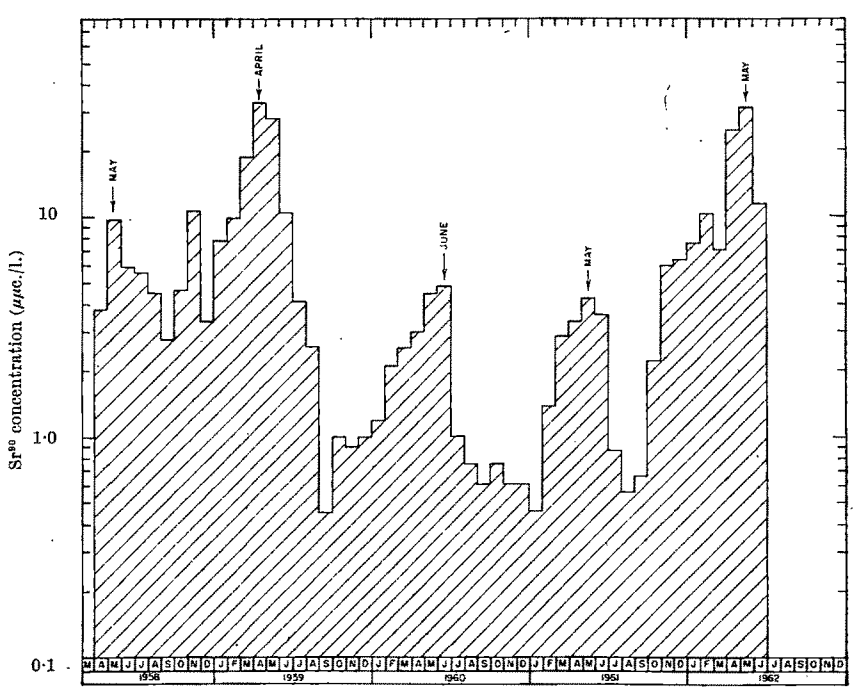


Fig. 1. Average monthly concentrations of strontium-90 in precipitation at Westwood, New Jersey (1958–62)

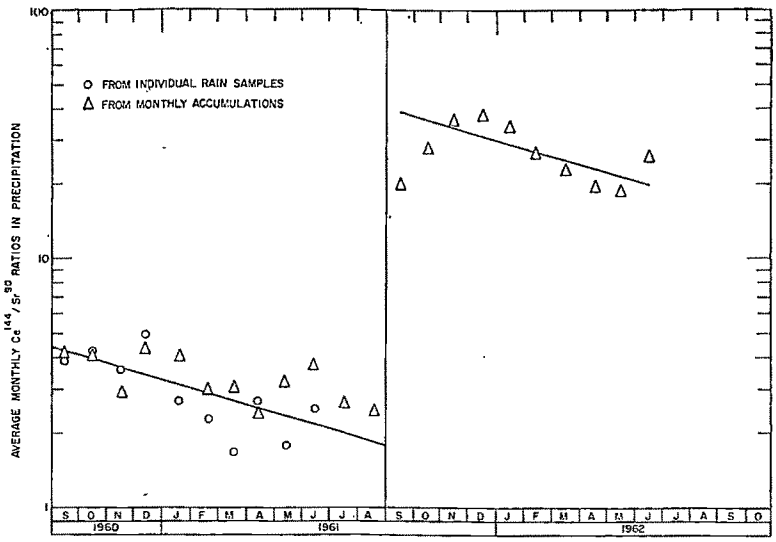


Fig. 2. Cerium-144/strontium-90 ratios in precipitation (1961–62)

was derived from the analysis of single monthly accumulations of precipitation while the second series was obtained from monthly averages of individual rainstorm measurements. Prior to September 1961, the results showed an apparent shot date of approximately May 1958 (assuming an initial ratio at shot date of about 39). The French tests in early 1960 were low yield and did not produce sufficient debris to cause any appreciable increases in the cerium-144/strontium-90 ratios in precipitation at Westwood, New Jersey[5]. The majority of the nuclear debris being deposited during the first half of 1961 can probably be assigned, therefore, to detonations occurring in mid-1958. The majority of the debris from the U.S.S.R. detonations of the autumn of 1958 had deposited in 1959. It is concluded the U.S. test series of mid-1958, with some contribution from United Kingdom tests, are the major source of the radioactivity deposited at Westwood in the January–June period of 1961. These findings are substantiated by studies of cerium-144/strontium-90 ratios in stratospheric air-filter samples[6]. Here the results suggest that most of the debris in the lower stratosphere in 1961 had originated in the rocket shots *Teak* and *Orange*, detonated in the summer of 1958. In fact it was calculated by the same authors[6] that about 68 per cent of the debris in the lower stratosphere between 10° N. and 50° N. latitude during June–September 1961 was produced by the rocket shots. This situation is to be compared with the previous year, 1960, when the material in the lower stratosphere did not contain as high a quantity of rocket shot debris but was primarily attributable to lower altitude bursts occurring in the equatorial regions of the Pacific in 1958.

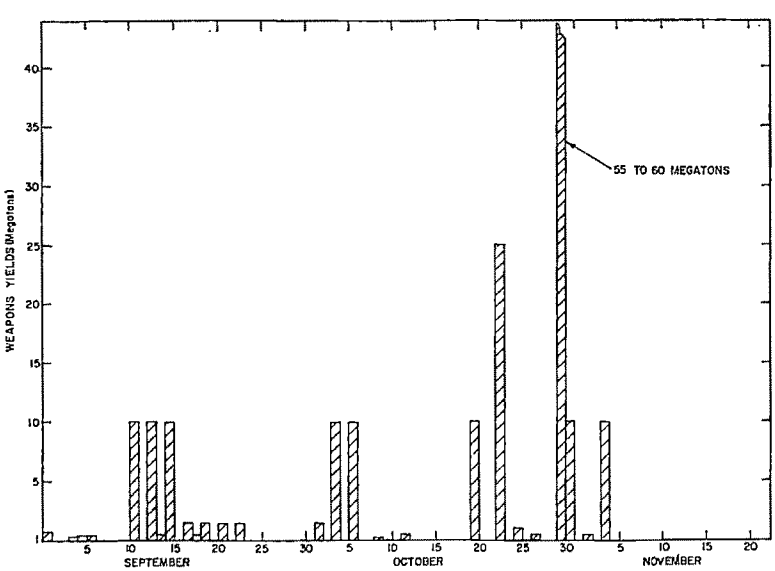


Fig. 3. U.S.S.R. atmospheric nuclear detonations in 1961

Two approaches are possible in determining what the fall-out deposition at Westwood would have been in the absence of further atmospheric testing. The first approach is dependent on a knowledge of previous year's fall-out and some information on the changes in radioactivity concentration in the source regions, that is, the lower stratosphere in the northern polar regions. A second method is to use the acquired information on nuclide ratios of short-lived isotopes to assess the fraction of strontium-90 attributable to the relatively 'old' debris.

Comparison of 1961 and Older Debris

It has already been mentioned that the major sources of debris for the deposition occurring in the first half of 1961 were the rocket shots *Teak* and *Orange* of 1958. In Table 1 it can be seen that the precipitation in 1961 and 1962 was quite similar. Thus if the frequency and types of rainfall and quantity of nuclear material in the source regions were the same in both years the total deposit of strontium-90 at Westwood in the first six months of each year would have been similar. According to Friend and Feely[6], the strontium-90 burden in the northern hemisphere stratosphere up to an altitude equivalent to 40 mb was 0·40 megacurie in early 1960 and 0·21 megacurie in June–September 1961. Approximately 0·27 megacurie of the 0·40 megacurie in the lower stratosphere was deposited in early 1961. Applying the same ratios to 1962 it would appear that we could have expected approximately 0·14 megacurie of the 0·21 megacurie strontium-90 lost from the northern hemisphere stratosphere. In other words, the fall-out in 1962 could have amounted to about 50 per cent of that experienced in 1961. Thus on a crude semi-quantitative basis, using the foregoing information and the results given in Table 1, a strontium-90 deposit of about 2 mc./mi.^{2} could have been expected in January–June 1962 in the absence of testing. As also can be seen in Table 1 the actual deposit was 19·6 mc./mi.^{2} and thus about 10 per cent of this should be relatively old debris.

In order to interpret the data on nuclear debris in precipitation at Westwood, particularly during the first five months of 1962, Fig. 3 has been plotted to show the extent of atmospheric nuclear testing in late 1961. Other testing conducted in 1961 and early 1962 was underground and should not have contributed to the radioactivity found in precipitation at Westwood during this period. The data for this plot were taken from the details announced by the Atomic Energy Commission and compiled by the U.S. Public Health Service in several reports[7]. (The U.S. Atomic Energy Commission has later advised that approximately 50 tests were conducted by the U.S.S.R. during September 1–November 4, 1961, but only 31 specific U.S.S.R. tests were announced during that period.) It has been arbitrarily

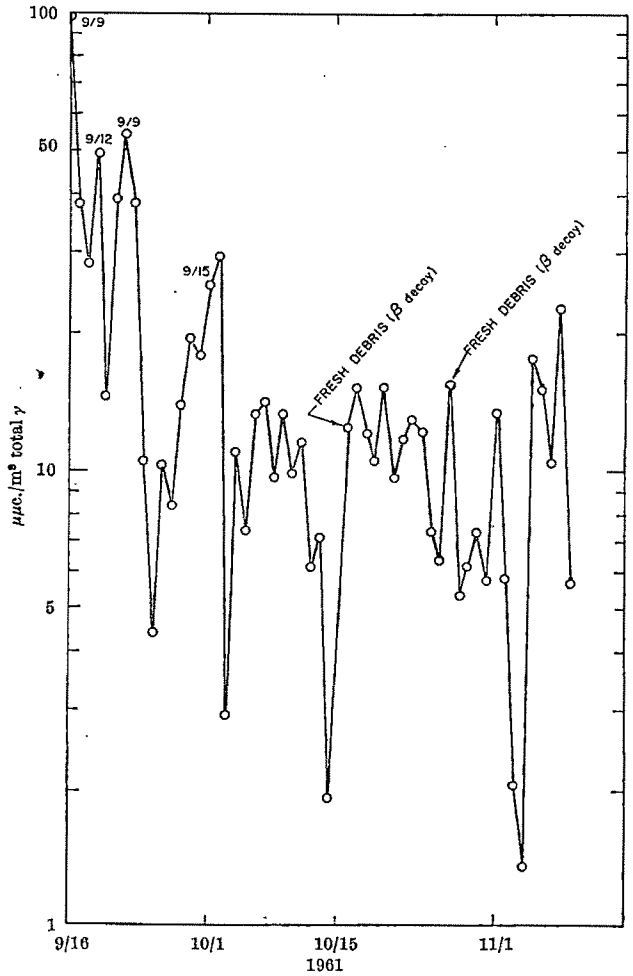


Fig. 4. Concentrations of radioactivity in air during September–November 1961

assumed that those detonations reported as possessing a yield of 'several megatons' actually had a yield of about 10 megatons.

The object of this plot was to obtain a view of the extent of the testing series and determine a date which could be taken as an average shot date for this particular series. It can be seen that the U.S.S.R. testing for larger weapons was distributed over the period September 10–November 5, 1961. The mid-point of this time span is about October 8, 1961. From the pictorial presentation this appears to be quite reasonable. Other estimates will be made from isotope ratio data.

Following the announcement of renewal of nuclear testing an air sampling programme was instituted in our laboratories at Westwood. Daily samples were collected during the period September 1–November 15, 1961, and analysed primarily by γ-ray spectrometry for barium-140, zirconium-95, ruthenium-103, iodine-131 and molybdenum-99. Various combinations of activity ratios give good indications of the date of origin for the debris.

In Fig. 4 the total γ-results on air filter samples are plotted as a function of time, and identification of shot dates has been made using molybdenum-99/barium-140 ratios. These dates are given for some samples.

The first significant quantities of activity to be detected at Westwood occurred on September 16, 17, 1961. Other sampling stations throughout the U.S. observed similar increases in air activities on or about the same time[8]. Radioactive debris layers over Flin Flon, Manitoba, Canada[9], were detected by balloon flights on September 14 and 15, 1961. Isotope ratios indicated that the material originated around September 9, 10, 1961. Reference to a compilation of tests shows the nearest detonations to have taken place on September 10, 1961, when two devices were exploded near Novaya Zemlya. The meteorological conditions leading to the arrival of this debris in the U.S. have been discussed fully elsewhere[8].

Subsequent analyses of filter samples during October 1961 gave ages varying from September 9 to September 15, 1961.

Isotope Ratios in Rain at Westwood

According to our results of activity measurements in precipitation the first rain to exhibit measurable quantities of 'fresh debris' occurred on September 14, 1961. This was a day or so earlier than our ground-level air measurements indicated for the arrival of U.S.S.R. material. Our best estimates of shot dates for this type of sample are obtained from barium-140/strontium-89 data. In this particular sample the isotope ratio had a value of 3·84 and assuming an initial ratio of 6·49 a shot date close to September 1, 1961, was calculated. This result is several days older than was indicated by molybdenum-99/barium-140 ratio in the air filter sample collected later.

In view of these observations it is felt that neither measurements of radioactivity in ground-level air nor in precipitation is a guaranteed method for monitoring the first arrival of debris. Movement of radioactivity in the upper troposphere may pass undetected in the absence of any precipitation.

Several nuclides had been analysed in individual rains during the period September 1961–June 1962. Here our discussion will be limited to three nuclides, strontium-90, strontium-89 and barium-140, where isotopic fractionation is less of a problem than with others such as cerium-141, cerium-144 and zirconium-95.

In Fig. 5 the barium-140/strontium-89 results from the analyses of individual rains are plotted as a function of time. Since neither of these nuclides was present in

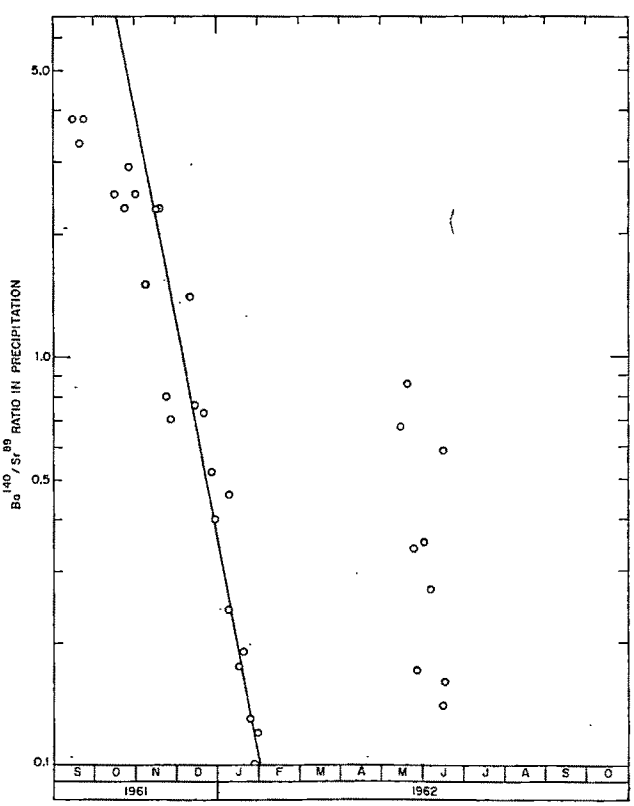


Fig. 5. Barium-140/strontium-89 ratios in precipitation (September 1961–June 1962)

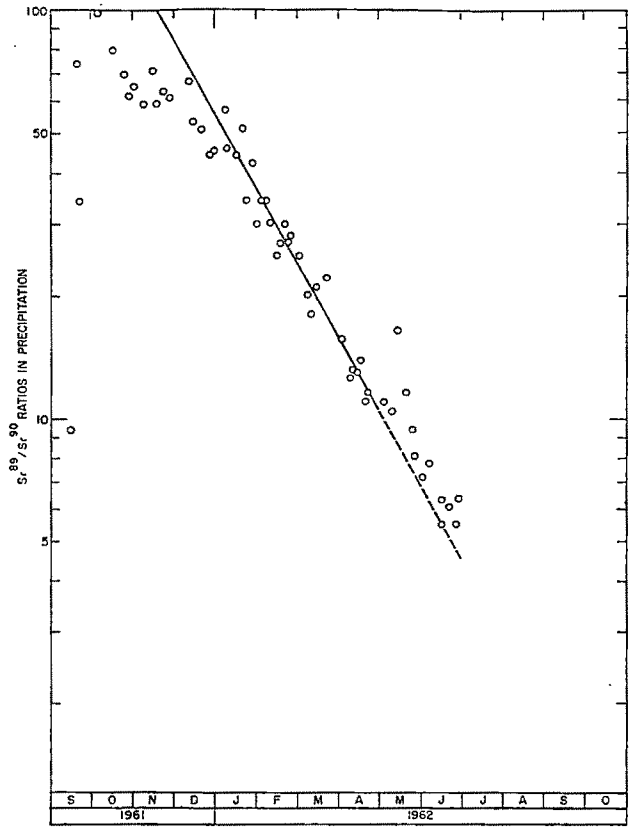


Fig. 6. Strontium-89/strontium-90 ratios in precipitation (September 1961–June 1962)

measurable quantities in precipitation prior to September 1961 and U.S. atmospheric testing did not commence until the end of April 1962, the results during this period are indicative of the behaviour of U.S.S.R. debris. In May 1962 evidence of debris originating at a source apparently different from that of the preceding months is clear in the results of this nuclide ratio (Fig. 5).

Following the two-month period September–October 1961 when the barium-140/strontium-89 ratios were somewhat variable, the results indicate a progressive decay with a half-life which is close to the theoretical decay of a well-mixed source of these nuclides (~17 days) (ref. 10). Such behaviour has been observed on several previous occasions[3,10]. Extrapolation of the smooth decay section of the curve to an initial ratio of about 6·7 yields a date close to the middle of October 1961. A very crude estimate of a mean shot date from the schedule of testing gave a date of about October 8, 1961, which is in quite good agreement with that obtained from the isotope ratio data. For the practical purposes of calculations a mean date of October 15, 1961, has been adopted in ensuing sections.

Fig. 6 shows the variability of the strontium-89/strontium-90 ratio in precipitation at Westwood from September 1961–June 1962. Features similar to those in the preceding curve for barium-140/strontium-89 are clearly visible in that a smooth decay in the ratio, close to the theoretical rate of decay, follows an initial disturbed period. There is a suggestion that the smooth decay commences a little later in time for the strontium-89/strontium-90 ratios than for the barium-140/strontium-89 ratios.

If the strontium-89/strontium-90 ratio decay curve is extrapolated to the mean shot date as previously determined a ratio of about 155 is obtained. Assuming, therefore, an initial ratio[10] at shot date of 176 the observed value of 155 on October 15, 1961, could result from the inclusion of close to 10 per cent of 'old' material. In other words, 10 per cent of the strontium-90 deposited at Westwood during the period December 1961–May 1962 was derived from debris pre-dating the 1961 U.S.S.R. tests.

The graph of barium-140/strontium-90 ratios shown in Fig. 7 can be used in the same manner as above. In this instance the theoretical ratio at shot date is 1,185 and the observed value on October 15, 1961, obtained from extrapolation of the straight line portion in Fig. 7, is about 1,000. Therefore, the percentage contribution of old material to the total deposit at these times is approximately 15 per cent.

It appears from independent estimates that the extent of old debris present in fall-out during the late winter of 1961 and early spring of 1962 is about 10–15 per cent of the total deposit. This estimate is expressed pictorially in Fig. 8.

Total Fall-out from 1961 U.S.S.R. Tests

One of the most interesting features of the 1961 U.S.S.R. test series is the total yield of the weapons detonated. It has been estimated by the U.S. Atomic Energy Commission that the yield was about 120 megatons with a fission yield of close to 25 megatons[2]. Using the generally agreed ratio 1 megaton of fission equivalent to 0·1 megacurie of strontium-90 the strontium-90 yield should be 2·5 megacuries. The rather slow rate of fall-out of this 2·5 megacuries is of special interest in view of the 25 and 55–60 megaton detonations which occurred during the series.

Kuroda and Nix[11] reported that the 1961 weapons were 'cleaner' on the average by a factor of five than those exploded in 1957 and 1958. However, no explanation of the fact was given. Machta, List and Telegadas[8] pointed out that the clouds from both the 25 and 55–60 megaton tests may have injected fission products to an altitude of 30–45 km.

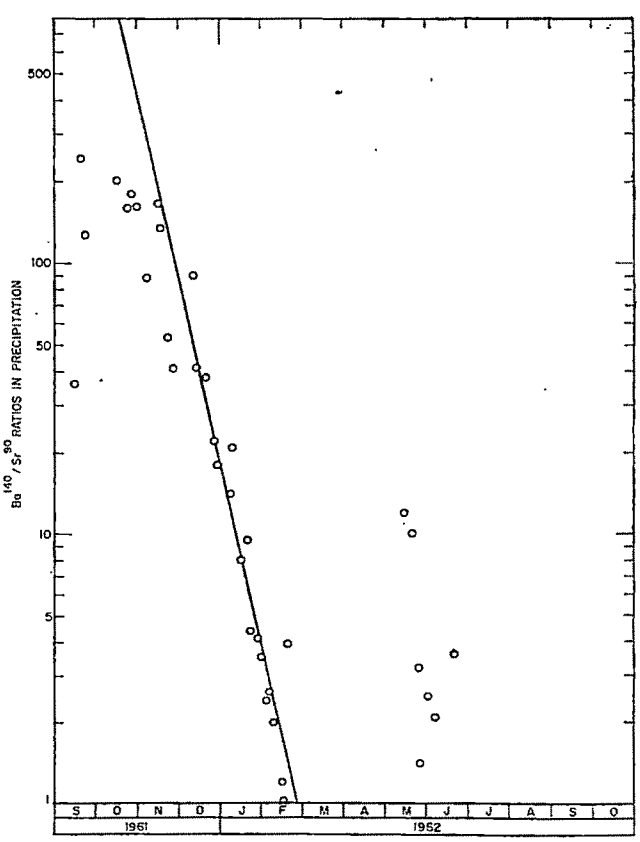


Fig. 7. Barium-140/strontium-90 ratios in precipitation (September 1961–June 1962)

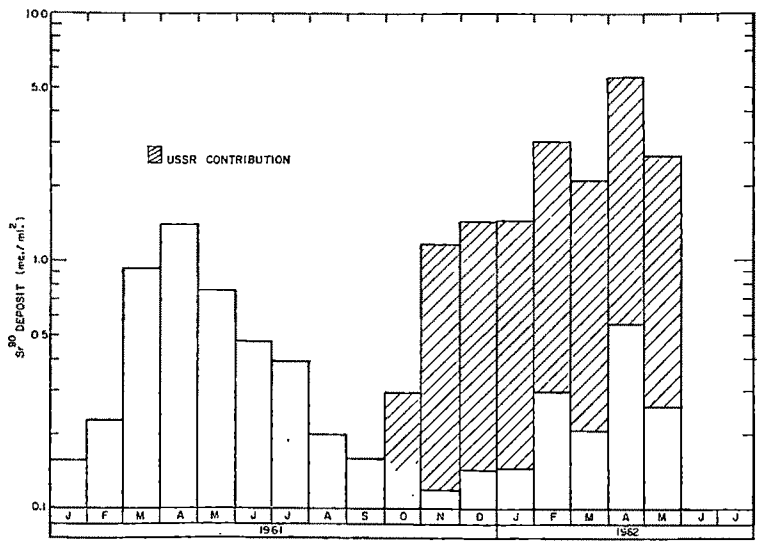


Fig. 8. Contribution of U.S.S.R. (1961) and 'old' debris to the total fall-out of strontium-90 in 1961–62

At this point in time two facts are needed to allow estimates of the rate of deposition of the U.S.S.R. strontium-90 to be made. The amount of strontium-90 deposited to date must be known together with the quantity remaining in the stratosphere. Pending the examination of world-wide fall-out collections some initial estimates of the former quantity may be made from the results already secured at our Westwood, New Jersey, laboratories. Preliminary estimates of the stratospheric inventory of U.S.S.R. material are available at this time.

Table 1 presents the quantity of strontium-90 deposited at Westwood in the first six months of the years 1959–1962. From strontium-89/strontium-90 ratios and an initial ratio of 176 at shot date a contribution in 1959 of 65 per cent 1958 U.S.S.R. debris was calculated. Based on this figure, the U.S.S.R. tests of 1958 would have contributed about 13 mc./mi.² out of a total of 20 mc./mi.² strontium-90 deposited at Westwood in the first half of 1959.

In 1962, on the other hand, the total fall-out of 19·6 mc./mi.² strontium-90 in the corresponding six-month period was shown to have been between 85 and 90 per cent from U.S.S.R. 1961 debris. If the lower value is used, to allow for some contribution from U.S. atmospheric tests from April 1962 onwards, the quantity of strontium-90 from the U.S.S.R. detonation would be about 16·6 mc./mi.².

The total fission yield of the 1958 U.S.S.R. series was given as 10–15 megatons with a resulting yield of 1·0–1·5 megacuries of strontium-90. Most of the debris from these tests was brought down in the spring of 1959 [3,6,11,12]. If we equate this yield, say, 1 megacurie, with the fall-out experienced at Westwood in 1959, 12 mc./mi.², and assume similar fall-out conditions in this area in 1961–62 it follows that the amount of U.S.S.R. debris brought down in 1962 is in the region of 1·4 megacuries.

Study of world-wide isotope ratios by Telegadas and Murayama[12] demonstrates that the fission yield in 1958 is closer to 0·6–0·9 megacurie of strontium-90. Using a mean value of 0·75 megacurie, it follows again that the U.S.S.R. tests of 1961 would contribute a total deposit of strontium-90 of close to 1·0 megacurie during 1962. A summary of these results is given in Table 2.

Table 2. Behaviour of U.S.S.R. Debris from 1958 and 1961 Test Series

Test series	Energy yield (megaton)	Estimated data Fission yield (megaton)	Sr-90 yield (megacurie)	Sr-90 deposits (megacurie)
1958	20–30	10–15	1·0–1·5	0·6–0·9*
1961	120	25	2·5	1·0†

* Total yield of 1958 U.S.S.R. Sr-90 on Earth's surface calculated from Sr-89/Sr-90 ratio in world-wide precipitation.

† Yield to date of 1961 U.S.S.R. Sr-90 on Earth's surface calculated from comparison of 1959 and 1962 depositions at Westwood, N.J.

In order to compare these results with the reported yield of 25 megatons of fission, or 2·5 megacuries of strontium-90, one must have some knowledge of the stratospheric inventory. It would be preferable to have this information for a given point in time, but unfortunately only a mean value of an inventory over a few months is available. Machta[13] has reported that during January–April 1962 the estimated amount of strontium-90 present in the stratosphere (up to 70,000 ft.) was 1·3 megacuries and even after the "Spring peak" of 1962 perhaps 1·0 megacurie of strontium-90 from the U.S.S.R. tests remained in the stratosphere. Coupled with a deposit of 1·0 megacurie of strontium-90 on the Earth's surface this gives for the 1961 tests a total yield of about 2·0 megacuries. This value, together with some possible undetected portion in the higher stratosphere above the ceiling of *U*-2 aircraft, is to be compared favourably with the reported yield of 2·5 megacuries of strontium-90.

Conclusions

An investigation has been made of the deposition of radioactive fall-out at Westwood, New Jersey, immediately prior to and immediately following the atmospheric Soviet tests of 1961. Before September 1961 the major source of radioactivity appeared to be the Pacific tests of 1958 while in the first six months of 1962 these same detonations seemed to have contributed about one-tenth of the deposition of strontium-90 at Westwood, New Jersey. The U.S.S.R. tests series of late 1961 would seem to be responsible for 90 per cent of the debris in 1962. The total deposits of strontium-90 during the first six months of 1962 and 1959 were almost equal at 20 mc./mi.². Knowing the contributions of U.S.S.R. debris to the total fall-out in 1959 and 1962, and the world-wide deposition of U.S.S.R. material from 1958 tests it is estimated that about 1 Mc. of 1961 U.S.S.R. strontium-90 has been deposited over the Earth's surface to date. It is also suggested that the total yield of 2·5 Mc. estimated by the U.S. Atomic Energy Commission is in reasonable agreement with our inventory.

Results from our rainfall studies indicate that nuclear debris from the 1961 U.S.S.R. test series is behaving somewhat differently from similar but earlier detonations. Other autumn test series conducted in Arctic regions had resulted in rapid deposition of the majority of the radioactivity in the northern hemisphere in the following spring. On this occasion it appears that only half the total yield would be released from the stratosphere in 1962, with the majority of the remainder most likely being deposited in spring 1963. It is probably a direct result of these high-yield weapons that the average 'residence time' for the debris from the 1961 series is longer than previously determined values for other test series. This strongly suggests that the early concepts of residence time is too elementary for an adequate description of the fate of nuclear weapons' debris.

The distinguishing of 1961 U.S.S.R. debris from the material originating in U.S. and later U.S.S.R. series, at some future time, presents a problem. Careful consideration will have to be given to the use of suitable isotope ratios and, in addition, the measurement of cadmium-109, a unique tracer, added to the U.S. high altitude detonation on July 8, 1962, may be used in similar ways as was tungsten-185 (refs. 14 and 15) and rhodium-102 (refs. 4 and 16).

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MINERALIZATION OF KERATIN AND ITS COMPARISON WITH THE ENAMEL MATRIX

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ALTHOUGH it has long been known (for example, ref. 1) that keratins of varying degrees of hardness are responsible for the shape and function of claws and nails by the differential wear of the tissue during use, the structural classification of these organs has been limited to the general description of a 'harder' unguis and a 'softer' sub-unguis. It is customary to regard the hardness of keratin as being due to the compactness of its cells, its fibrils, microfibrils and other protein units and to the presence of abundant cystine residues. The purpose of this article is to present evidence in support of the hypothesis that a principal factor in determining the form and function of hairs, hoofs, claws, beaks and enamel is the pattern of hardening of keratins with calcium and other salts. The broad picture of an exoskeleton of keratin, mineralized according to its position and use is in conformity with the situation in other epidermal structures; there is a parallel case in the crustacean cuticle, for example, where calcium carbonate and phosphate and other salts are laid down to strengthen the tissue.

A biophysical, biochemical and cytological survey (some aspects of which will be reported elsewhere) has been carried out on a wide variety of natural and calcined mammalian, avian and reptilian keratins, and the results suggest that all keratins contain insoluble calcium and other salts which are deposited by, and usually within, the epidermal cells in selected parts of each tissue. In the case of enamel, the X-ray diffraction evidence points to some affinity of the mature enamel proteins with the keratins, and enamel may be regarded as the most highly calcified but least keratinized member of the group.

It is well known that X-ray diffraction patterns of some keratins contain 'salt' reflexions, and it has been shown that in baleen the inorganic pattern is given by bone salts[2] laid down in an oriented partnership with keratin instead of collagen[3]. Recently, similar 'apatite' reflexions have been reported by Earland, Blakey and Stell in Indian rhinoceros horn[4] and in platypus hair, lion whiskers, goose feather calamus and in some human fingernails[5]. In the survey presented here, the nature and extent of mineralization varied widely, ranging from sparse deposits rich in calcium and phosphorus giving clear X-ray diffraction patterns of hydroxyapatite to dense deposits poor in phosphorus giving no inorganic pattern. In the air-dried native tissues, the calcium salt varied in composition, concentration, crystallinity, location and orientation. Those keratins which did not give an X-ray diffraction pattern of inorganic salt were calcined at 900° C for 2 h; with a few exceptions (some hairs) the diffraction patterns of the water-insoluble part of the ash contained reflexions characteristic of one or more of the hydroxyapatite/CaO/$Ca_3(PO_4)_2$ lattices described by Carlström[6] for calcined bone and enamel; added reflexions suggesting other salts were frequently present. The analyses of a representative selection of specimens is set out in Table 1.

For convenience, the keratins have been divided broadly into three groups according to the nature of the mineral which is deposited.

Group I : Hairs, Quills, Feathers, Horns

The feature common to all members of this group was the lack of histological identification of the inorganic deposits, even though the gross calcium concentration was found to be as high as 1 per cent and the total mineral content often exceeded 2 per cent. The X-ray diffraction evidence was inconclusive. Several specimens of hairs gave 'salt' reflexions which coincided with the strongest reflexions from the specimens of powdered bovine enamel used for comparison, but the details of the fainter wide-angle spacings were obscured by scatter from the sample. One batch of hairs from the sole of the feet of the polar bear (*Thalarctos maritimus*), however, gave a more readily identifiable pattern of bone salts, with the *c*-axis of the crystallites oriented parallel to the fibre axis. Nevertheless, not more than 0·1 per cent calcium phosphate (calculated as $Ca_{10}(PO)_4(OH)_2$) could be present in most of the hairs, where the proportion of the salt was usually not more than 25 per cent of the ash. In spite of the low proportion of calcium and phosphorus, however, the water-insoluble fraction of the ash gave X-ray diffraction patterns with many reflexions closely resembling those from calcined bone (Fig. 1*a*, for example). On the other hand, the diffraction patterns from powdered calamus of some flight feathers gave a clearer indication of hydroxyapatite and the proportion of calcium phosphate present in the ash was usually in the region of 60 per cent.

Differences were observed in the amount of calcium and phosphorus in similar keratinous structures in the same animal; for example, mature flight feathers were more heavily mineralized than down feathers. In horse tail hairs, however, differences were found not only in the ash, calcium and phosphorus content of medullated hairs as compared with non-medullated hairs, but also in the distribution of mineral within each type of hair. Medullated and non-medullated hairs were removed from several white horse tail switches and analyses for ash, calcium and phosphorus were carried out on bundles of fibres cut into 5-cm sections from the tips of the hairs backwards. The results, which are expressed in graph form in Fig. 2, show clearly that while the percentage of calcium is much the same for both medullated and non-

Table 1. Representative Analyses of Keratins

Subject	Tissue	Matrix	'Salt' X-ray diffraction pattern of native tissue	Ash (per cent)	Ca (per cent)	$Ca_{10}(PO_4)_6(OH)_2$ (per cent)
Group I						
Man	Scalp hair	α-keratin	—	0·26	0·015	0·016
Domestic horse	Tail hair	α-keratin	—	0·93	0·022	0·14
Wildebeeste (*Connochaetes taurinus*)	Tail hair	α-keratin	*HA*?	1·45	0·43	0·05
African cattle	Body hair	α-keratin	*HA*?	1·75	0·87	0·09
Pigeon (*Columba livia*)	Flight feather	Feather keratin	—	0·65	0·18	0·09
Pigeon (*Columba livia*)	Down feather	Feather keratin	—	0·34	0·24	0·05
Pigeon (*Columba livia*)	Flight calamus	Feather keratin	*HA*	0·20	0·05	0·12
Domestic ox	Horn	α-keratin	—	0·22	0·15	0·06
African rhinoceros (*Rhinoceros bicornis*)	Horn	α-keratin	—	2·70	0·21	0·05
Group II						
Domestic ox	Hoof (hard unguis)	α-keratin	*HA*	0·91	0·20	0·28
Domestic sheep	Hoof (hard unguis)	α-keratin	*HA*	0·83	0·17	0·21
Domestic sheep	Hoof (soft sub-unguis)	α-keratin	—	0·16	0·12	0
Man	Fingernails	α-keratin	—	0·84	0·11	0·28
Leopard (*Felis pardus*)	Claw (soft area)	α-keratin	—	0·27	0·005	0·012
Leopard (*Felis pardus*)	Claw (hard area)	α-keratin	—	1·30	0·92	0·12
Bear (*Ursus ferox*)	Claw	α-keratin	*HA*	2·60	0·90	0·30
Pigeon (*Columba livia*)	Claw	Feather keratin	*HA*	0·60	0·32	0·60
Group III						
Pigeon (*Columba livia*)	Beak tip	Feather keratin	*HA*	0·55	0·28	0·40
Sei whale (*Balaenoptera borealis*)	Baleen (total)	α-keratin	*HA*	3·6	1·4	3·5
Sei whale (*Balaenoptera borealis*)	Covering horn	α-keratin	*HA*	1·8	0·5	1·3
Sei whale (*Balaenoptera borealis*)	Fringe fibres	α-keratin	*HA*	14·2	5·8	14·5
Fin whale (*Balaenoptera physalis*)	Baleen (fringe fibres)	α-keratin	*HA*	3·7	1·3	3·3
Lesser rorqual whale (*Balaenoptera acutorostrata*)	Baleen (fringe fibres)	α-keratin	*HA*	1·1	0·4	1·0
Greenland whale	Baleen (fringe fibres)	α-keratin	—	1·0	0·4	0·5
Ox	Molar enamel	"α-keratin"	*HA*	94·1	49·5	86·0
Rabbit	Femoral bone	Collagen	*HA*	68·2	23·1	73·0

HA = Equivalent spacings of powdered bovine enamel. Calcium estimation by the method of Weatherell[21], phosphate estimation by the method of Gomori[22]. The percentage $Ca_{10}(PO_4)_6(OH)_2$ is calculated from maxima Ca^{++} or PO_4^{---} available.

medullated hairs, non-medullated hairs contain appreciable amounts of phosphate and both the ash content and the calculated amount of calcium phosphate rise steeply in the thin worn tips of the fibres. It would appear that the non-medullated hairs have a denser core of amorphous calcium, and other salts, than the medullated hairs, perhaps related to the function of the fibres in the switch.

Group II : Hoofs, Nails and Claws

In this group, which might be called the 'digit keratins', the proportion of calcium in the ash was generally higher than that present in the subjects in Group I and the mineral deposits were clearly visible under the optical microscope as clusters and layers of particles, which were distributed in various patterns in each tissue. A number of specimens gave X-ray diffraction diagrams which only suggested that hydroxyapatite might be present. In most cases (hoof unguis, for example) the reflexions were faint and difficult to measure, but some samples (a few claws) gave 'salt' patterns which were sharp and distinct. On the other hand, many well-calcified hoofs and claws gave no indications of crystalline substances, judging from their X-ray diffraction patterns.

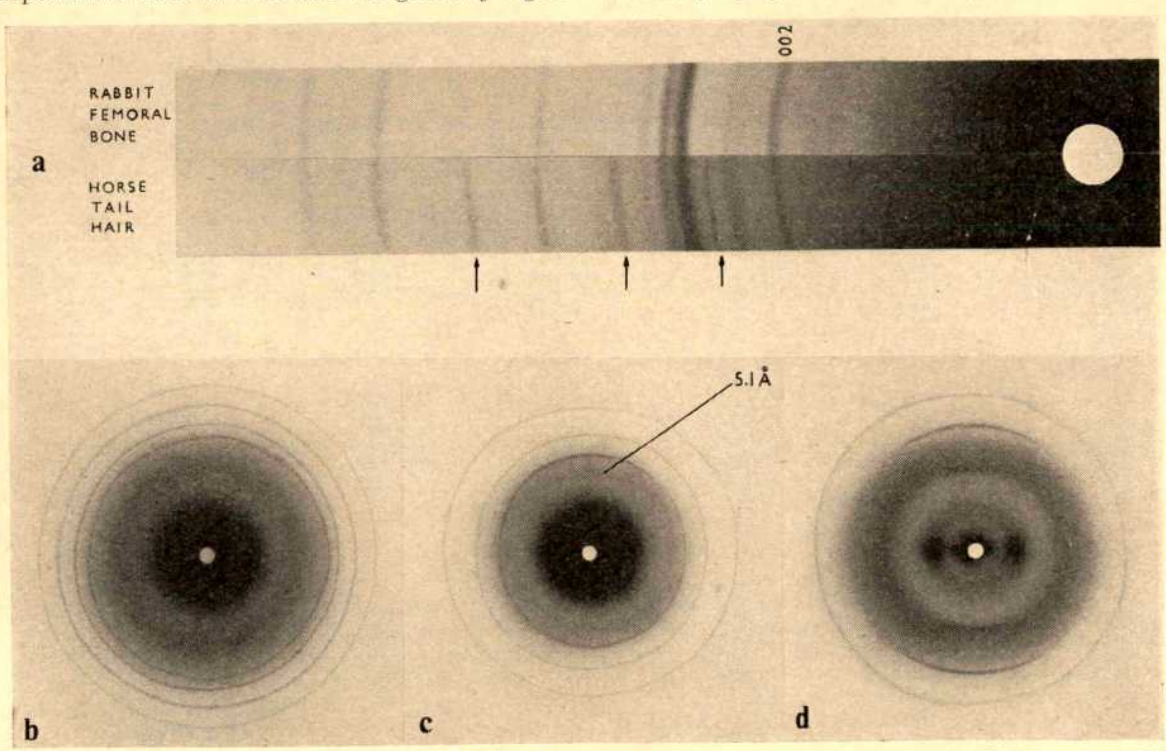


Fig. 1. X-ray diffraction patterns from keratins and enamel matrix. All specimens were examined by copper *Kα* radiation, collimated by glass capillary, using a flat-film camera with a 4-cm specimen-to-film distance
(*a*) Comparison of patterns from specimens of rabbit femoral bone and horse tail hair, both calcined at 900° C for 2 h and rinsed briefly with ion-free water. The 002 reflexion is marked for reference; added reflexions from the horse tail hair are indicated by arrows. (*b*) Calcified region of bear (*Ursa ferox*) claw, beam perpendicular to the claw axis. (*c*) Stretched and dried fibres drawn from partially decalcified films of enamel matrix from the erupted incisor teeth of new-born calves, fibre axis vertical. The equatorial spacing at about 10 Å has been obliterated by the exposure necessary to demonstrate clearly the reflexion at 5·1 Å, which is marked for reference. (*d*) Fringe fibres from the baleen of the fin whale (*Balaenoptera physalis*), fibre axis vertical

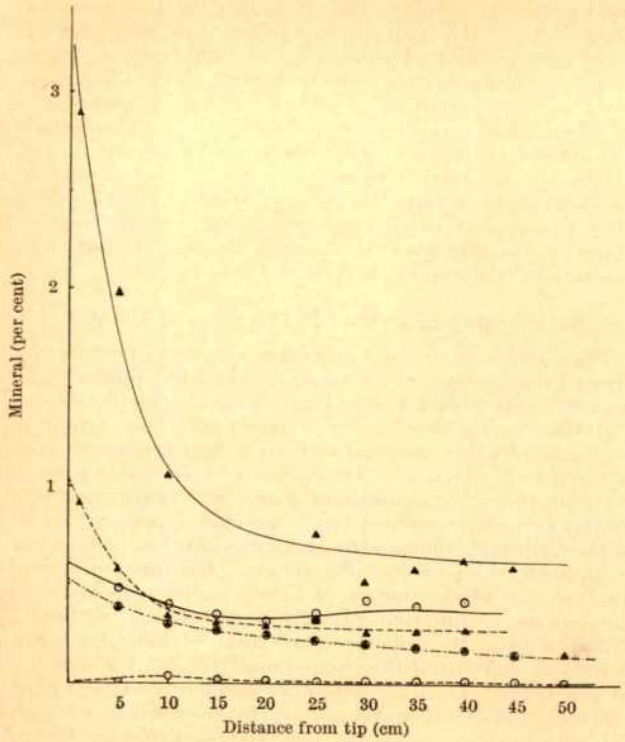


Fig. 2. Distribution of mineral salts in horse tail hairs. ▲, Non-medullated hairs; ○, medullated hairs. —, total mineral (ash); —·—, Ca^{++}; — — —, calcium phosphate calculated as $Ca_{10}(PO_4)_6(OH)_2$

In some instances, the phosphate concentrations were so low that less than 10 per cent of the calcium salt could be accounted for as $Ca_{10}(PO_4)_6(OH)_2$. No carbonate was detected in these phosphate-deficient specimens; the insoluble portion of the ash gave characteristic 'calcined bone' X-ray diffraction diagrams (similar to the pattern from horse tail hair shown in Fig. 1*a*).

In the hoofs that were examined, the calcification zone was always visible to the eye as a white line or patch confined to the least worn (middle) region of the hard wall (unguis). In cattle from the Leeds area the proportion of salt in the centre of the wall keratin was not high, but in many wild ungulates, such as the Klipspringer (*Oreotragus oreotragus saltratrix*), the very hard hoof tips were heavily mineralized. Under the optical microscope, the particles of calcium salt were usually observed grouped around the horn tubules. A typical pattern of mineralization in bovine hoofs is illustrated in Fig. 3*a*. In detail, shown in higher magnification in Fig. 3*b*, the particles showed up in various forms, mostly within the cells, which were sometimes filled completely with salt. In other areas, the deposits were elongated, flattened and irregular in shape; or scattered as a fine dust round the perimeter of the cells. All these structures (and often the whole cells) stained strongly for calcium. Treatment with 0·1 N hydrochloric acid or 0·2 M ethylenediamine tetraacetic acid (sodium salt *p*H 7·4) removed the particles completely, leaving only the small, easily identified melanin granules in those parts which happened to contain pigment.

In human finger- and thumb-nails, the calcified structures present appeared to be similar to those found in hoofs, except that the pattern of calcification varied considerably, often changing from thin 'woven' layers of sparsely distributed, mineralized cells to sheets and patches of dense salt in the space of a hundred microns or so of tissue (Fig. 3*c*).

A most striking example of a functional pattern of intracellular calcium deposits was found in leopard claws, where the mineralized zone is clearly visible as an opaque band narrowing in an arc to the tip of the claw (Fig. 3*d*). In transverse section (Fig. 3*e*) the mineralized cells are arranged into a T-shaped pattern, with the long

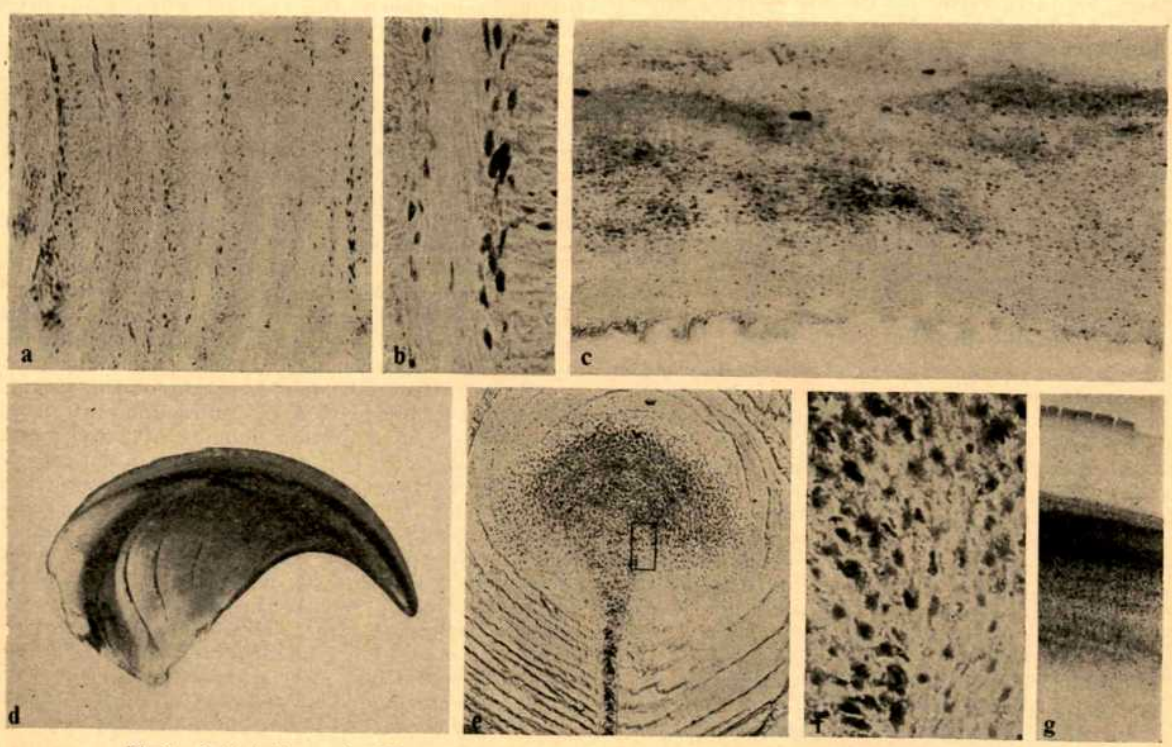


Fig. 3. Optical micrographs of Group II keratins. Each specimen has been cleared by immersion in clove oil (*a*) Longitudinal section of tubules in the calcified zone of the unguis of domestic cattle hoofs (× *c.* 55). (*b*) Enlarged detail of particle arrangement from (*a*) showing general shape of the deposits (× *c.* 300). (*c*) Transverse section of human thumbnail showing variations in density and distribution of the calcium deposits (× *c.* 50). (*d*) Leopard claw, bisected and viewed by transmitted light (× *c.* 1·5). (*e*) Transverse section tip of leopard claw. The annular fracture lines have been produced by the knife during sectioning (× *c.* 60). (*f*) Detail of mineralized cells outlined in (*e*) (× *c.* 420). (*g*) Longitudinal section of bear claw showing dense calcification zone (× *c.* 20)

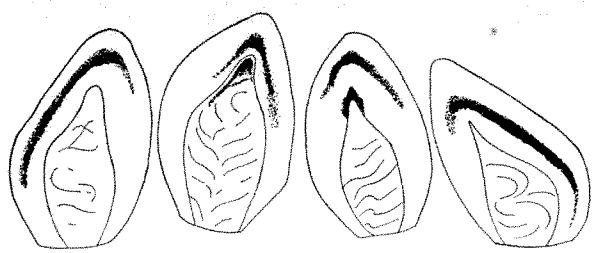


Fig. 4. Distribution of calcified deposits in rabbit claws. Right hind-foot viewed from below, sections cut about 5 mm from the claw tips. The keratin wears down to the outer edge of the mineral deposits. Note that in the outer central digit the anterior part of the 'soft' sub-unguis is hardened with salts also

limb of the T forming a rigid keel or strut to the concave (ventral) surface of the claw. The calcium salts appear to be laid down within the cells in positions corresponding to nuclei. In detail, these dense 'nuclear' particles are often surrounded by numerous smaller granules (Fig. 3*f*). Judging from the disposition of the calcified zone in the claw and from the fracture lines shown in Fig. 3*e*, it is clear that, as the cat sharpens its claws, the outer sheets of softer, less-mineralized keratin are flaked away, exposing the hardened core, which then maintains the hook-like shape of the organ by the resistance to wear of the projecting strut on the concave surface. In spite of the heavy impregnation of the central cells with salts and the hundred-fold increase in calcium content from the 'soft' to the 'hard' parts of the claw (Table 1), no reflexions suggesting crystalline inorganic substances have been found in the X-ray diffraction diagrams of any specimen of this or related types of claw. The relatively low phosphate concentrations suggest that other calcium salts or complexes may play a part in hardening the tissue.

In claws of the bear, on the other hand, a similar dense calcified zone (Fig. 3*g*) was found to give sharp 'salt' reflexions in the X-ray diffraction diagram (shown in Fig. 1*b*) indicating crystallites of similar, or larger, size than those found in enamel. In this subject, mineral particles were observed so closely packed together that in places the local concentration of salt was estimated to be about 50 per cent.

In other claws, several zones of calcification were traced in each specimen. The rabbit, for example, appears to possess a highly complex calcification pattern in its claws, each of which contains a different arrangement of salts. In the hind feet, the densest deposits were found dorsally on the two central digits but they extended down the outer sides of the claws on the two outer digits; the overall effect on the closed paw is an effective 'scoop' of salt-hardened keratin (Fig. 4).

Group III : Bird Beaks and Baleen

The distinguishing characteristic of this group was that most of, or all, the mineral fraction was in the form of calcium phosphate; where X-ray diffraction patterns indicated a crystalline salt, it was present as hydroxyapatite and usually oriented with respect to the axis of the organ.

Baleen is the best example which has been found so far of oriented deposits of bone salts in the keratins. The whalebone plate is a complex structure composed of rows of hard fibres embedded in a softer matrix; the whole is enclosed in a thick sheath of tough covering horn. As the closely packed plates wear, the fibres fray along the lingual margin to form the curtain of bristles with which the whale strains its food. The baleens from all the rorqual whales showed evidence of oriented deposits of hydroxyapatite, although the amounts of calcium and phosphorus were found to be different in each species of whale and always greater in the fibres than in the covering horn (sei whale, Table 1). On the other hand, no crystalline inorganic salts were detected in any part of Greenland whale baleen, even though the amount of calcium and phosphorus was the same as that found in the lesser rorqual whale. As in the case of the rorqual baleens, however, more calcium and phosphorus was found in the fibres than was found in the covering horn. All the baleen fibres were found to be unevently calcified, the calcium salts being laid down in concentric layers which increased in number and density towards the central tube. The evidence suggests that irrespective of the crystallinity of the calcium phosphate, the way in which the solid baleen plate wears away into a triangular shape with a fringe of fibres on one side could be decided by the distribution of calcium salts alone (cf. Figs. 2 and 3*d*).

Relationship of Enamel to Mineralized Keratin

The exception to the apparently universal partnership of hydroxyapatite (or crystallographically similar substances) with collagen has been enamel, which contains plate-like crystallites of larger size than those found in bone and dentine, associated with a sparse matrix often referred to as 'keratin'. The nature of the enamel protein is debatable, since amino-acid analyses[7–9] of the insoluble matrix of mature enamel have included 5 per cent or so of the hydroxyproline in the assay and this has been taken to indicate the presence of collagen. Recent amino-acid analyses[10–12] of the matrix of fœtal enamel suggesting an anomalous proline-rich protein (or proteins) markedly different from the substances of mature tissue have not helped to establish if the enamel matrix is truly a member of the keratin family. Attempts to identify the enamel matrix by X-ray diffraction have been even less successful; the protein has been described as being in the β- (ref. 13) or cross-β- (refs. 14–16) configuration; or in no recognizable form[17]. The evidence is inconclusive but it could be that the mineral salts in enamel are deposited in a collagen-free matrix.

Although the enamel matrix is flimsy and difficult to prepare for X-ray diffraction examination, it is possible to remove thin membranes of (predominantly) enamel protein by the cautious decalcification of appropriate tissue, particularly the erupted incisors of new-born calves[15]. Thin, dry films of such preparations have not been found to give clear X-ray diffraction patterns; a sharp meridional reflexion at 4·2 Å (lipid) was frequently observed in films viewed with their edges parallel to the X-ray beam. Otherwise, the pattern was of non-specific protein; no continuous, or meridionally disposed, reflexion at about 4·65 Å suggesting the β- or cross-β-state was observed in any preparation. It is therefore likely that previous reports[13–16] might be the results of artefacts caused by denaturation.

On the other hand, it was found that when the wet enamel membranes were stretched in moist air, they extended easily into fibres. A few of these fibres contained reflexions suggesting collagen (presumably contaminating material from dentine), but when carefully prepared, particularly from the labial margin of the tooth, both wet and dry fibres gave X-ray diffraction patterns indicative of the α-configuration, judging from the meridional reflexion at 5·1 Å and a broader equatorial reflexion at about 10 Å. In some cases it was found possible to partially decalcify the enamel in such a way that the remaining enamel crystallites were stretched with the fibres to give an oriented pattern of hydroxyapatite superimposed on a typical α-keratin-like diagram (Fig. 1*c*), altogether closely comparable with the diffraction pattern from fin whale baleen (Fig. 1*d*). Mature enamel, therefore, may contain a small proportion of protein in the α-state which can be detected best after stretching the enamel matrix.

This result might be expected from the amino-acid analyses of the mature enamel protein. The whole enamel matrix from fœtal teeth contains too much proline to be readily compatible with a stable α-helix[18], but there is no evidence that the proline-rich protein (or proteins) persist during enamel maturation, and it is reasonable to suppose that in enamel, as in other typical 'α-keratins', there are

changes in the cell contents as the microfibrils are formed. The ameloblasts may not synthesize fibrous proteins as completely as other mammalian epidermal cells and hence the portion of protein in the α-configuration may be less.

Conclusions

The evidence presented above seems to leave little doubt that the mineralization, particularly the calcification, of keratins is a widespread and complex phenomenon associated closely with the form and function of horny tissues. It is impossible to generalize about the inorganic hardening process which controls the rate of wear of the epidermal 'skeleton'. The mechanism seems to function on a cell-to-cell basis according to local demands—the worn horse tail is the result of different mineralizations of hairs, the rabbit's claws are armoured for their collective purpose by the co-operation of many regions of calcified cells.

The gross assay of keratin for minerals is at best a pointer which gives us no clues as to the distribution of the hardening cells. The X-ray evidence, too, must be treated with caution; less than 0·2 per cent of crystalline salt can produce a distinctive pattern, often in subjects containing the major bulk of their inorganic substances in some other amorphous form.

While the X-ray diffraction patterns from most keratins containing crystalline salts suggest consistently that calcium phosphate in the form of hydroxyapatite might be present (and the similarity between the calcined keratin and bone patterns emphasizes this possibility throughout) it is only in the Group III keratins that organized deposits of bone salt alone have been demonstrated beyond doubt. In the Group I keratins, the nature of the mineral is debatable. The calcium and other salts may be present in the form of small, diffuse, sub-crystalline particles, but it is equally likely that some, or all, of the hardening ions and complexes will be cross-linked with the protein matrix.

In the Group II keratins, where there are many examples of 'anomalous' calcium salts, it is clear that the deposits are laid down inside the cells. While intracellular calcification does not normally take place in bone and most invertebrate shells and tests, it has been reported in renal disease[19] and in Protozoa[20] and may be more common than has been supposed hitherto.

The heterogeneous character of calcification in the keratins suggests that even in enamel, where the 'keratin' is replaced almost entirely by calcium phosphate, there are probably areas of different hardness depending on the function of each type of tooth. The history of wearing baleen, or sharpened claws, is doubtless repeated to some degree in every keratinous tissue; to what extent the pattern of hardening is determined by selection or by environment is a matter which requires further investigation.

Finally, the occurrence of well-oriented deposits of hydroxyapatite in the keratins raises wider issues about calcification. Calcium phosphate has been examined principally in bone (and in the analogous dentine), where the association of small inorganic crystals of specific size and composition with collagen has led to numerous theories as to the role of this protein in the initiation, growth and orientation of the mineral phase. There is a need to re-examine the present theories of calcification now that hardened vertebrate tissues other than bone and enamel have come to light. Work is in progress to this end and will be reported in due course.

I wish to thank Dr. K. M. Rudall for much valuable information, the staff of the British Museum of Natural History for specimens and useful advice, and Mr. F. B. Preston for assistance; this work is part of a programme supported by grant *DE*–01345–02 from the U.S. National Institutes of Health.

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NEWS and VIEWS

Virology and Bacteriology at Birmingham : Prof. N. P. L. Wildy

Dr. N. P. L. Wildy has been appointed professor of virology and bacteriology at the University of Birmingham. He is forty-three years of age and was educated at Eastbourne College, Caius College, Cambridge, and St. Thomas's Hospital. He served for two years in the R.A.M.C., principally with the Royal West African Frontier Force, both in West Africa and India. In 1947 he was senior medical officer of Sierra Leone and of Gambia. After hospital appointments in medicine, he joined the Department of Bacteriology, St. Thomas's Hospital Medical School, first as a research fellow and during 1952–58 as lecturer and senior lecturer. At this period he spent a year as a British Memorial Fellow in virology at the Walter and Eliza Hall Institute in the University of Melbourne. Since 1959 until now he has been assistant director of the Medical Research Council Experimental Virus Research Unit in the University of Glasgow. Dr. Wildy is the author of some thirty papers, principally on the structure of viruses, particularly those of a symmetrical configuration as the icosahedral form of adenovirus. In these investigations, Dr. Wildy has developed new methods of delineation by electron microscopy. Dr. Wildy will continue this work in Birmingham, as well as directing the Department of Virology and Bacteriology in the Medical School and acting as a consultant in bacteriology to the Queen Elizabeth Hospital.

Physics at Birmingham : Dr. S. E. Hunt

Dr. S. E. Hunt has been appointed head of the Department of Physics in the College of Advanced Technology, Birmingham. Dr. Hunt was head of the Nuclear Physics Section at the Associated Electrical Industries (A.E.I.) Research Laboratory, Aldermaston. He was born in 1922 and was educated at Belle Vue Grammar School, Bradford, and then read science in the University of Liverpool. His studies were interrupted by War Service in the Royal Air Force and he graduated at Liverpool with first-class honours in physics in 1947. He joined the A.E.I. Research

Laboratory at Aldermaston in 1947, and in 1952 obtained a degree of Ph.D. at the University of Reading. He was elected Fellow of the Institute of Physics in 1957, and in 1958 was appointed visiting professor in modern physics in the University of Algiers. Dr. Hunt's main research interest has been in low-energy nuclear physics and he has a considerable list of publications in this field, and on the development of Van de Graaff accelerators. He has also published a number of papers on thermo-nuclear work and during 1960–61 acted as head of the Thermo Nuclear Section at A.E.I., Aldermaston. He was also in charge of Radiological Protection for the A.E.I. Woolwich Group of Companies.

Theoretical Physics at Bristol : Dr. J. M. Ziman

Dr. J. M. Ziman, whose appointment to the newly created chair of theoretical physics in the University of Bristol has recently been announced, studied in New Zealand and Oxford. He graduated from Victoria University College, Wellington, New Zealand, in 1945, and obtained an M.Sc. in physics there in 1946, after which he went to Balliol College, Oxford, to read mathematics. He took his Oxford B.A. in 1949, and D.Phil. in 1952, and continued as a postdoctoral research fellow in Oxford until 1954, when he moved to Cambridge to join Sir Nevill Mott, who had himself just left Bristol to become Cavendish professor in the University of Cambridge. Prof. Ziman's early work was on antiferromagnetism, on the hydrodynamics of liquid helium, and on thermal conductivity in solids. More recently his research interests have extended to cover the whole field of transport phenomena in solids, particularly metals, and to liquid metals. His book, *Electrons and Phonons*, is already a classic. He has had success as leader of a theoretical research group on these topics in Cambridge. He has also shown a wide interest in the more general academic affairs of the University, as joint editor of the *Cambridge Review*, and as author of articles in the national weeklies. His move to Bristol will strengthen the already traditional interest there in the properties of solids and liquids, pioneered by Lennard Jones and Mott. Prof. Ziman is at present on leave of absence in Australia and is expected to take up his duties in Bristol in October 1964.

Microbiology in the University of London : Prof. B. A. D. Stocker

Dr. B. A. D. Stocker has been appointed to the new Guinness chair of microbiology of the University of London at the Lister Institute of Preventive Medicine. Dr. Stocker, who is forty-six, qualified in medicine in 1940, from King's College, London, and Westminster Hospital Medical School. In 1946, after service in the R.A.F.V.R. Medical Branch, he returned to Westminster Medical School, to the Pathology Department. During 1948–53 he worked in the Department of Bacteriology in the London School of Hygiene and Tropical Medicine, spending 1951–52 as a Commonwealth (now Harkness) Fund Fellow in the Department of Microbiology at New York University College of Medicine. In 1953 he was appointed head of the Guinness–Lister Research Unit in the Lister Institute. The Institute owes the establishment and maintenance of this unit, for research in microbiology, to the generosity of the Directors of Messrs. Arthur Guinness, Son and Co., Ltd.

The work of the Unit in bacterial genetics and physiology has elucidated the phenomena of abortive transduction and somatic antigen conversion effected by phage in *Salmonella*, the genetics, physiology and biochemistry of bacterial flagella and motility, and the transmissible determinants of production of colicine; recently these determinants have been used to obtain fertility in *Salmonella*, and all of many different loci have been found to fit into a single closed-loop linkage group. The new chair, which is named to commemorate the contribution of Messrs. Arthur Guinness to microbiological research at the Institute, is the first chair of microbiology to be established in the University; Prof. Stocker will continue to direct the Unit's researches, and to teach graduate students.

Mathematics at Manchester : Prof. J. S. Griffith

The Councils of the University of Manchester and the Manchester College of Science and Technology have approved the appointment of Dr. J. S. Griffith to a newly created chair in mathematics in the Faculty of Technology. Dr. Griffith, who is thirty-four years of age, entered Trinity College, Cambridge, with an open major scholarship in natural science in 1946. He was awarded a senior scholarship at Trinity in 1948 and obtained a distinction in Part III mathematics in the following year. During 1949–51 he worked on the course for a Part II natural science in biochemistry and was awarded a research scholarship at Trinity College. In 1951 and 1952 he was engaged in research in the field of theoretical chemistry and in October 1952 was awarded a senior Royce Boyle studentship which he was permitted to use for a year of research at Oxford in Prof. C. A. Coulson's Department. In 1953 he was engaged at the Admiralty Research Laboratory, Teddington, and in 1954 was awarded the Stokes studentship by Pembroke College, Cambridge. For the next five years he was engaged in research at Cambridge on various aspects of theoretical chemistry, molecular physics and biophysics, receiving in 1956 the Berry–Ramsey fellowship in mathematics at King's College, Cambridge, which he still holds. He became professor of chemistry in the University of Pennsylvania in 1960, returning to Cambridge in 1961 to undertake further research. He was awarded the Marlow Medal of the Faraday Society in 1961 and was awarded an Sc.D. at Cambridge in the same year. He has recently completed a three-months period as professor of chemistry at Stanford University, California. Dr. Griffith is the author of two books and has published a considerable number of papers in various scientific journals. He will take up his new appointment in September.

Genetics at Canberra : Prof. D. G. Catcheside, F.R.S.

The Australian National University, Canberra, has established a chair and Department of Genetics within the John Curtin School of Medical Research, and Prof. D. G. Catcheside has been appointed as the first professor. The John Curtin School of Medical Research at present consists of Departments of Biochemistry, Experimental Pathology, Medical Chemistry, Microbiology, Physiology and Physical Biochemistry, so the addition of a Department of Genetics will add considerable strength to the research structure of the School. Prof. Catcheside, who is fifty-six, has been professor of microbiology in the University of Birmingham since 1956. During 1952–55 he was professor of genetics in the University of Adelaide. He held a visiting professorship at the California Institute of Technology in 1961. Educated at the Strand School and the University of London, Prof. Catcheside held teaching appointments in the Universities of Glasgow, London and Cambridge from 1928 until 1950. He was reader in cytogenetics at Cambridge during 1950–51, and a Fellow of Trinity College, Cambridge, during 1944–51. Until comparatively recently Prof. Catcheside carried out genetical and cytological work with flowering plants, particularly the evening primrose. During this period he made the notable discovery that the position of a gene in the chromosomes of a plant may profoundly alter its activity. His later experiments were concerned with the effect of radiations on the genetic material. For about the past ten years he has examined the genetics of the bread mould, *Neurospora*, making important discoveries regarding the interactions of gene products in the cell. He is the author of a book, *The Genetics of Micro-organisms*, and was elected a Fellow of the Royal Society in 1951 for his outstanding research. He became a Foundation Fellow of the Australian Academy of Science in 1954.

Developments in Television Services in the United Kingdom

In a written answer in the House of Commons on July 9, the Postmaster-General, Mr. J. R. Bevins, stated that he had approved, in principle, proposals by the British Broadcasting Corporation to build additional 405-line stations in Band III to serve north-east Wales, south-west Scotland, east Lancashire, mid-Lancashire and south-west Lancashire, and to proposals by the Independent Television Authority to serve the Bedford/Peterborough, Central Berkshire, east Lancashire, Scarborough, Dundee and Caithness/Orkney areas. The last-mentioned six stations would bring the programmes of independent television to nearly a million new viewers and would improve reception for 600,000 others. The north Wales station would complete the main network of stations transmitting the B.B.C.'s distinctly Welsh programmes, and the additional station to serve south-west Scotland would enable this area to receive programmes transmitted in Scotland while continuing to receive those available on the B.B.C. English network. The remaining three stations were designed to improve reception in Lincolnshire and Lancashire, which is at present spoilt by periodic interference from Continental stations operating on the same channels. The low-power Band I satellite stations to serve Grimsby and Blackpool would no longer be necessary, but the B.B.C. stations for north-east Wales and south-west Scotland and the I.T.A. station for the Bedford/Peterborough area would need to use Channel 6. The Mullard Observatory at Cambridge was conducting a sky-survey of the northern hemisphere using frequencies within Channel 6, and until this survey was completed at the end of 1967 it would be necessary to restrict transmissions from these stations on Channel 6 to the twelve-hour period noon to midnight.

Oil in Navigable Waters

The Oil in Navigable Waters Bill, which received its second reading in the House of Lords on June 28, gives effect to the amendments to the International Convention for the Prevention of Pollution of the Sea by Oil, 1954, agreed by the Convention of March–April 1962. This, as Lord Aberdare explained, was attended by 55 countries, and the amendments have already been ratified by France, Poland and Sweden, and the Bill will enable the United Kingdom Government to ratify them also. Clause 1 relates to sea areas in which the release of oil is prohibited and eliminates the distinction between tankers and other ships. One of these areas extends some 1,000 miles westward into the Atlantic from Iceland to Cape Finisterre, including the whole of the Bay of Biscay, the English Channel, the North Sea, the Irish Channel and the Baltic Sea. Clause 2 makes it an offence for any United Kingdom registered ship of 20,000 gross tons or more, for which the building contract is placed after the clause becomes effective, to discharge persistent oil anywhere at sea. The intention is that ships of this size built in future should include arrangements for retaining oil separated from water in tank washings, oily ballast water and oily residues, so that clean water only is discharged at sea and oily waste deposited on arrival at port. Discretionary discharge in special circumstances outside the prohibited areas is allowed provided the master reports the fact to the Minister of Transport. Naval vessels are still excluded, but the provisions of the Bill now apply to other Government ships such as cable-layers and weather ships. The Marquess of Willingdon warmly welcomed the Bill on behalf of the Fauna Preservation Society and the World Wild Life Fund. Lord Hurcomb referred to the difficulty caused by some authorities abroad who charged dues on the separated oil.

Seismological Computing Centre, Edinburgh

The U.S. National Science Foundation has awarded 280,700 dollars to the University of Edinburgh to assist in the establishment of a Seismological Computing Centre within the Department of Astronomy. The award will be supplemented by a grant of £10,000 capital and £6,500 recurrent from the British Treasury. It is hoped that the project will be the beginning of a continuing International Service. The awards arise from recommendations which were made by the Committee for the International Seismological Summary, which pointed out the inability of existing institutions to cope with the increasing flow of data from the seismograph stations of the world. The first objective of the new Centre will be to use the station readings to determine the positions of origin of all the earthquakes which can be detected, at present estimated at some 30,000 events per annum. This activity will provide the basis for numerous research and engineering projects, ranging from the study of the deep interior of the Earth to the investigation of local tectonics and the estimate of earthquake hazards in developing countries. The establishment of the new Centre will make Edinburgh one of the focal points of world seismology. As such, it should provide great stimulus to the existing Seismological Research Group at the Royal Observatory which is led by Dr. P. L. Willmore, and which is a joint project of the University, the Department of Scientific and Industrial Research, and of the Scottish Home and Health Department (see also *Nature*, **195**, 1250; 1962).

Graduate School of Engineering Studies at Trinity College, Dublin

A Graduate School of Engineering Studies will be inaugurated in Trinity College, Dublin, in October. It will provide a full-time course of one year in computer applications leading to the degree of master in science. The course is designed with the emphasis on the mathematical side of computers for graduates in mathematics, physics or engineering, and on the computer as a management tool for graduates in other fields. The School will also provide short courses in engineering mathematics, computers, properties of materials, aircraft structures and photogrammetry, and seminars will be organized on research topics by members of the staff or visiting teachers. Most of the research work related to industry which is being carried out in the Engineering School at present will be transferred to the new Graduate School. The director of the School will be the professor of engineering, Dr. William Wright. Giving information about the purposes of the School recently, he said that the rapid technological advance of the present day increased the need for specialization in the various branches of engineering taken at university-level. At the same time, it is becoming more and more necessary to provide advanced instruction beyond the undergraduate stage. The Engineering School at Trinity College recently has introduced new specialized undergraduate courses in civil engineering, mechanical and production engineering, and electronic engineering. A course in aerodynamics will begin in the autumn. The new Graduate School will have the two main functions of continuing the education of new graduates and providing refresher courses for older engineers with the aim of bringing them up to date in new branches of technology relevant to their fields of work.

The Volcanic Eruption on Tristan Da Cunha, 1961–62

In October 1961, after two months of earth tremors, an eruption occurred a few hundred yards from the only village situated on a low-lying coastal plain on the north-west side of the island. This resulted in the immediate evacuation to Great Britain of the entire population of nearly 300 islanders and Europeans. This eruption aroused considerable interest among volcanologists mainly because: (*a*) Tristan had previously been considered to be an extinct volcano; (*b*) the type of eruption, that is, the extrusion of a solid tholoid followed by extremely viscous blocky lava flows, had rarely been observed during growth on a truly oceanic island. The Council of

the Royal Society considered these factors warranted immediate investigation by scientists while the eruption was still proceeding, and in November 1961 formed a committee to consider the possibility of dispatching scientists to the island.

During December 1961 two geologists made a brief reconnaissance visit to Tristan da Cunha aboard H.M.S. *Jaguar* by courtesy of the Royal Navy (*Nature*, **193**, 719; 1962). This brief visit was followed in January 1962 by a twelve-man expedition which spent seven weeks on the island (*Nature*, **194**, 1119; 1962). This expedition included four geologists, one botanist and one zoologist, and the summary of their preliminary results was reported at a discussion meeting of the Royal Society in February of 1963. A film which was largely taken by the members of the Royal Society expedition shows the development and growth of the volcano from the beginning of the eruption in October 1961 until March 1962, when active growth had virtually ceased. It also indicates some of the effects of the volcano on the flora and fauna of the island. The film is of approximately 20-min duration, in colour, sound on film, and is available on hire at a charge of 25*s*. from the Executive Secretary, The Royal Society, Burlington House, W.1.

Royal College of Science and Technology, Glasgow: Biological Engineering Research

The Department of Mechanical Engineering of the Royal College of Science and Technology has received a grant of £50,000 from the Medical Research Council to support the further development of biological engineering research. 'Biological engineering' is a collective phrase used to describe the application of a wide range of specialist aspects of the physical sciences and of technology, in collaboration with the medical disciplines, to investigation of problems relating to the human body. There is already very considerable activity in biological engineering in Britain and abroad; yet the field is so wide and the problems so numerous that the present effort will have to be considerably intensified if the full potential of this collaboration is to be realized. Work in this field commenced at the Royal College of Science and Technology some six years ago with the application of refrigeration techniques in hypothermia, followed at a slightly later stage by the experimental studies of skin tensions in the human body. Since then, the College's research activity has greatly intensified in close co-operation with surgeons and physicians in Glasgow's teaching hospitals, combined teams drawn from the professions working in the College engineering laboratories, the hospital wards, the operating theatres and the medical research departments. The success of this co-operation has led to the creation of a College-based Biological Engineering Unit under the direction of Dr. R. M. Kenedi. The work will be carried out under the guidance of a Steering Committee composed of representatives of the Medical Units concerned and of the College.

British Food Manufacturing Industries Research Association

The annual report for 1962 of the British Food Manufacturing Industries Research Association has recently been published. The research programme has been largely concerned with problems which arise in factories dealing with cocoa and chocolate, sugar confectionery, meat and fish products, preserves, pickles and sauces, oils and fats and baker's prepared materials. Apart from contributions to the general literature, the Association published during the year six research reports and nineteen technical circulars. The 'analytical service' examined a total of 1,190 samples, of which 30 per cent were meat products for assessment of the proportion of meat present and 20 per cent were sugar products and chocolate for general composition. The special section dealing with extraneous materials handled 275 samples, of which 40 were rejected as failing to conform with U.S. requirements. Also 52 of the 81 samples examined for the Howard mould count failed to conform to presumptive U.S. tolerances. In addition, the 'bacteriological service' examined 978 samples, including 548 curing brines. The new library and information office came into full operation in September. The newly appointed director of research, Dr. C. L. Cutting, states in his report: "It should not escape official notice that although the largest single item amounting to between 20 and 25 per cent of the national outlay is food at £5,000 million a year, the total of about £5 million expenditure on technological research by the food manufacturing industry and Government combined is woefully inadequate compared with a number of other industries that are surely no more vital than food".

Further Education for School Leavers

A new booklet, entitled *Further Education for School Leavers*, which the Ministry of Education is sending to every secondary school in England and Wales, is intended to help teachers, and particularly careers masters and mistresses, to advise boys and girls as to the extent and variety of courses now available for those who wish to continue their education after leaving school (Pp. 40. London: Ministry of Education. 1963). The first two chapters deal with full-time courses for the sixth-form and fifth-year leaver, respectively; the third deals with other opportunities in further education, and the fourth with opportunities for girls, particularly in commerce, in science, in librarianship, management and catering, and in nursing. Enrolment, fees and grants are dealt with in a fifth chapter and lists of Colleges of Technology and of publications are appended. The booklet deals with the technical colleges as well as the universities, including also the social sciences, art, agriculture and commerce.

Science Journalism

The first Inter-American Seminar on Science Journalism was held in Santiago, Chile, during October 16–18, 1962, under the sponsorship of the Department of Scientific Affairs, Pan American Union, and of the Technical Centre of the Inter-American Press Association. The main purposes of the Seminar, the report of which is now published (Pp. iii + 30. Washington, D.C.: Department of Scientific Affairs, Pan American Union, Pan American Bldg. 1963. 35 cents), were to examine the techniques of science journalism, discuss some aspects of Latin American science, and promote better understanding among scientists and journalists. Besides abstracts of addresses given at the Seminar, the report includes the recommendation of the three committees appointed to deal with educational, information and administrative matters, respectively. The Committee on Educational Matters recommended universities to include in the programmes of schools of journalism and communications, curriculum courses in science journalism or chairs widening the traditional concepts that relate this work to humanism and philosophy. The universities and schools of journalism are also recommended to conduct seminars for professional journalists and schools of journalism in accordance with the science journalism programmes. The Committee also recommends to the Department of Scientific Affairs of the Pan American Union a programme including publication of a list of science writers, promoters and reporters in the hemisphere, publication of text-books popularizing science, translation and publication of didactic text-books on journalism, organization of an Inter-American course for professors of scientific journalism, interchange of schools of journalism curricula, establishment of awards to outstanding science journalists, and possibly the foundation of a Latin American association of science journalists.

Synthesis of Normally Dispersed Wave Trains by means of Linear System Theory

Stephan Mueller and Maurice Ewing have published a thesis with this title as contribution No. 559 of the Lamont Geological Observatory, Columbia University, Palisades, New York. Examples of natural wave propagation processes with normal dispersion are seismic surface waves and explosion sounds in shallow water. If transient signals of this kind are analysed by a new technique, it is possible to display directly the group travel time as a function of frequency. A quantitative description of this empirical dependence in the frequency domain yields an explicit system function, the inverse transform of which, for regular dispersion, has the form of an Airy function in the time domain. For inverse dispersion the solution leads to a Bessel–Clifford function, while for wave propagation processes associated with minimum group travel time the convolution integral of these two types of functions is obtained. These system transfer functions include instrumental distortion caused by the amplitude and phase characteristics of the recording system. The most important system property is the relation between the phase characteristics deduced from the group travel time and the logarithmic amplitude function of wave propagation systems with energy losses due to leakage. By means of the known transfer function the system response due to various physically plausible input functions can be synthesized. If these theoretical results are compared with actual observations, it is possible to draw certain conclusions with regard to the transient excitation at the focus of the disturbance. The deductive method described, therefore, not only permits us to make statements about the behaviour of wave propagation systems in the complex frequency domain, but also allows us to find some essential criteria concerning the little-known mechanism of the focal transient.

Small-Animal Anæsthesia

A SYMPOSIUM organized by the British Small Animals Veterinary Association (BSAVA) and UFAW (Universities Federation for Animal Welfare) at the Middlesex Hospital Medical School during July 23–24 achieved outstanding success and is likely to lead to studies, far better co-ordinated in the future than in the past, of the techniques for anæsthetizing laboratory animals and animals dealt with in veterinary practice. The Organizing Committee, comprising Mr. Oliver Jones of the Zoological Society, who acted as chairman, Mrs. Joyce Wright of the BSAVA, who acted as organizing secretary, Dr. Phyllis Croft and Miss Felicity Charig of UFAW, and Dr. Lane-Petter of the Medical Research Council, brought together a remarkable array of experts to deal with the techniques of anæsthetizing primates, reptiles, amphibians, aquatic animals including fish and porpoises, rodents and lagomorphs, birds including fowls and cage birds, the smaller ungulates, and carnivores including, among others, stoats and weasels. The proceedings were opened by Dr. S. J. Folley and closed by Prof. J. G. Wright, a historic figure in this field who directed particular attention to the risks incurred in the use of muscular paralysants as adjuncts to anæsthesia. The proceedings, which will be published by the Pergamon Press, exhibited a combination of technical expertise and unashamed humaneness which cannot fail to stimulate thought and research in the important and difficult field with which they deal.

U.S. Air Force Office of Scientific Research: Research Grants and Contracts

THIRTY-SEVEN universities and sixteen research firms in the United States and Europe were awarded research grants and contracts, amounting to more than three million dollars, during the month of May, by the U.S. Air Force Office of Scientific Research. The May grants and contracts bring the total for this fiscal year to more than twenty-eight million dollars. The primary mission of the Air Force Office of Scientific Research is "to support research through grants or contracts based on unsolicited proposals both within the United States and throughout the free world". Grants in Europe, Africa and the Near East are administered by the European Office of Aerospace Research, Brussels.

The Jane Coffin Childs Memorial Fund for Medical Research

THE Jane Coffin Childs Memorial Fund for Medical Research supports investigations in the fundamental aspects of neoplastic growth, and awards post-doctoral fellowships to candidates of outstanding potential in the same field. Applications for grants-in-aid are reviewed by the Board of Scientific Advisers and the Board of Managers of the Fund three times a year, in the fall, winter and spring. Final dates for receipt of applications are September 1, November 1 and March 1. Fellowship applications are reviewed at the winter meeting of the Fund, the final date for applications being October 1 of each year. Grants-in-aid and fellowships are not restricted to U.S. nationals. Information may be secured from the Office of the Assistant Director, 333 Cedar Street, New Haven 11, Connecticut.

Registrar General's Quarterly Return for England and Wales

THE *Registrar General's Quarterly Return for England and Wales* for the last quarter of 1962 (H.M.S.O., 1962. 2*s*. 6*d*. net) makes it possible to calculate the natural increase for that year. The total number of live births was 840,557, the largest number since 1947. There were 557,446 deaths, in absolute numbers also the largest figure since 1940. Natural increase therefore amounted to some 283,000 persons, a figure which is very considerably in excess of the average of 203,000 in the period 1952–60 and nearly 30,000 higher than the figure of 252,000 for 1961. The acceleration in rate of population growth in Britain is well brought out by these data. It has become customary to have a new record low in the infant mortality rate each year, and 1962 is no exception, though the decline was marginal from 21·6 to 21·4 per 1,000, indicating perhaps that the minimum rate likely to be achieved under present conditions is slowly being reached. Stillbirths have decreased more significantly from 19·1 to 18·1 per 1,000, but neonatal and perinatal rates have not yet become available.

Effects of Atomic Radiation

DR. C. AUERBACH has written to the Editor of *Nature* stating that, in her review of the second report of the United Nations Scientific Committee on the Effects of Atomic Radiation (*Nature*, 198, 343; 1963), she warned readers that most of the literature references in the chapters were wrongly numbered. "The Secretary of the Scientific Committee on the Effects of Atomic Radiation has pointed out to me that this warning rests on a misconception, and that the correct use of the reference numbers is explained in a note on p. ii of the report. Unfortunately, this note is not prominently displayed and has been overlooked by most readers. I, therefore, wish to draw attention to it. I should, however, like to add that the present procedure for locating literature references is very cumbersome: the reference number does not refer to a page but to a paragraph in one of the appendixes, and since there are no running headlines it takes some time and effort to find the relevant paragraph. I feel that the usefulness of this extremely useful publication would be greatly enhanced by (1) references to literature by page numbers, (2) running headlines showing appendix and paragraphs, (3) a full index. The latter is of special importance. Many items are discussed in more than one appendix, and at present location of any particular piece of information is a time-consuming job."

University News : London

The title of professor of clinical cardiology has been conferred on Dr. J. F. Goodwin in respect of his post at the Postgraduate Medical School of London. The title of professor of physics has been conferred on Dr. M. J. Seaton in respect of his post at University College. The following titles of reader have been conferred: Dr. D. E. Barton (statistics), in respect of his post at University College; Dr. A. F. Seager (mineralogy), in respect of his post at Birkbeck College; Dr. D. A. Walker (botany), in respect of his post at Queen Mary College.

The title of professor of experimental veterinary medicine has been conferred on Dr. F. R. Bell, in respect of his post at the Royal Veterinary College. The title of reader in anatomy has been conferred on Dr. R. W. Guillery, in respect of his post at University College, and that of reader in physiology on Mr. O. C. J. Lippold, in respect of his post at University College.

Announcements

Dr. F. H. K. Green is retiring from his position as scientific secretary of the Wellcome Trust on August 31. He will be succeeded by Dr. P. O. Williams, at present deputy scientific secretary.

The Robert Cormack Bequest Committee of the Royal Society of Edinburgh has awarded a Robert Cormack Fellowship to Miss Helen M. Hollingsworth, Silverwood, Sywell, Northampton, to enable her to undertake research at the University Observatory, St. Andrews, under Prof. W. N. Stibbs, on radiative transfer in stellar atmospheres and interiors. The Fellowship is tenable for three years and is the seventh award under the Bequest, which is administered by the Society for the purpose of promoting astronomy in Scotland.

The College of Physicians of Philadelphia has awarded the Alvarenga Prize for 1963 to Prof. Rupert E. Billingham, professor of zoology, Wistar Institute, University of Pennsylvania, for his outstanding work on tissue transplantation immunity and immunological tolerance. The Alvarenga Prize was established by the late Pedro Francisco DaCosta Alvarenga, of Lisbon, Portugal, an associate fellow of the College of Physicians of Philadelphia, to be awarded annually by the College on the anniversary of his death, July 14, 1883.

A symposium on "Changing Food Habits" will be held at Queen Elizabeth College, University of London, on September 24. Further information can be obtained from J. C. McKenzie, Nutrition Department, Queen Elizabeth College, Campden Hill Road, London, W.8.

A symposium on "Progress in the Biological Sciences in Relation to Dermatology" will be held in the School of Clinical Research and Postgraduate Medical Training, University of Cambridge, during September 30–October 5. Further information can be obtained from the Secretary of the Medical School, Tennis Court Road, Cambridge.

The Central Electricity Generating Board is organizing a symposium on "Environmental Monitoring", to be held at Berkeley, Gloucestershire, during October 3–4. Further information can be obtained from the Secretary, Central Electricity Generating Board, Room 728, Friars House, 157–168 Blackfriars Road, London, S.E.1.

An informal discussion on "Solvation of Molecules and Ions", arranged under the auspices of the Faraday Society, will be held at Bradford Institute of Technology during September 26–27. Further information can be obtained from Dr. W. R. Moore, Department of Chemical Technology, Bradford Institute of Technology, Bradford 7.

An international summer course on "Advances in Experimental Embryology", sponsored by the NATO Advanced Study Institutes Programme, will be held at the Zoological Station, Naples, during September 30–October 30. Further information can be obtained from Prof. G. Reverberi, Istituto di Zoologia, Università di Palermo, via Archirafi 18, Palermo.

A second regional meeting on "Particle Size Analysis", arranged by the Whiting and Industrial Powders Research Council, is to be held on October 2, 1963, at Sheffield. For further details and tickets please apply to the Director of Research, Industrial Powders Division, Whiting and Industrial Powders Research Council, The Hall, Welwyn, Hertfordshire.

A conference on "Plasma Physics", arranged by the Institute of Physics and the Physical Society, will be held at the Culham Laboratory of the U.K. Atomic Energy Authority during September 24–27. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

The fifth International Congress on Electro-Heat, arranged by the International Union for Electro-Heat, will be held at Wiesbaden during September 30–October 5. Further information can be obtained from the Secretary, British National Committee on Electro-Heat, c/o the British Electrical Development Association, 2 Savoy Hill, London, W.C.2.

A programme of displays and lectures on "Electronics Research and Development for Civil Aviation", organized by the Ministry of Aviation in association with the Electronic Engineering Association, will be held at the Ministry's research establishment at Farnborough and Malvern during September 23–October 1. Further information can be obtained from the Ministry of Aviation, Strand, London, W.C.2.

An international conference on "Elementary Particles" will be held in Sienna during September 30–October 5. The programme will include sessions on: weak interactions; electromagnetic interactions; particles and isobars; GeV physics; future of old and new theoretical ideas; general accelerator programme in Europe. Further information can be obtained from E.N.D. Steel, Scientific Conference Secretariat, CERN, Geneva 23.

A three-day laboratory course on "Radioactive Analysis of Water and Effluents" will be held in the Manchester College of Science and Technology during September 25–27. The course is designed to give practical experience in those radiation measurement techniques which are suitable for the monitoring of radioisotopes at low concentration-levels, and will provide an opportunity to use the latest equipment available. Further information can be obtained from the Registrar, Manchester College of Science and Technology, Sackville Street, Manchester 1.

The special series of postgraduate level courses is to be held in the Liverpool College of Technology for the fifth consecutive year. In the coming session the short full-time courses will cover topics such as radiological protection, radiobiology and radiometric analysis. The two-weeks' full-time courses in radiological protection provide an introductory survey of this increasingly important subject and are of assistance to medical officers and safety officers concerned with the new regulations under the Factories Act and the Radioactive Substances Act of 1960, which is to be implemented from December. The course in radiometric analysis is designed to be of assistance to the analytical chemist who is concerned with the public assessment of radiation hazards in the environment. The courses in radiobiology provide an up-to-date account of advances in this rapidly expanding and important subject. Opportunities for research in all these fields will also be available. Further particulars of all courses are set out in a twelve-page leaflet now available from the Principal Lecturer for Nuclear Studies, College of Technology, New Building, Byrom Street, Liverpool 3.

HUMIDITY AND MOISTURE

THE symposium on "Humidity and Moisture—Measurement and Control in Science and Industry" attracted more than 850 scientists and engineers from five continents to Washington, D.C., during May 20–23. The symposium was sponsored by the U.S. National Bureau of Standards and the U.S. Weather Bureau, both of which are agencies of the U.S. Department of Commerce, together with the Instrument Society of America, the American Meteorological Society, and the American Society of Heating, Refrigerating and Air-Conditioning Engineers. These five organizations joined in sponsoring the symposium because of their interest in humidity and moisture, ranging from the meteorologist's interest in humidity of the atmosphere to the engineer's concern with humidity in cryogenic materials.

The more than 230 papers presented in thirty-two sessions dealt with topics ranging from humidity standards and fundamentals, through means of measuring and controlling humidity and moisture, to specific applications. These applications varied from measurement of the humidity of air for meteorological purposes to determination of moisture in grain—an important factor in storage. Of particular interest were papers on applications in biology and medicine, describing the use of controlled humidity in treatment and the relationship of symptoms to humidity-level.

Moisture has always been the essential, though often invisible, ingredient in most processes of science, industry and agriculture—in fact, virtually every field of human endeavour. To-day humidity measurements are being extended in two dimensions—to include more processes, and to obtain more accurate values. It is now sometimes necessary to know to an accuracy of a fraction of 1 per cent how much moisture is included in a cryogenic material, a refrigerant or a storage enclosure.

All five of the organizations sponsoring the symposium are concerned with problems of measuring and controlling humidity and moisture. Both the Weather Bureau and the American Meteorological Society deal with humidity measurements for weather prediction, including the recording and transmission of humidity data from unattended stations. The American Society of Heating, Refrigerating and Air-Conditioning Engineers is concerned with measurement and control of humidity not only for personal comfort but also in industrial and agricultural processes and in the preservation of materials. Members of the Instrument Society of America are interested in the design and applications of hygrometers, psychrometers, and other humidity transducers. Finally, the National Bureau of Standards is faced with the need for new techniques in measurement of humidity and for more accurate and easily used standards of humidity.

The symposium was opened on May 20 with a plenary session presided over by W. A. Wildhack, associate director of the National Bureau of Standards and chairman of the general committee of the symposium. The main address was delivered by Dr. J. H. Hollomon, assistant secretary of commerce for science and technology. Dr. Hollomon noted that man's advances in science and technology now permit him to exercise control over his environment, including humidity and moisture. He saw man's own abilities as a neglected or undeveloped natural resource, noting that the number of engineers who will graduate in the United States in 1963 is the same as five years ago. The remainder of the plenary session was devoted to papers on basic concepts and of general interest in the field of humidity. A second plenary session held later consisted of papers surveying the state of the hygrometry art in selected fields.

At a banquet held on the evening of May 21 the assembly of scientists was addressed by officers of the five sponsoring groups. J. H. Fox, president of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, described the activities of his Society, emphasizing the research activities it supports, such as investigations of the effect of humidity on milk production and the habitability of survival shelters. He expressed the Society's concern that the number of students reading applied sciences is decreasing, although the number of college registrants is rising.

The American Meteorological Society was represented by its vice-president, Dr. Helmut E. Landsberg, who commented briefly on personalities and instruments prominent in the history of humidity measurement. The wide variety of indicating materials used in early hygrometers was a surprise even to those well versed in hygrometry.

The activities of the Instrument Society of America were noted by its president, Nathan Cohn, who also suggested that honour is due to the only American whose name has been given to a fundamental electrical unit. Mr. Cohn described Joseph Henry's concern that scientists be able to communicate and co-operate with each other and cited the Symposium as one which would have had Henry's approval.

F. W. Reichelderfer, chief of the U.S. Weather Bureau, discussed humidity as being of widespread interest as a factor in forming the world's weather. He described the World Meteorological Organization as a particularly effective means of transmitting useful meteorological data.

Dr. A. V. Astin, director of the U.S. National Bureau of Standards, noted the necessity that measurements made by all scientists and engineers be compatible and interchangeable. He emphasized the Bureau's concern with devising standard measurement techniques that can be applied at local levels.

Fundamentals

The sessions of the symposium devoted to fundamentals included papers and discussion on the fundamental laws, principles and relationships governing the behaviour of water vapour–gas mixtures, mainly moist air, and on the properties of water as basic to instruments and processes involving humidity. With the increasing demand for more accurate humidity measurements, a need has arisen for theoretical foundations provided by real gas behaviour, rather than an assumed ideal gas. The papers which were presented provided important new information on real gas behaviour of mixtures of which water vapour is an important constituent, and on the transport and thermodynamic properties of moist air.

Humidity Measuring Instruments

The water-vapour content of gases is measured by a wide diversity of instruments, the methods spanning a broad realm in the fields of physics, chemistry and engineering. A major portion of the symposium was devoted to important methods used in modern hygrometry.

Three sessions were concerned entirely with electric hygrometry, which is utilized in many areas of science and industry because of its high sensitivity, its use of small sensors, and its adaptability to recording or remote indication. Among the sensors considered were those based on electrolytic solutions, carbon films, polyelectrolytic films, ceramics, thin vacuum deposited salt films, oxide films and plastics.

Psychrometry has a history which goes back to the early part of the nineteenth century. Still a major method for measuring the humidity of air, it finds extensive application in meteorology, air conditioning, and industry. Both theoretical and experimental papers treated the use of psychrometers under such unusual conditions as temperatures below freezing and hot, arid atmospheres. Other electric hygrometric instruments that were described included those using the Peltier effect, spectroscopic methods, infra-red and ultra-violet radiation, and, operating in another area of the spectrum, mierowave refractometers and hygrometers.

In dew-point and frost-point hygrometers humidity is measured as the temperature at which dew or frost is deposited on a cooled surface, usually a highly polished mirror. Theoretical aspects of this method of measurement and improved versions of dew-point and frost-point instruments were described. Considerable interest was shown in hygrometers utilizing the Peltier effect to cool the mirror and others providing high accuracy with automatic operation and continuous recording.

Unusual measuring methods were also described, among them a chemical technique for measurements of low water-vapour pressure. Another paper described an instrument detecting the heat generated with water absorption by a desiccant and still others the design of cells which absorb and electrolyse water vapour for measurement of humidity as a function of the electrolysing current. Critical discussions of the advantages and shortcomings of thermal conductivity methods and hair hygrometers were presented in review papers.

Moisture Measurements

The latest advances in the art of measuring moisture in solids and liquids also had a place in the symposium, since moisture critically affects the behaviour of many materials. A session devoted to physical and chemical methods included papers describing gas chromatography, spectrophotometry, chemical extraction, and infra-red analysis. Further sessions treated dielectric, resistive and capacitance methods, as well as nuclear methods, nuclear magnetic resonance in particular. The papers described equipment, methods of calibration, and typical uses and presented data on accuracy.

Standards

The sessions on humidity standards were of special interest to staff members of the National Bureau of Standards, several of whom presented papers on this subject, and in general to scientists needing accurate hygrometry devices. Two of the papers described for the first time the use of the gravimetric hygrometer in making humidity measurements to a degree of accuracy suitable for the most demanding humidity calibrations. Other papers described pneumatic bridges developed at the National Bureau of Standards which make possible calibration-level hygrometric determinations as a function of the pressure differential across the bridge. Also of interest in this area were papers discussing the use of fixed humidity points in hygrometer calibrations.

Applications

The sessions concerned with the application of humidity and moisture measurements included the topics of meteorology, agriculture, biology and medicine, air-conditioning, humidification and de-humidification, environmental chambers and radio propagation and atmospheric refraction. In these sessions, particularly, scientists and engineers had an opportunity to learn about each other's measurement problems and to exchange information among widely separated fields and disciplines.

The part played by humidity in meteorological phenomena was covered in detail by the papers presented. The distribution of moisture in the atmosphere was discussed, and special techniques reported for its determination in the stratosphere. A 'state-of-the-art' survey on the application of hygrometry to meteorology was also presented.

Humidity and moisture are important in agriculture in many ways. Examples discussed were storage of agricultural products, particularly grain, flour and tobacco; curing peanuts and tobacco; the physiological responses of dairy cattle to humidity; the moisture content of butter, dehydrated foods and dry milk; and the moisture content of soils and its influence on plant growth.

The session on biology and medicine included papers on the effects of humidity on man, plants and biological systems. Humidity affects personal comfort, and imposes a stress on those working in hot environments; when controlled it can be used in treating lung disorders, for example.

The papers presented concerning air-conditioning, humidity control, humidification, and de-humidification were of a highly practical and engineering nature. Typical of the problems discussed were air-conditioning calorimetry, moisture in small refrigerating systems, residential humidification, chemical de-humidification, moisture control in dry-cleaning solutions, monitoring moisture in sealed electronic modules, and humidity levels in environmental cabinets and chambers.

Propagation of radio signals, observations of missile trajectories, and monitoring atmospheric turbulence depend to a large extent on the refractive index of the atmosphere which, in turn, is a function of the ambient vapour pressure. Research in this area also was presented. A paper on the potential of radio refractometry for humidity investigations and one on the use of radio refractometers to measure water vapour turbulence were presented. Papers on the use of radio, optical and microwave techniques for humidity measurements also were delivered.

The papers presented at the symposium will be published by the Reinhold Publishing Corp., New York, and are expected to become available early next year.

REPROCESSING FOR IRRADIATED FUELS

A SYMPOSIUM on "Aqueous Reprocessing Chemistry for Irradiated Fuels" was held in Brussels during April 23–26, under the auspices of the European Nuclear Energy Agency of the Organization for European Co-operation and Development and of the European Company for Chemical Processing of Irradiated Fuels (Eurochemic).

The object of the conference was to compare the progress made by the European countries which participate in Eurochemic, with the status of work in the same field in the United States and the United Kingdom. About half the twenty papers presented were concerned with research and development work being carried out either at Mol, Belgium, or elsewhere in Europe for the Eurochemic plant which is under construction at Mol. Other papers from French and Italian authors were related to possible new reprocessing plants in these two countries. As descriptions have been published in recent years on the major reprocessing plants of the United States and the United Kingdom, contributions from these countries were largely limited to surveys of recent developments.

It is apparent that on the Continent, as elsewhere, a solution of tributylphosphate in a hydrocarbon diluent is

regarded as the most reliable solvent extraction system for the recovery of uranium and plutonium from irradiated fuels. The degree to which this system is capable of technical improvement is now largely limited to ways in which the effluents of moderate radioactivity can be reduced in volume, for example, by using only one cycle, or be made capable of being evaporated to small bulk: in papers concerned with the latter aim, the results of research on the substitution of ferrous sulphamate by uranium (IV) nitrate as the reducing agent for the reaction plutonium (IV)→plutonium (III) were described. Otherwise, as was brought out in the contribution by Dr. R. Rometsch, the research director of Eurochemic, the main load that now falls on a research and development team is concerned with the problems involved in 'decladding' the wide variety of fuel elements used in Western European reactors and in bringing their contained fuel into solution of such a composition as to be suitable for the recovery of the fissile elements by solvent extraction.

There is now little work being done on ion exchangers for reprocessing: in the period 1950–60 they were developed and sometimes installed for the final purification of plutonium and uranium-233. The present tendency is to establish for these purification steps a solvent extraction process using a tertiary (or quaternary) amine, in a suitable organic diluent. The results from several laboratories on amine systems indicate that sufficient knowledge has already been accumulated for an amine-cycle to be capable of being successfully applied for this purpose. The two advantages of an amine, compared with the conventional tributylphosphate, for this particular purification-cycle consists in the better decontamination from uranium, and the greater reduction in the volume of the plutonium nitrate stream that the amine can provide.

Although the symposium dealt mainly with aqueous methods, there was a review by Dr. M. D'Hont (Nuclear Energy Centre, Mol, Belgium) on the alternatives, for example, reprocessing by melt refining or by fractional volatilization from halide systems. It is noticeable that these and similar non-aqueous methods, though a popular field for research at the time of the Geneva Conferences of 1955 and 1958, are losing favour. Their development costs seem likely to be prohibitive for any purpose other than an established fuel-cycle operating on a large number of reactors.

The final session was devoted to a discussion on the future role of reprocessing in fuel-cycles. It was introduced by Dr. F. L. Culler (Oak Ridge National Laboratory), who quoted his investigation on a scheme for the distillative purification of sea-water using, as a source of power, very large reactors (each of 8,700-MW thermal) fuelled with natural uranium, and cooled and moderated with heavy water: the lowness of the reprocessing cost (calculated to be about 4 dollars/kg of uranium) for the fuel-cycle concerned was primarily due to its large throughput (about 10 tons of uranium per day). This illustrated the important point that the throughput of a plant can influence costs to a greater degree than possible savings from improved technology. In the discussion which followed, two points of particular interest emerged. First, in the early stages of the expansion of the nuclear reactor industry, storage of irradiated fuels until such time as adequate quantities have accumulated to feed a large reprocessing plant may be preferable, on economic grounds, to the proliferation of many small plants: it would follow as a corollary of this policy, that the number of teams engaged on reprocessing research and development that should support such reprocessing would be relatively few in number. Secondly, for large reprocessing plants to be generally accepted, the problems in the transport of irradiated fuel both by water and on land must be overcome: these problems can be particularly severe where international boundaries have to be crossed.

About 100 persons drawn from fifteen countries as well as from the International Atomic Energy Agency and from Euratom attended the symposium, in addition to the forty scientists and technologists from Eurochemic. The meeting provided the first large gathering of the reprocessing experts of Western Europe and it was appropriate that the delegates could see at Mol the emergence of the Eurochemic plant. Dr. T. J. Barendregt and the others responsible under Dr. E. Pohland, the director-general of Eurochemic, for this plant have had to overcome many problems, new in plant construction, due to the components being supplied by one or other of the various countries which participate in Eurochemic. The plant represents a pioneering effort, new in Europe, from which benefits should accrue to many future co-operative enterprises.

J. M. Fletcher

FLOW STUDY OF POLYMER MELTS USING THE INSTRON RHEOMETER

At the meeting of the Cambridge Rheology Club held on March 4, Mr. G. G. Zahler (Instron, Ltd.) spoke on "Flow Study of Polymer Melts using the Instron Rheometer". First, he briefly discussed general flow-characteristics of polymer melts showing that temperature, speed of flow and stress measurement should be accurately controlled, instrument geometry must be accurate and accessible. A rheometer must cover shear rates from at least 1 to 10,000 reciprocal seconds in order to simulate normal processing conditions from compression moulding to injection moulding.

The Instron rheometer, which is an extrusion type and was originally designed by Monsanto Chemicals (United States), is driven by an Instron model *TT-C* tensile testing machine. This machine covers load ranges from 2 g to 10,000 lb., using strain-gauge load cells, to an accuracy of 0·5 per cent on all ranges. Speeds are governed by a synchronous motor and positional servo-system, being instantly selectable through pushbuttons over a range of 10,000 to 1.

The extrusion assembly consists of a barrel surrounded by four heaters connected to a controller giving proportional, integral and differential control from 10° C above ambient to 340° C within a limit of error of ± 0·5° C. Capillaries of different diameters, lengths and entrance angles are inserted into the lower end of the barrel. A plunger is forced through the barrel by the moving crosshead of the tensile testing machine, the load on the plunger is recorded automatically by the load cell on a strip chart recorder. Tests over wide ranges of speed may be carried with one barrel loading. As plunger speed is known, plunger load is measured and dimensions of the extrusion assembly have been accurately determined, shear rate and shear stress can be readily calculated or read directly from nomographs.

Mr. Zahler discussed possible errors such as due to the capillary aspect ratio, plunger friction force, fluid compressibility and temperature rise along the capillary, etc. He showed that all these effects were either negligible or could be easily corrected.

It was suggested that it is possible to measure the dynamic shear modulus (for visco-elastic flow) by steady-state flow in the capillary rheometer. This theory was substantiated by results obtained experimentally. Another parameter that can be measured is the activation

energy of viscous flow. Rheopectic and thixotropic characteristics of materials can also be investigated.

The next meeting of the club will be held on October 28 at the Technological Research Station, Spillers, Ltd., Station Road, Cambridge, when Dr. J. H. C. Vernon (Ministry of Aviation) will speak on "The Rheology of Solid Propellants for Rocket Motors".

E. Mary Nicholas

THE AUSTRALIAN ATOMIC ENERGY COMMISSION

THE tenth annual report of the Australian Atomic Energy Commission covers the year ended June 30, 1962, in which the expenditure was £2,803,787 and capital expenditure £905,275. Revenue from sales, including lease of reactor space, was £83,858, an increase of £28,615 for the year. The cost of the exploration for uranium carried out by the Bureau of Mineral Resources and by Territory Enterprises Pty. Ltd., was £194,000.

On March 23, 1962, two major new buildings were opened by the Prime Minister, the Rt. Hon. R. G. Menzies. One was the Reactor Physics Building, housing the low-power research reactor Moata, and the other the building devoted to Radiation Biology and Health Physics. The *Hifar* Experimental Building, a new Low-Level Radiochemistry Building, and extensions to the Isotope Handling Building, also were completed during the period under review. In a separate suite at the rear of the main block of the Radiochemistry Building is housed the total-body monitor, the radiation detector of which consists of a large scintillation crystal (8 in. diameter and 4 in. thick) of sodium iodide, suspended over the abdomen of the subject seated in a reclining chair. Two heavily shielded rooms with facilities for the irradiation of animals or biological specimens form part of the ground-floor of the building. One room contains a 10-c. cæsium-137 source for low dose-rate exposures and the other a 250-kV X-ray plant for the delivery of doses of much higher levels.

The reactor research programme was mainly concentrated on the feasibility study of a high-temperature gas-cooled reactor, in which the fuel is dispersed in the moderator. Both beryllium metal and beryllium oxide have been investigated as moderators, with carbon dioxide as coolant. Most work has been done on the metal system, but because of the poor economics of the fuel-cycle and the temperature limitations of the metal fuel, the oxide or ceramic system is now receiving greater consideration. The beryllium oxide moderated, all-ceramic fuel reactor seems to hold promise of some long-term advantage and it is believed that, provided certain design problems can be satisfactorily solved, a beryllium oxide based, high-temperature gas-cooled reactor should yield lower plant and equipment costs than most other gas-cooled systems. It would appear that with steels at present available the maximum temperature of the carbon dioxide coolant would be about 750° C, which is a considerable advance on British gas-cooled stations of present design. To assess the value of the proposed type of reactor as a power producer, a design study for a power plant of net electrical output of 300 MW has been started. The most difficult mechanical engineering problem involved is the production of the all-ceramic core of the reactor for as yet nobody has made any serious attempt to produce a satisfactory core based on beryllium oxide for power-station reactors. Several fuel cycles are being considered, with most attention being directed to the uranium-233, thorium, beryllium system, but because of lower initial cost and greater availability of fuel the plutonium-enriched, natural uranium cycle may be preferred for Australia.

In the annual report for 1961, reference was made to the investigation being carried out on the compatibility of steels with carbon dioxide. This is now essentially complete. Carbon steels, low alloy steels, and stainless steels have been studied at high temperatures and pressures, and for various moisture contents of the gas, and firm recommendations can now be made for the most satisfactory materials for use in the temperature range 400°–750° C in the various parts of the gas circuit of a reactor. At the Lucas Heights Research Establishment some research on semi-conductor radiation detectors has been carried out. Silicon-surface barrier counters can now be produced quickly and economically, and their applications to α-particle spectrometry and for scanning neutron flux are being studied. Radiation standards at the microcurie level for cobalt-60, gold-198, sodium-24, iridium-131, antimony-124 and phosphorus-32 have been developed and intercomparisons with standards of other laboratories are to be undertaken.

For neutron diffraction experiments, there will shortly be available, attached to the reactor *Hifar*, two powder spectrometers, two single crystal spectrometers, and one long-wave-length spectrometer. The spectrometers were all designed and built at the Lucas Heights Establishment, and will be of use to the university groups from Western Australia who are interested in the positions of hydrogen atoms in organic crystals, and from the University of New South Wales who are investigating the defect structure of titanium–zirconium–oxygen alloys, the structure of inorganic crystals containing hydrogen and the structure of magnetic transition-metal alloys. The physics division is now operating a neutron time-of-flight spectrometer and a second one is in the design stage. They are to be used for the measurement of the distribution of neutron energies within reactor cores.

There are well-established facilities for the production, handling and dispatch of radioisotopes at the Lucas Heights Establishment, and the annual demand for those produced at the Establishment has increased four-fold. Short-lived isotopes, including sodium-24, potassium-42, copper-64 and gold-198, which are valuable in medical work and industrial investigations, were supplied for the first time. The first cobalt-60 was removed from the reactor *Hifar* during September 1961, after approximately 320 days irradiation. Eighty-eight per cent of the product had a radioactivity of more than 50 c. per gram, with a maximum of 105 c. per gram. 2,600 c. of the 8,270 c. in the batch were used to fabricate the first Australian-made teletherapy source, which was delivered to St. Vincent's Hospital, Sydney, during November 1961. The sales value of the 454 shipments of imported radioisotopes was £14,400 and the value of radioisotopes produced was £12,646. An order for 5,000 c. of cobalt-60 for delivery in 1962–3 was received from the Japan Radioisotope Association, and there are prospects of additional orders from Japan and countries in South-East Asia. The Radioisotope Advisory and Consulting Service of the Commission has carried out a number of interesting and important field and factory investigations using radioactive isotope techniques and these are described in some detail in the annual report.

In May 1962 the Minister for National Development announced that the Government had decided to continue uranium treatment operations at Rum Jungle after the completion of the Combined Development Agency contract. This decision was made in the light of the importance of Rum Jungle to the economy of the Northern

* Commonwealth of Australia. Tenth Annual Report of the Australian Atomic Energy Commission for the year ended 30th June 1962. Pp. 88. (Coogee, N.S.W.: Australian Atomic Energy Commission, 1962.)

Territory. Uranium oxide will be available at a competitive price, but the Government is prepared to stock-pile the output for a number of years should this be necessary. Production figures were the highest so far recorded. Mary Kathleen Uranium, Ltd., is expected to complete its contract with the United Kingdom Atomic Energy Authority by the end of 1963. The Radium Hill mine and its associated chemical treatment plant at Port Pirie were closed during December 1961. Exploration for uranium is now virtually confined to existing producers, and the Commonwealth's offer to pay rewards for the discovery of uranium in Australia and Territories has been withdrawn, with the final reward instalment during the year of £14,800 in respect of the Eva deposit at Pandamus Creek, N.T.

Visitors to the Commission and to the Research Establishment during the year under review included Sir William Cook, Sir John Cockroft, Air Chief Marshall Sir Claude Pelly, of the United Kingdom Atomic Energy Authority; Dr. G. A. Robb of the New Zealand Atomic Energy Committee; Dr. P. C. Abersold of the United States Atomic Energy Commission and Congressmen J. E. van Zandt and O. E. Teague; Dr. G. A. Siwabessy of the Indonesian Institute of Atomic Energy; Dr. J. P. Hugo of the South African Atomic Energy Board; and Mr. F. R. Herrington and Mr. C. H. Hetherington of Atomic Energy of Canada, Ltd. Training for fellows of the International Atomic Energy Agency at the Research Establishment was continued and a voluntary contribution was made to the general fund of the Agency to assist in its technical assistance programme. The Commission published a large number of photographs, leaflets and booklets to satisfy the lively public interest in atomic energy, in addition to its more scientific and informative quarterly journal *Atomic Energy*. Several major exhibitions were presented, including one at the Sydney Trade Fair, and at the Sydney Town Hall during the convention of the Australian Welding Institute, and on nuclear power in Perth during May and in Adelaide during June.

The research programme at Lucas Heights is supplemented by university research contracts. All the universities in Australia together with the Commission are members of the Australian Institute of Nuclear Science and Engineering, and each university has research projects supported by Institute grants. Approximately fifty research projects involving co-operation with the Research Establishment were being sponsored during the period covered by the annual report. Details are given in a separate section and in an appendix to the report.

LONG-DISTANCE RADIO COMMUNICATION AT HIGH-FREQUENCIES

PENDING the application of satellite relays to intercontinental communications, the world's long-distance radio services have been developed on high-frequencies or short waves, which are reflected from the ionosphere in their transmission around the Earth. A survey of the present state of technique in this field and of modern trends in equipment was conducted by the Electronics Division of the Institution of Electrical Engineers at a convention on "High-frequency Communication" on March 25–27 *.

This convention was attended by some 290 participants, including a number of overseas visitors, and more than 50 papers were presented at the eight technical sessions. The texts of all contributions and formal papers included in the programme were issued in a preprinted booklet, and many of the papers were marked as having been accepted for publication in a future issue of the *Proceedings of the Institution*.

On the first day, two sessions were held dealing respectively with the propagation of high-frequency waves by way of the ionosphere, and with the design of aerial systems for the most efficient communication over distances both below and above 1,000 km. On the succeeding days, the papers read and discussed related to the various systems used in modern radio-telegraph communication and to the special technical problems associated with the development of both fixed and mobile equipment. While much ingenuity has gone into the advance design of signalling techniques and the automatic correction of any errors which occur in transmission, the limiting factor in most long-distance radio links lies in the mode of transmission of high-frequency waves by way of the ionosphere.

For many years now, using a network of ionospheric observatories, conditions in the ionosphere have been investigated by the well-known technique of sounding at vertical incidence. The results of these soundings, which in most cases are made day and night throughout the year, are exchanged on an international basis, and co-ordinated at certain recognized institutions. From these records of past measurements, forecasts are made of the high-frequency radio transmission conditions likely to prevail in the future in various parts of the world. Such forecasts have in general been made for periods of from one to six months ahead; but, as the operation of the observatory network extended over one or two sunspot cycles, each of about eleven-year periods, it has become possible to produce charts for the epochs of maximum and minimum solar activity. Interpolation between these epochs was assisted by the continuing measurements at a number of key stations throughout the world. While such arrangements are satisfactory for the long-term planning of communication circuits, the performance of these have always been subject to erratic, and in many cases unexpected, variations in the ionospheric conditions, and these have prevented the use of many radio circuits to their full capacity.

With the view of overcoming this limitation, an investigation has been in progress in some countries of methods of sounding the ionosphere under oblique incidence conditions, and, so far as is practicable, over the path of the actual communication circuit in question. Such 'oblique ionosphere sounding' as it is termed, is effected by using a transmitter emitting pulses on a series of increasing frequencies, with synchronizing arrangements for maintaining the receiver at the remote station in tune with the same series of frequencies. A paper describing this technique under the title "Oblique Sounding and H.F. Communications" by R. D. Egan and D. S. Pratt was presented at the convention. In the arrangement described, the sounder transmitter is set to begin sending pulses on a predetermined frequency at a particular time within 1 msec after each pulse, the frequency is increased in a series of rapid steps so as to cover the entire high-frequency band of 2–32 Mc/sec in a few seconds. At the receiver, the frequency is kept in step with that of the transmitter by electronic digital control after a starting signal initiates the sequence of frequency stepping. By this means oblique incidence ionograms are produced from which the upper and lower limits of frequency which can be propagated over the chosen path are readily obtained. The authors of the aforementioned paper also describe an extension of their research to the direct control of a communication circuit on a 24-h basis, by locating a sounder transmitter at the communication sending site near Anchorage, Alaska, and a sounder

* Institution of Electrical Engineers: Electronics Division. Convention on H.F. Communication March 25–27, 1963—*Proceedings*. Pp. 444. (London: Institution of Electrical Engineers: 1963.)

receiver at the communication receiving station near San Francisco, California.

Following the publication of the technical paper referred to here, *The Times* of June 19 gave some details of an ionosonde developed by E.M.I.-Cossor Electronics Ltd., Halifax, Nova Scotia; and demonstrated recently to Commonwealth representatives, the Services and other organizations interested in long-distance radio communication. The equipment was shown in operation between a sending station in Nova Scotia and the Post Office radio-receiving station at Cooling Marshes, near Rochester, Kent. At the sender, the frequency was swept in a series of 128 steps from 1·8 to 28·8 Mc/s, and the receiver was synchronized with the aid of standard time-signals. The received signals were displayed on a cathode-ray tube which, with the changing conditions in the ionosphere, indicated the most suitable frequency at any moment for transmission over this trans-Atlantic path. In addition to the direct assistance given to high-frequency communication, the ionosonde can be used for the scientific investigation of ionospheric conditions.

R. L. Smith-Rose

NEW MATERIALS TESTING LABORATORY FOR THE CEMENT AND CONCRETE ASSOCIATION

In a recent issue of the *Magazine of Concrete Research* (15, No. 43; March 1963), J. D. McIntosh gives an interesting account of the imposing new laboratory erected for the Cement and Concrete Association at its research station at Wexham Springs, Stoke Poges, Slough, Bucks. The interest lies not so much in the fact that this is a much desired extension to the existing facilities at Wexham Springs, designed to re-house various departments hitherto operating in temporary accommodation, but in what may be termed the very 'tight' specification to which the building had to conform. The primary function of the new laboratory is research on the engineering properties of concrete and its constituent materials, including soil-cement; all necessary facilities for studying these problems and for structural model testing had to be provided and somewhat stringent measures had to be taken to ensure that working conditions, for example, high degree of temperature control and correct relative humidity, in many of the rooms, were satisfied. In this, air-conditioning of much of the building plays an important part; it influenced the siting, planning and method of construction of this laboratory. "Particular problems included reducing nuisance from the dust and noise of many operations, preventing the spreading of shock and vibration to the rest of the structure, planning a convenient flow pattern for routine operations while allowing adequate versatility for other techniques, and filtering 'live' cement from liquid waste. Although all the desirable refinements were not included at the time of erection, provision was made, where possible, for them to be incorporated later".

Thus the design of a materials-testing laboratory calls for solution of many problems not encountered in ordinary building, or at least much less critically. Every eventuality seems to have been foreseen in the present instance. Although ideally premises in which such fundamental research can be carried out should provide controlled conditions which can be taken for granted, any breakdown in vital equipment should be limited in its effect by appropriate planning in advance. While on one hand the layout should be designed in such a way that frequently recurrent operations can be reduced to a fool-proof routine, the building of this nature must be readily adaptable to new processes or testing procedures. It certainly seems from Mr. McIntosh's detailed description of this new laboratory, coupled with the site-plan, floor plans and other illustrations in his paper, that the latest Wexham Springs project will eventually, if it has not already done so, fulfil the ideals which its sponsors and its architect had in mind. It certainly provides a most intriguing prototype for other buildings destined for similar purposes in the future and as it is intended that visitors will be encouraged to "see something of the laboratories without hindrance to the work in hand" (by provision of large windows between corridors and rooms and special circulation spaces), there will be no obstacle to architects and others concerned with design of special buildings for scientific work obtaining first-hand visible and highly valuable information on basic requirements.

H. B. Milner

ISOSTATIC RECOVERY IN SCOTLAND

By D. F. W. BADEN-POWELL

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Some years ago evidence was published for a correspondence in height and fossil content of some British raised beaches and those of the Mediterranean area[1,2]. If the correlations based on that work are correct, there is obviously a contradiction with the theory that northern Britain has been rising during the Holocene as a result of isostatic recovery or that recent extensive tilting has occurred in England. In other words, if some British raised beaches which were originally horizontal are now tilted, their heights cannot be used as evidence of correlation with the Mediterranean area. Recent work on the Pleistocene marine fauna of the Nar Valley Clay of western Norfolk and on an almost identical fauna from other parts of Britain has shed new light on recent crustal movements as a whole, and some widely held opinions on this subject must now be changed.

Jamieson[3] seems to have been one of the first geologists to suggest that many Scottish raised beaches might be due to local uplift, and W. B. Wright[4] later enlarged Jamieson's ideas by assuming that most of the Scottish beds are late- or post-glacial and are not represented in the south of England, whereas the English fossil beaches were supposed to be much more ancient. This idea has been accepted so generally that it is often forgotten that it was challenged by Depéret[5,6], who considered that some of the marine beds in the north can be correlated with those of southern England, and that it is not necessary to invoke any form of crustal movement to explain their distribution. Another nearly related problem is that of the supposed recent tilting of the southern parts of Britain. The original levelling of England and Wales was done by the Ordnance Survey during 1840–60, and when the re-levelling was carried out between 1912 and 1921, a discrepancy of nearly 2 ft. was found near Harwich and the Essex coast, as though there had been a submergence here since the original survey. Sir Charles Close[7] thought this discrepancy was due to changes in sea-level caused by local meteorological effects and not to vertical crustal

movement; but Longfield[8] revived the idea that there might have been real subsidence of this part of the coast. One way of solving these two related problems is to find out whether it is possible to correlate the raised beaches of the north with those of the south by palæontological means; it cannot be stressed too strongly that answers to these questions can never be found by a consideration of heights alone.

The immediate problem was to find a marine horizon which could be traced through by its fossils from the unglaciated part of southern Britain to Scotland where uplift was supposed to have taken place. The opportunity occurred when recent work was done on the Nar Valley Clay of western Norfolk, and Dr. R. G. West and Miss Stevens generously sent me marine shells which they had collected, and these, together with my own collection, were compared with specimens in the British Museum (Natural History), the Geological Survey Museum, and the museums at Norwich, King's Lynn and Wisbech, and the great help given me by Dr. West, Miss Stevens and the authorities in these Museums is gratefully acknowledged.

In her paper on the Nar Valley Clay, Miss Stevens[9] proved by an investigation of the plant remains that this clay is to be correlated with the Clacton Channels on the Essex coast, and an investigation of the marine molluscs from the two sites strongly confirms this opinion. The fauna from Clacton indicates a distinctly warmer climate than that of to-day (Baden-Powell in ref. 10) and the same is true of the Nar fossils. The chief characters which are found in the Clacton and Nar deposits include a lack of extinct forms such as *Nucula cobboldiae*, *Tellina obliqua* and *Nassa reticosa*, which occur in older deposits like the Corton Beds of East Anglia, and the nearest approach to an extinct form in the Nar Valley fauna is *Turritella pliorecens*, which is found together with the recent living form *T. communis*, which already appears at this horizon. Only very few shells indicate a cold climate in the clay, but on the other hand three warm indicators are relatively common; *Tapes decussatus*, *Nassa pygmaea* and *Bela septangularis*, which now live mainly southward from Britain. This assemblage often includes the small cockle *Cardium exiguum* (actually found at Clacton but not so far in the Nar Valley Clay).

The next stage in correlation was to inquire whether this special type of fauna can be found in other parts of Britain. Turning to southern England, it became evident that the shells from a raised beach in Croyde Bay, near Barnstaple, in Devon, and the Selsey Mud Deposit from Sussex agree well with Clacton and Nar in the lack of the extinct shells mentioned and the warm climate represented by the fauna, and it seems reasonable to connect the high-level raised beaches of Sussex with the mud deposit, as suggested by Clement Reid[12]. There is not space here to discuss these various sites in detail, but there are also at least three localities in Scotland where a fauna has been found which is very like that of Clacton and Nar, namely, in Caithness[13], the Isle of Lewis[14] and Kintyre[15]; the shells from these three areas agree with Clacton and Nar in the absence of the extinct shells *Nucula cobboldiae*, *Tellina obliqua* and *Nassa reticosa*, by the presence of *Cardium exiguum* and by the occurrence of warm indicators. Furthermore the *Turritella* shells from both Caithness and Lewis include examples of the form *pliorecens* (originally identified by me as *Turritella tricarinata*), as in the Nar Valley; unfortunately I have not yet had an opportunity to examine examples of *Turritella* from the Kintyre beds. The assemblage from all these places is so similar that it seems highly probable that these deposits were all formed during one marine submergence, and the lack of certain extinct species, combined with the presence of *Turritella pliorecens* with common warm indicators, strongly suggests a Tyrrhenian age for this set of deposits. This age has been suggested for the Sussex beds in an earlier paper[2] (but I must correct my statement in that paper that the Corton Beds are Tyrrhenian, and admit that they are older than was previously thought; this present revision of these faunas has shown that the Corton Beds belong to some phase of the Sicilian–Milazzian group of deposits).

An attempt was then made to find out whether crustal movements have taken place since these Tyrrhenian beds were laid down, working on the assumption that the original Tyrrhenian shore-lines were fairly level. Unfortunately the old beach lines have been seldom preserved, but measurements of the present height of these deposits, which contain a recognizable fauna at least, makes it possible to decide between the rival theories of W. B. Wright and Depéret which are discussed earlier in this communication. The following list shows the heights which are associated with the Tyrrhenian fossils so far:

List of Tyrrhenian sites with amount of submergence (ft.).

Clacton Channels, to 27.
Nar Valley clay, to 65.
Caithness boulder clay, no height possible, because fauna collected so far from glacially disturbed material.
Lewis, measured to 60, and glacially disturbed to 180.
Kintyre, to 135 and ? 200, but may all be glacially disturbed.
Barnstaple, measured to 40, and ? to 80.
Sussex, shingle beaches to 90 and ? 140.

The Caithness shells are so far only from erratics of sea-bottom in the Caithness Boulder Clay; but the Lewis fauna was collected from an interglacial deposit in place[11]; as noted in the list, the height and great thickness of the Kintyre shell beds[15] makes one suspect that they are involved in a push-moraine. The Barnstaple raised beach disappears upwards under a great thickness of local Head, and an enormous amount of excavation would be necessary to prove its real or maximum height; the altitude of the Sussex raised beaches is known accurately; but as they consist partly of huge shingle banks, it is difficult to find out the exact amount of submergence represented. Two of these sites were discussed by Miss Stevens[9], who suggested that late Pleistocene subsidence in East Anglia could account for the discrepancy between the height of the Nar Clay and that of the Clacton Channels, and compared a possible gradient from north-west to south-east with the suggested downward movement of the Essex coast. A strictly local subsidence here is possible, but it seems better at this stage to concentrate on the evidence from Britain as a whole, as is done in this article.

At first sight the heights given in the preceding list may seem extremely vague, but they illustrate two points:

(1) If the correlations suggested here can be accepted, the Tyrrhenian beaches of southern and eastern England are at the same order of height as those in the north, and not at or below sea-level, as predicted by W. B. Wright.

(2) A comparison of the heights for the Isle of Lewis and Sussex show that no extensive tilting like that suggested by Longfield seems to have taken place since Tyrrhenian times. It therefore seems certain that any isostatic adjustment which may have affected Britain did not take place along the lines postulated by W. B. Wright; on the other hand, Depéret seems to have gone too far in suggesting that there has been no movement at all. More detailed conclusions will only be possible after further investigations have been made of the heights of the later Monastirian and Holocene beaches.

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A GENERALIZATION OF THE PHASE-LAW OF LUNAR GEOMAGNETIC TIDES

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LUNAR geomagnetic variations, or 'geomagnetic tides', are generally supposed to be caused mainly by a semi-(lunar-) diurnal motion of ionospheric layers. The electrical conductivity of these layers is believed to vary with solar time (apart from other variations) in such a way that a maximum is reached about local noon, with low values prevailing during the night hours. The shape of the daily variation of conductivity cannot be assumed to be strictly symmetrical, nor is it centred exactly at noon; this is probably true of both the transverse and Hall conductivities. It is, therefore, impossible to represent the daily variation of conductivity as a function of solar time t by a simple superposition of cosine waves of equal phase. Instead, a more general scheme is required[1] involving terms both in sin t and cos t.

Chapman's phase-law states that the main part of the lunar diurnal variation L of any geomagnetic element is a superposition of harmonic terms $L_\varkappa$ the phases of which change systematically in the course of a month:

$$L = \sum_\varkappa L_\varkappa = \sum_\varkappa l_\varkappa \sin [\varkappa\tau - (2 - \varkappa)\nu + \varepsilon_\varkappa] \qquad (1)$$

τ being lunar time, and:

$$\nu = t - \tau \qquad (2)$$

a measure of the moon's phase. This law was originally found empirically[2,3] and later shown to be in accordance with the assumptions mentioned already, if the motion is supposed to be a simple function of lunar time of the form:

$$M_2(\tau) = m_2 \sin (2\tau + \lambda_2) \qquad (3)$$

and the conductivity:

$$K(t) = a_0 + \sum_\varkappa a_\varkappa \cos \varkappa t + b_\varkappa \sin \varkappa t \qquad (4)$$

a function of solar time. If only terms up to $\varkappa = 2$ are considered in expression (4), multiplication with expression (3) in combination with expression (2) leads to five terms $L_\varkappa$ in expression (1), up to a 6-hourly lunar variation L_4 (and including a fortnightly term L_0); these are the lunar magnetic tides generally investigated. Chapman and Bartels[1] have pointed out, however, that other terms are likely to exist, and that there was observational evidence to support this view.

In an examination of the magnetic east component at Batavia (now Kuyper, near Djakarta) it had been recognized[4] as early as in 1936, though not understood at that time, that the oscillations $L_\varkappa$ found for individual moon-phases $\nu \neq 0$, when reduced to New Moon by shifting the waves by $+(2-\varkappa)\nu$, do not coincide completely. The residuals are greater than the mean errors and show a systematic grouping according to ν, the age of the Moon. These residuals have been examined more closely in the meantime[5] and it has been found that they represent waves of the form:

$$\Xi_1 = \xi_1 \sin (\tau + 3\nu + \gamma_1) \qquad (5)$$

and

$$\Xi_2 = \xi_2 \sin (2\tau + 4\nu + \gamma_2) \qquad (6)$$

The numerical values of their amplitudes and phases are given in Table 1.

Both the main terms and the partial tides as given in Table 1 are still subject to minor corrections (for example, for the length of the lunar day as distinct from the solar day). The amplitudes of L_1 and L_2 differ slightly from the values originally found[4]; this is due to a correction for seasonal change of the S_q-variation which was applied in the recent re-examination; the errors for the amplitudes and phases are probable errors; they are quoted here from ref. 4, as they have not been determined again. It is noteworthy that in this particular set of data, Ξ_1 has nearly half the amplitude of L_1.

Table 1. Geomagnetic partial tides Ξ_1 and Ξ_2, as compared with main terms L_1 and L_2, Batavia (now Kuyper, near Djakarta) east component, southern summer (Nov. 11–Feb. 20), 1906–1929, excluding disturbed days with magnetic character figure > 1·1

	Main terms		Partial tides		Ratios	
	L_1	L_2	Ξ_1	Ξ_2	Ξ_1/L_1	Ξ_2/L_2
Amplitude (unit: 0·01 γ)	150 ± 17	279 ± 17	70	43	0·47	0·15
Phase	255° ± 10°	+86° ± 3°	171°	29°		

In analogy to similar terms appearing in general tidal theory, waves such as Ξ_1 and Ξ_2, being components of the 'geomagnetic tides', may be called 'geomagnetic partial tides', although it should be kept in mind that they do not stem from air oscillations of period $(\tau+3\nu)$ and $(2\tau+4\nu)$ superposed on expression (3); they originate in the conductivity factor (4) rather than in the factor representing the motion of the air. In fact, it is physically most unlikely that the daily variation of the conductivity should have a form that could be fitted by only a diurnal and a semi-diurnal term. If a term with $\varkappa=3$ is introduced in expression (4), multiplication by expression (3) gives the component expression (5), and a term $\varkappa=4$ in expression (4) similarly gives expression (6). However, as will be shown here, Ξ_1 and Ξ_2, if deduced from data comprising one season only, as in this case, will contain a portion not stemming from the process here invoked.

Martyn[7], in his work on lunar tides of the ionosphere at Huancayo, Peru, has found similar features in $h^{\max}F2$, and f^0F2, and Tschu[8] has pointed out that these 'lunar-solar' ionospheric variations contain a term equivalent to our geomagnetic partial tide Ξ_2.

As shown by Chapman and Bartels, a still more complete picture is obtained if the motion is also supposed to have a more general form than the simple 12-hourly wave defined by expression (3). Even though there is little doubt that the air motion is mainly semi-diurnal, a small 24-hourly component is likely to exist. In fact, Doodson's harmonic development[9] of the tide-generating potential shows that there is a diurnal term depending only on τ, the mean lunar time, and not on any other one of the remaining five arguments involved in tidal theory; (its argument-number, in Doodson's notation, is therefore {155·555}). The amplitude of this tide is about 0·7 per cent of that of the main semi-diurnal term M_2, and about 1·8 per cent of O_1, the largest one of the diurnal terms involving other arguments. However, as Bartels[10] has pointed out, the circumstance that the amplitude of lunar diurnal movements in the ionosphere are much bigger than at ground-level suggests the possibility of an independent origin of L in the ionosphere; the relative importance of the amplitude factors may, therefore, be different in the ionosphere.

If, then, a term:

$$M_1(\tau) = m_1 \sin (\tau + \lambda_1) \qquad (7)$$

is included, the product of expressions (3) plus (7) by (4), comprising terms with $\varkappa \geqq 3$, will contain other partial waves, in addition to expressions (5) and (6). For example, the 24-hourly wave will contain the further components:

$$L_1{}^* = l_1{}^* \sin (\tau + \varepsilon_1{}^*) \qquad (8)$$

and

$$\Xi_1{}^* = \xi_1{}^* \sin (\tau + 2\nu + \alpha_1{}^*) \qquad (9)$$

If the geomagnetic partial tides here discussed can be revealed in empirical data, it seems justifiable to restate

Chapman's phase-law in a generalized form. This is conveniently done by converting all formulæ to solar time t by means of expression (2). Then the classical phase-law describes terms $L_\varkappa(t)$ the phases of which decrease by 4π during a month, regardless of $\varkappa$, whereas the partial tides $\Xi_\varkappa$ have phases increasing by 4π during the month; both types stem from the semi-diurnal component of the air motion, and are, therefore, the predominant feature of L. The partial tides $L_\varkappa{}^*$ and $\Xi_\varkappa{}^*$, in turn, arising from the 24-hourly component of the air movement, have phases that decrease by 2π, and increase by 2π, respectively, in the course of each lunation.

Table 2. Geomagnetic partial tides Ξ_1, $\Xi_1{}^*$, and $L_1{}^*$, as compared with the main term L_1, Greenwich, west declination, all months, 1848–63 and 1868–1914, including (most of the) disturbed days; deduced from results obtained by Chapman (ref. 3)

	Main term	Partial tides		
	L_1	$L_1{}^*$	Ξ_1	$\Xi_1{}^*$
Monthly phase change if expressed in lunar time	-2π	0	$+6\pi$	$+4\pi$
Amplitude (unit 0·01γ)	49	0·4	11	14
Phase at new moon	131°	256°	14°	151°
Magnitude in units of main term L_1	1·00	0·008	0·22	0·29

Three of these four types of waves can be shown to be present in Chapman's results which he used in his paper[3] on the lunar variation of magnetic declination at Greenwich, based on more than 20,000 days. As an example, Fig. 1 reproduces Chapman's diagram of the variation of the phase Θ_1 of the 24-hourly wave during one lunation, with the straight line representing his phase-law. This picture is here supplemented by a curve which includes the geomagnetic partial tides Ξ_1 and $\Xi_1{}^*$; the fourth component, $L_1{}^*$, was found to be negligibly small in this particular case. To obtain the new curve, which obviously fits the observed phases better than the straight line, the amplitudes of the 24 empirical waves for each individual moon-phase ν had of course to be taken into account. Fig. 2 gives a harmonic dial, or "dial-gram"[6], in lunar time, of the 24-hourly wave of the same Greenwich data; the observed points (numbered by Arabic figures), which would lie on a circle described clockwise in one lunation, if only the wave L_1 according to expression (1) existed, show a considerable variation of the amplitudes. This is due to the presence of the vectors for Ξ_1 and $\Xi_1{}^*$, which describe three, and two, counterclockwise rotations, respectively, during one month. The sum $L_1+L_1{}^*+\Xi_1+\Xi_1{}^*$ is represented by the continuous curve; also shown, in the lower left insert, is the superposition of $L_1+\Xi_1+\Xi_1{}^*$ for new moon ($\nu=0$). Table 2 gives the amplitudes and phases of these waves.

Although the ratio ξ_1/l_1 is less than half that found for Batavia (Table 1), it is still surprisingly high, and the phase differences between L_1 and Ξ_1 are also similar.

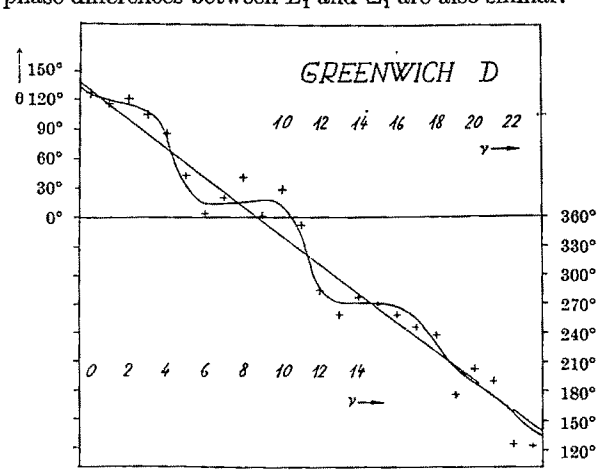


Fig. 1. Phase change, during a lunation, of the 24-hourly lunar geomagnetic tide of east declination, Greenwich, after Chapman. Crosses: observed phases; straight line: Chapman's phase-law[3]; curve: generalized phase-law including partial tides

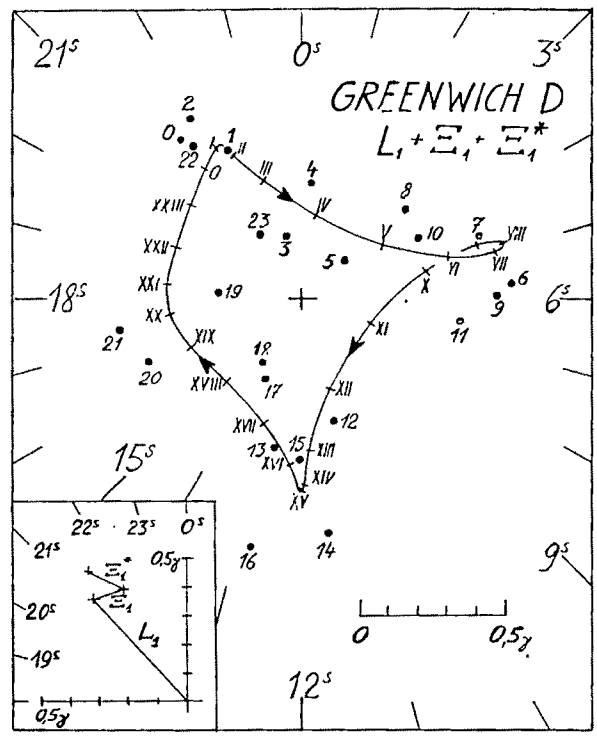


Fig. 2. "Dial-gram" of the same waves as Fig. 1, in lunar time. Dots: observed data; curve: best fit by waves given in Table 2. Inset: main term plus two major partial tides at new moon

It has already been mentioned that waves of type Ξ_1 and Ξ_2, if obtained from data belonging to one season only, will contain a portion of a different origin, in addition to the one arising from the combination of expressions (3) and (4). This portion will be produced by the diurnal tide O_1, which we may suppose not to be negligible at ionospheric levels, and will cause an air-motion of the form:

$$M_1{}^{(o)}(\tau) = m_1{}^{(o)} \sin(\tau - s + \lambda_1{}^{(o)}), \qquad (10)$$

where $s=\nu+h$ is the Moon's mean longitude, and h the Sun's mean longitude. Again, the geomagnetic effect will be obtained by multiplication with expression (4); the expressions resulting in this way have the form:

$$Z_\varkappa = C_{\varkappa+1} \sin(\varkappa\tau + (\varkappa+2)\nu + h - \delta_1) + C_{\varkappa-1} \sin(\varkappa\tau + (\varkappa-2)\nu - h + \delta_1) \qquad (11)$$

As the arguments contain h, both terms of the right hand side would average out in the course of a year, provided that the coefficients $C_{\varkappa+1}$ and $C_{\varkappa-1}$, involving the coefficients of the conductivity function (4), be constant or their annual variation be symmetrical. This, however, cannot be taken for granted, nor do the data generally cover the whole year, that is, the complete range of h; Table 1, for example, comprises only a range of $0{\cdot}56\pi$ in h, while Chapman's data used in Table 2 cover the full cycle of 2π. For a group of days belonging to only a season, then, the average of the waves $Z_\varkappa$ will not vanish and will have a resulting phase-angle involving δ_1 and depending on the length of the season analysed. The resulting form of the first term of $Z_\varkappa$ has an argument like expressions (5) and (6), and this will therefore contribute to $\Xi_\varkappa$, whereas the second term is analogous to expression (1) and contributes to $L_\varkappa$.

The partial waves here discussed are of some theoretical interest in so far as the components $L_\varkappa$, $L_\varkappa{}^*$, $\Xi_\varkappa$, and $\Xi_\varkappa{}^*$ are associated with harmonic coefficients of the conductivity function (4) of order $(\varkappa-2)$, $(\varkappa-1)$, $(\varkappa+1)$, and $(\varkappa+2)$ in diverse combinations. This may open a possibility for determining these coefficients of the conductivity function. From a practical point of view, the

geomagnetic partial tides are of importance because they account for a considerable part of the variability of the main terms that has been considered as statistical noise so far.

A search for similar features in the remaining terms of Chapman's data, and at other geomagnetic stations, including southern hemisphere observatories up to middle latitudes, is under way.

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IMPLICATIONS OF SIMILARITY IN RARE-EARTH FRACTIONATION OF NAKHLITIC METEORITES AND TERRESTRIAL BASALTS

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THE absolute abundances of the 14 rare-earth elements and yttrium and scandium have been determined[1,2] by neutron activation analysis[3,4] in two nakhlitic achondrites, Nakhla and Lafayette. Rare-earth element, yttrium and scandium abundances in parts per million are given in Table 1. Normalized abundances have also been listed in columns 3 and 5 of Table 1 for comparative purposes, and the normalized abundances of the rare-earth elements, yttrium and scandium found in 16 chondritic meteorites[3,5] have been tabulated in the last column.

Table 1. Absolute and Normalized Abundances of the Rare-Earth Elements, Scandium and Yttrium in Nakhlitic Achondrites as Determined by Neutron Activation*

REE†	Nakhla‡ Absolute (p.p.m.)	Nakhla‡ Relative	Lafayette§ Absolute (p.p.m.)	Lafayette§ Relative	Average in 16 chondrites¶
La	1·57 ± 0·05	1·00	1·76 ± 0·05	1·00	1·00
Ce (141)	5·98 ± 0·06	3·95	5·50 ± 0·16	3·12	2·77 ± 0·22
(143)	6·40 ± 0·19		5·46 ± 0·13		
Pr	0·67 ± 0·03	0·43	0·80 ± 0·03	0·45	0·41 ± 0·05
Nd	3·18 ± 0·2	2·02	3·35 ± 0·006	1·90	1·86 ± 0·16
Sm	0·73 ± 0·02	0·47	0·85 ± 0·02	0·48	0·66 ± 0·05
Eu (152m)	0·20 ± 0·01	0·124	0·24 ± 0·01	0·134	0·24 ± 0·03
(152)	0·19 ± 0·01		0·23 ± 0·01		
Gd	0·94 ± 0·03	0·60	0·92 ± 0·04	0·52	0·98 ± 0·12
Tb	0·109 ± 0·002	0·069	0·120 ± 0·003	0·068	0·16 ± 0·02
Dy	—	—	0·89 ± 0·14	0·51	0·99 ± 0·13
Ho	0·140 ± 0·002	0·089	0·146 ± 0·004	0·083	0·24 ± 0·03
Er (169)	0·36 ± 0·02	0·22	—	0·23	0·69 ± 0·06
(171)	0·32 ± 0·01		0·40 ± 0·01		
Tm	0·047 ± 0·003	0·030	0·057 ± 0·006	0·032	0·102 ± 0·008
Yb (169)	0·21 ± 0·01	0·143	—	—	0·56 ± 0·05
(175)	0·24 ± 0·01		0·36 ± 0·01	0·205	
Lu	0·044 ± 0·001	0·028	0·051 ± 0·002	0·029	0·103 ± 0·012
Y	3·17 ± 0·15	2·02	4·4 ± 0·2	2·50	6·5 ± 0·6
Sc	53·6 ± 1·1	34·2	77·6 ± 1·5	44·1	26·6 ± 2·4

* Values for elemental abundances were calculated by assuming that meteoritic and terrestrial isotopic ratios were identical for the rare-earth elements. This assumption has been verified for at least five rare-earth element ratios, especially for $^{168}Yb/^{174}Yb$, which is strongly dependent on nucleosynthetic models. Errors are primarily one standard deviation due to counting statistics.
† Parentheses show pairs of radionuclides that were measured.
‡ Values first reported by Schmitt (ref. 1).
§ Values first reported by Schmitt (ref. 2).
¶ Relative rare-earth element, scandium and yttrium abundances were taken from Schmitt, Smith and Haskin (ref. 5).

In Fig. 1 the ratios of the normalized weight per cent of the rare-earth elements, yttrium and scandium in Nakhla and Lafayette (see columns 3 and 5 of Table 1) to the average normalized weight per cent of the rare-earth elements, yttrium and scandium in 16 chondrites (see column 6 of Table 1) have been plotted as a function of the rare-earth element, yttrium and scandium ionic radii of Ahrens[6]. This geochemical procedure, modified after that introduced by Coryell *et al.*[7], clearly demonstrates fractionation of the rare-earth elements, yttrium and scandium in any given system relative to the rare-earth elements, yttrium and scandium in chondritic meteorites, which are considered the best representatives of primordial solar matter[8,9] for non-volatile chemical elements. For comparison, smooth curves have been drawn through the corresponding ratio values of Kilauea basalt[10] and through the average values in seven different sediments[5,11].

In Fig. 2, smooth curves have been drawn through ratio values for the rare-earth elements, yttrium and scandium obtained[2,3] in three 'calcium-poor' achondrites, Johnstown, Shalka and Norton County, and in three 'calcium-rich' achondrites, Nuevo Laredo, Pasamonte and Juvinas. Essentially, no rare-earth element and yttrium fractionation relative to the chondrites has been found in the calcium-rich achondrites.

On an absolute basis, the abundances of the rare-earth elements and yttrium in Nakhla and Lafayette are only ~ 10 per cent different from each other; however, on a normalized basis, which is a more severe criterion of fractionation, the rare-earth elements and yttrium agree within the 95 per cent confidence-level, with minor exceptions for the elements, cerium, ytterbium and scandium. These deviations have been discussed elsewhere[3].

Fig. 1 and Table 1 clearly show that the rare-earth elements and yttrium in Nakhla and Lafayette have been fractionated to nearly the same degree relative to the primordial rare-earth element distribution, while the element scandium has been enriched in these nakhlitic achondrites by ~ 6 relative to scandium in chondritic meteorites. The difference in scandium fractionation may be attributed to the geochemical differences[12] of scandium compared with rare-earth element and yttrium properties. Furthermore, the fractionation of the rare-earth elements and yttrium in these two nakhlitic achondrites is very similar in shape to that found in a Kilauea basalt (Fig. 1). For the light rare-earth elements, lanthanum to europium, the differences in fractionation between nakhlitic and Kilauea basalts vary smoothly from 0 per cent (by definition) for lanthanum to ~ 15 per cent for europium; for the heavy rare-earth elements, gadolinium to lutetium, the fractionation difference widens uniformly to ~ 40 per cent for the element lutetium. The smooth trend in rare-earth element fractionation, which is observed in Kilauea basalt, has been confirmed by Chase *et al.*[13], who determined three rare-earth elements, lanthanum, europium and dysprosium, in three oceanic basalts.

The rare-earth element and yttrium distribution curve of the nakhlites does not match well the corresponding distribution curve in seven sediments; the latter is considered[5] to represent best the rare-earth element and yttrium distribution in the Earth's crust. Furthermore, from Fig. 2, it is evident that rare-earth element and

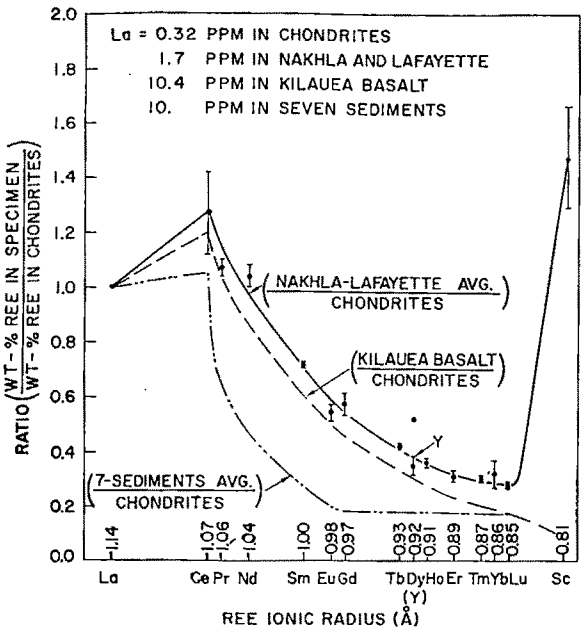


Fig. 1. Ratios of the average normalized weight per cent of the rare-earth elements, yttrium and scandium in Nakhla and Lafayette to those in sixteen chondrites (solid curve) as a function of ionic radius. For comparison, dashed curves have been drawn through corresponding ratios for Kilauea basalt (*Iki*-22) and through the average ratios in seven different terrestrial sediments

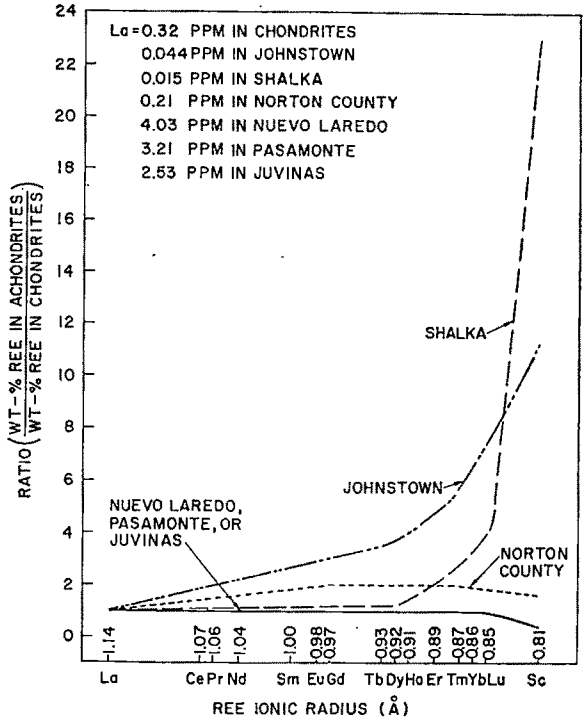


Fig. 2. Ratios of the normalized weight per cent of the rare-earth elements, yttrium and scandium in three calcium-poor achondrites, Johnstown, Norton County, and Shalka, and in three calcium-rich achondrites, Nuevo Laredo, Pasamonte, and Juvinas, to those in sixteen chondrites as a function of ionic radius

yttrium fractionation differs markedly in the calcium-poor achondrites, whereas no fractionation for the rare-earth elements and yttrium has been found in the three calcium-rich achondrites.

In an earlier report[2] we have shown that the rare-earth element, yttrium and scandium fractionation results presented in Table 1 and depicted in Figs. 1 and 2, together with abundances of the rare-earth elements, yttrium and scandium determined in a total of 27 meteorites which cover the entire spectrum of meteorites, are consistent with the concept of a single parent meteoritic body[2], perhaps intermediate in size between those suggested by Ringwood[14] and Fish *et al.*[15]. With increasing radius the various shells are: iron-nickel core, pallasitic, calcium-poor achondritic, chondritic, mesosideritic, calcium-rich achondritic, Type II carbonaceous chondritic, and finally, nakhlitic achondrites scattered over the surface of the meteoritic body. Volcanic action, terrestrial-like[14] on the parent meteoritic body, may have produced the similarity in the fractionation of rare-earth elements and yttrium to the Kilauea basalt. Goles[16] has suggested that, according to the model of Fish *et al.*[15], the basaltic or nakhlitic meteorites could be chilled and embedded just below, or in, the carbonaceous meteoritic shell.

These nakhlitic meteorites account for only ~ 0·3 per cent of observed meteoritic falls; furthermore, Prior[17] reported that Nakhla resembled a fine-grained basalt. Mason[18] states that the two meteorites Nakhla and Lafayette are mineralogically indistinguishable. If the volcanic root originated[2] in the calcium-rich achondritic (4·8 per cent of observed falls) shell, only ~ 0·3/4·8, or 6 per cent of the calcium-rich achondrites in that shell may be enriched in the heavy rare-earth elements and yttrium. With no convection, about 2 of the 37 calcium-rich achondrites observed may show an enrichment factor. With sufficient convection in this shell, changes in rare-earth element and yttrium abundances would not be reflected in the calcium-rich achondrites, since the data are good ~ ±5 to ~ 10 per cent.

Many other tests may provide corroborative evidence for the above suggestions, for example, macroconstituency and trace elemental content, cosmic-ray exposure ages, gas-retention ages, etc. A cosmic-ray exposure age similar to ordinary chondrites would be more compatible with Goles's suggestion[16]. On the other hand, a shorter cosmic-ray exposure age (compared with ordinary chondrites) and similar to carbonaceous chondrites, which have been postulated for surface matter[15], would suggest[19] that nakhlitic meteorites may be of surface lunar origin as proposed by Urey[20]. Therefore, if a lunar origin is postulated, and the rare-earth element distribution in chondritic meteorites is primordial, volcanic action may have prevailed on the Moon in order to produce the smooth fractionation so similar to terrestrial basalts. Such fractionation on a lunar surface might conceivably occur during the crystallization of the melt, generated by the heat associated with lunar crater formation[21].

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DETERMINATION OF GOLD AND OF SILVER IN SOLUTION BY ATOMIC ABSORPTION SPECTROSCOPY

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GOLD. Following the development of a method for the determination of silver in lead sulphide concentrate[1], attempts have been made to apply atomic absorption spectroscopy to the determination of gold in metallurgical samples.

Lockyer and Hames[2], working with dilute aqueous solutions of the noble metals, reported only moderate sensitivity for gold, together with pronounced drift of transmission readings as their burner warmed up. More recently, Gatehouse and Willis[3] reported a detection limit of 0·3 p.p.m., indicating an approximately six-fold reduction in sensitivity compared with that for silver using their equipment. They did not obtain the drift in transmission readings reported by Lockyer and Hames.

The work reported here was done on the equipment described formerly[1] except for the substitution of a stainless steel burner head having 20 × $\frac{1}{8}$ in. diameter collinear holes. With this head, the total flame-length was 10 cm. The limit of detection for aqueous gold solutions was 0·3 p.p.m. In agreement with Gatehouse and Willis, no transmission drift was observed.

This detection limit for gold clearly indicated the need for sensitivity enhancement if atomic absorption spectroscopy were to be applied to the determination of gold down to the 0·5 dwt. gold/ton (approx. 8×10^{-5} per cent by weight) level.

From preliminary trials on aqueous gold solutions, solvent extraction offered the advantage of both preconcentration and of sensitivity enhancement by appropriate choice of solvent[4]. Of those tested, methyl isobutyl ketone appeared the most promising, having the least effect on the combustion characteristics of the flame. Moreover, flame background at the resonance wave-length of 2428 Å was slight and reproducible.

Gold (III) ions readily form complexes with the halides. That with bromide ion has been much favoured for subsequent treatment. Solvent extraction for 30 sec with methyl isobutyl ketone of aqueous solutions containing the bromaurate anion in the presence of excess hydrobromic acid showed organic phase maximum absorption when the axis of the optical path was 8·5 mm above the burner top. No detectable concentrations of gold were found in the aqueous phases from these extractions.

Enhancement of sensitivity for gold was determined by repeated comparisons between aqueous and organic media solutions. Typically, 3·00 ml. aliquots of 1, 3, 4, 5 and 10 p.p.m. aqueous auric ion solutions were rendered 3 molar in hydrobromic acid in a total aqueous volume of 30 ml. Extractions were made with 10·00 ml. methyl isobutyl ketone, the separated organic phases then being atomized into the coal-gas/air flame for measurement. For comparison, a portion of each of the aqueous standard solution stocks was atomized without prior extraction. Typical results are given in Table 1.

The average slopes of the calibration curves of closest fit to these results are: organic extracts 0·056 absorbance units/p.p.m. gold; aqueous solutions 0·011 absorbance units/p.p.m. gold, or an uncorrected increase in sensitivity of 5·1 : 1. The volume of the organic phase was never less than 9 ml. after a period of 1 min from completion of extraction. Solubility data for water indicate an equilibrium volume of 9·4 ml. at 20° C.

Neither lead nor tellurium ions interfere with either the extraction or the subsequent absorption measurement. This has led to the use of co-precipitation of gold with lead sulphide as a means of collection and separation from the bulk of the ions present in solution.

Sensitivity is such that sufficient gold is present in 5 g of sample to permit its determination at the 0·5 dwt./ton-level, once adequate dissolution methods have been found. The combined precision of measurement and of collection of gold by lead sulphide is within ± 1 per cent relative, thus comparing favourably with the fire-assay method. Conditions for the quantitative collection of gold from metallurgical samples are currently being established for both lead sulphide and tellurium metal, while the rationale of solvent extraction from chloride and cyanide solutions is also being examined.

Silver. In a recent paper on the determination of silver in lead sulphide concentrate[1], the final solution for atomization was approximately 3·25 molar with respect to hydrochloric acid. Although the corrosive effect of this concentrated acid solution on the standard EEL atomizer could be overcome by using a platinum iridium atomizer capillary, other means were sought to overcome this. This article records one of a number of methods developed for this purpose.

Forward *et al.*[5] have shown that certain of the alkylpolyamines form stable, highly soluble complexes with lead ions. Their solubility data suggested the means for keeping lead salts in solution and, at the same time, for reducing the acidity to levels where atomizer corrosion was no longer a problem.

The reagent selected for testing was diethylene triamine, available in 'laboratory reagent' grade. Preliminary qualitative tests showed that the reagent would readily dissolve the equivalent of the lead ion content of 1-g samples of lead sulphide concentrate after the normal hydrochloric and nitric acid digestion.

The resultant solutions, whether made acid, neutral or alkaline with the reagent, were stable and did not re-precipitate lead salts on long standing or extensive dilution. Similar tests with solid silver chloride suspension in hydrochloric acid showed that this compound was also readily dissolved by addition of diethylene triamine. Such solutions were stable under both acid and neutral conditions, although incipient precipitation occurred when they were rendered alkaline with the amine.

Synthetic samples of lead sulphide concentrate were prepared from A.R. grade lead acetate trihydrate, silver free, to which measured amounts of a standard silver ion solution were added. Precipitation of the sulphides, followed by quantitative solids separation, drying to and

Table 1. Sensitivity Enhancement by Solvent Extraction

Gold conc. in aqueous standard (p.p.m.)	Nominal gold conc. in organic phase (p.p.m.)	Transmission (per cent)	Absorbance
0	0	97·5	0·011
1	0·3	95·0	0·022
3	0·9	88·8	0·052
4	1·2	84·8	0·072
5	1·5	81·0	0·092
10	3·0	68·0	0·168
0	—	100·0	Nil
1	—	97·9	0·009
3	—	93·6	0·029
4	—	91·2	0·040
5	—	88·5	0·053
10	—	77·6	0·110

Hollow-cathode tube supplied by Ransley Glass Instruments, Melbourne; lamp current, 8 m.amp; monochromator spectral band-width = 2 Å; solution up-take rate approximately 50 per cent greater for the organic solvent solutions than for the aqueous solutions.

weighing at atmospheric equilibrium enabled calculation of the silver contents.

The standard silver solution was prepared from spectroscopically pure silver wire. The filtrates from sulphide precipitation, diluted to a standard volume of 500 ml., were examined by atomic absorption spectroscopy for silver. None was ever found. The detection limit for silver with the absorption equipment used was $0{\cdot}0_5$ p.p.m.

These synthetic samples were used to test the diethylene triamine method by dissolving 1 g portions in 100 ml. of 10 N hydrochloric acid to remove the bulk of the sulphides, adding 16 N nitric acid as oxidant, followed by evaporation to 20–25 ml. volume. After cooling, the solutions were transferred to 100 ml. volumetric flasks, 8 ml. diethylene triamine added slowly, with vigorous agitation, both to complex precipitated lead salts and to reduce the acidity to approximately *p*H of 1. After making to final volume, the absorption of each was measured concurrently with those of similarly prepared standard silver solutions.

Measurement was made using the original equipment with a 10-cm coal-gas/air flame, air-pressure 6 p.s.i.g., lamp current 6 m.amp, *DU* monochromator slit-width 0·05 mm., at the silver resonance wave-length of 2381 Å. The optical axis traversed the flame at the optimum value of 8·5 mm above the burner top. Coal-gas flow-rate was the minimum consistent with stable combustion. Typical results are given in Table 2.

Table 2. Use of Diethylene Triamine for the Determination of Silver

Synthetic Sample No.	Theoretical assay (oz. Ag/ton)	Atomic absorption assay (oz. Ag/ton)	Ag recovery (per cent)
I	$22{\cdot}4_5$	$22{\cdot}0_1$	98·0
		$22{\cdot}4_0$	99·8
		$22{\cdot}6_5$	100·9
III	$22{\cdot}5_7$	$22{\cdot}4_1$	99·3
		$22{\cdot}2_8$	98·7
		Mean =	99·3

During the period of development of this method, several hundred atomizations were made without any noticeable corrosion of the atomizer, while the use of a test solution completely free from insoluble lead salts was an incidental advantage.

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COHERENCE TIME MEASUREMENTS OF LIGHT FROM RUBY OPTICAL MASERS

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THE coherence time[1] of the light pulses emitted by a ruby optical maser can be inferred from measurements of the spectral distributions made by several workers[2–6]. However, objections can be raised against the interpretation of most results reported so far. We have attempted to measure the coherence time directly in the following experiment, by a method which avoids most of these objections. We have detected the beat notes in the photoelectric fluctuations resulting from the superposition of light beams from two independent ruby masers on a photocell (cf. experiments of Forrester[7,8]). A preliminary account of this work was given at the Third International Conference on Quantum Electronics[9]. A similar spectral analysis for two continuously operating He–Ne optical masers has already been reported by Javan, Ballik and Bond[10].

Most workers[2–5] attempting to measure the spectrum of light from a ruby maser have made use of high-resolution interferometric or dispersive methods; but, because of the extremely narrow spectral width of the light, transient features of the spectrum (cf., for example, ref. 11) are easily lost in such experiments. Values obtained for the line-widths of individual components of the maser emission have ranged from a few[2] to many megacycles[4]. Berkley and Wolga[12] have examined the interference produced by two parts of a divided wave-front from a ruby maser, and have reported that the fringe visibility falls to below 1 per cent for delay times of order 0·1 μsec. However, it is doubtful whether this is a measure of the coherence time. On the other hand, McMurtry and Siegman[6] have measured the spectrum of beats between axial modes from a single ruby maser (cf. also Herriott[13]). On the assumption that phase correlation between modes is negligible, the widths of individual components were shown to be of the order of 2 mc/s, which suggests that the coherence time is $\sim 0{\cdot}5$ μsec and primarily limited by the finite duration of each pulse or 'spike'. However, phase relations between modes of a He–Ne maser have recently been found[14] and indeed are predicted theoretically for gaseous[15] and solid-state[16] optical masers. The possibility of similar effects in ruby cannot therefore be completely ruled out. The beats produced by two independent beams should therefore provide a less ambiguous measure of the coherence time.

Consider two plane, fully polarized maser beams incident on photocells singly and in superposition. The two complex classical wave amplitudes which we denote by $V_a(t)$ and $V_b(t)$ can be expanded in the form[17]:

$$V_a(t) = \sqrt{I_a(t)}\,\exp\,[2\pi i\nu_a t + i\varphi_a(t)]$$
$$V_b(t) = \sqrt{I_b(t)}\,\exp\,[2\pi i\nu_b t + i\varphi_b(t)] \qquad (1)$$

where ν_a and ν_b are the centre frequencies of the two light beams, $\varphi_a(t)$ and $\varphi_b(t)$ are their phases, and $I_a(t)$ and $I_b(t)$ are their instantaneous intensities (given by $V_a^*(t)V_a(t)$ and $V_b^*(t)V_b(t)$ respectively). $\varphi_a(t)$, $\varphi_b(t)$, $I_a(t)$ and $I_b(t)$ can be shown to be nearly constant within a time shorter than the coherence time of the light[17]. The beams, when incident singly and perpendicularly on two photocells, will give rise to the instantaneous photoelectric signals:

$$S_a(t) = \alpha D I_a(t)$$
$$S_b(t) = \alpha D I_b(t) \qquad (2)$$

respectively, where α is a constant representing the quantum sensitivity of the photodetector (assumed constant over the spectral range of the light) and D is the width of the accepted beam. The photocurrent $S_c(t)$, at the output of a third photocell, resulting from the superposition of the two light beams, will be:

$$S_c(t) = \alpha \int_x^{x+D} |V_a(t) + V_b(t+\tau)|^2\,dx \qquad (3)$$

where x is a point on the photocathode corresponding to an optical path difference $c\tau$, as measured from an arbi-

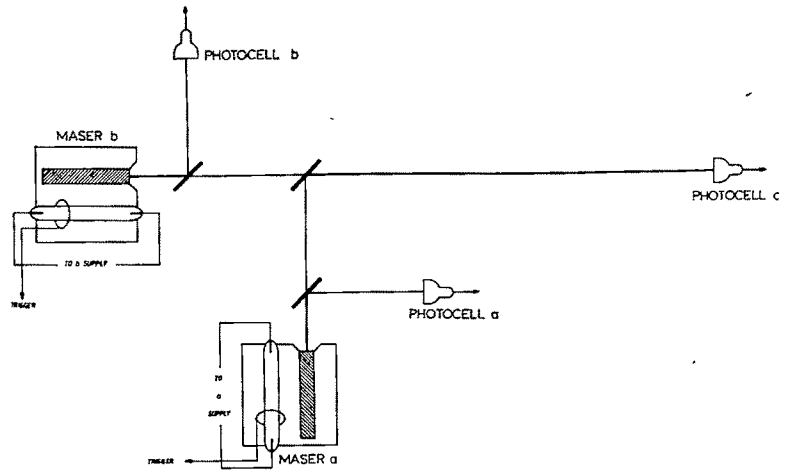


Fig. 1. Overall arrangement of the system

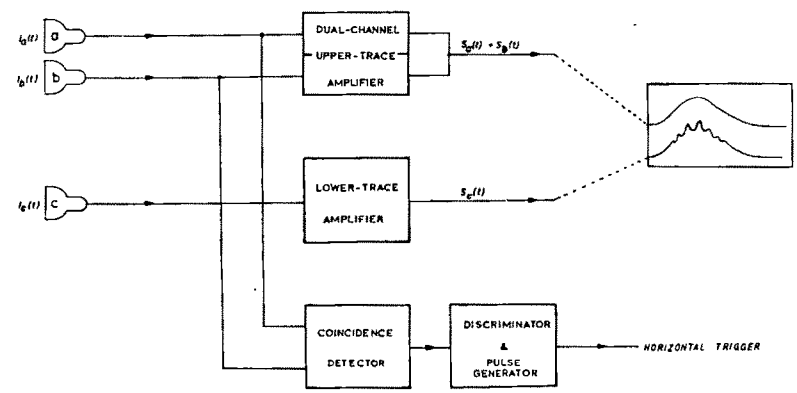


Fig. 2. Layout of the apparatus

trary origin. In the evaluation of $S_c(t)$ it is necessary to take into account a possible inclination β between the wave-fronts and an inclination β' between one of the wave-fronts and the photocathode. From equations (1), (2) and (3) we arrive at (cf. also equation 5 in ref. 18):

$$S_c(t) = S_a(t) + S_b(t) + 2k(\beta,\beta')\sqrt{S_a(t)S_b(t)}\cos[2\pi(\nu_a - \nu_b)t - \Phi(\beta,\beta') + \varphi_a(t) + \varphi_b(t)] \quad (4)$$

where:

$$k(\beta,\beta') = \frac{\sin\Phi(\beta,\beta')}{\Phi(\beta,\beta')},$$

$$\Phi(\beta,\beta') = \pi(\nu_a - \nu_b)\beta' D/c + \pi\nu_b\beta D/c$$

and c is the velocity of light.

From equation (4) we can see that the superposition signal is modulated sinusoidally at the difference frequency $|\nu_a - \nu_b|$. As long as the phases $\varphi_a(t)$ and $\varphi_b(t)$, together with the factor $\sqrt{S_a(t)S_b(t)}$, are constant the beat note remains steady. In the absence of other effects, the duration of a steady beat note is therefore a measure of the coherence time.

From the factor $k(\beta,\beta')$ in equation (4) it is evident that the wave-fronts must be nearly parallel $\left(\beta \ll \frac{c}{D\nu_b}\right)$ for $k(\beta,\beta')$ to be close to unity. On the other hand, the orientation of the photocathode (that is, the value of β') is critical only for very large difference frequencies $|\nu_a - \nu_b|$.

In practice the interpretation of the beats obtained with ruby maser light is complicated because the photoelectric signals tend to be heavily modulated in intensity by the envelope of the spikes. Moreover, high-frequency oscillations of the instantaneous light intensity $I(t)$ are sometimes observed[19] and these may mask the modulation due to the difference frequency. To avoid ambiguities in the identification of the beats, the intensity of the superposed beam and the sum of the individual beam intensities are recorded simultaneously in this experiment.

The system illustrated in Fig. 1 is arranged to detect chance coincidences of spikes after both masers are triggered simultaneously. The two light beams are sampled individually at the photocells a and b and are then combined by a beam splitter that directs the superposed beams to photocell c. The actual apparatus is reminiscent of a Michelson interferometer. The beams are superposed to within 0·03 mm and aligned to within 0·1 milliradians by means of the fine adjustments provided.

Small diameter (2·5 mm) ruby rods were chosen for this experiment to improve the chances of obtaining simple modes. The modes of one rod were controlled by flexing it in the way described by Lipsett and Strandberg[20]. With these precautions, nearly identical beams could be produced.

Fig. 2 is a block diagram of the electronic circuits. Photocells a and b serve the dual purpose of feeding a coincidence detection and discrimination circuit that triggers the oscilloscope sweep, and of providing the algebraic sum $S_a(t) + S_b(t)$ for the upper trace of the oscilloscope. The superposition signal $S_c(t)$ appears directly on the lower trace. Comparison of the two traces allows the cosine term of equation (4) to be identified with little ambiguity.

The maximum frequency $|\nu_a - \nu_b|$ that the system can detect is more than 60 mc/s, and is limited mainly by the response of the oscilloscope. Frequencies below about 10 mc/s tend to be obscured by the spike envelopes. The centre frequencies of the fluorescence lines will differ by almost 4 gc/s per °C temperature difference between crystals[21]. In addition, each spike may or may not be due to oscillation in more than one axial mode and the frequency may vary from one spike to the next. Moreover, because of thermal effects, the oscillation frequency can sweep through several megacycles during a single spike[2]. However, since both rubies experience similar temperature changes this should not affect our results, at least to a first order. Frequency shifts due to the effect of anomalous

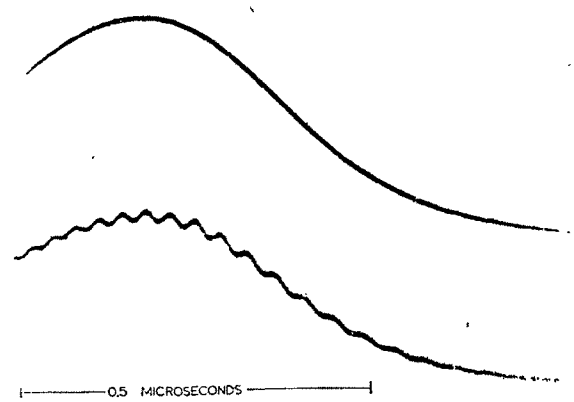


Fig. 3. Oscilloscope trace of the sum of the intensities of the maser beams (upper) and the intensity of the superposed maser beams (lower)

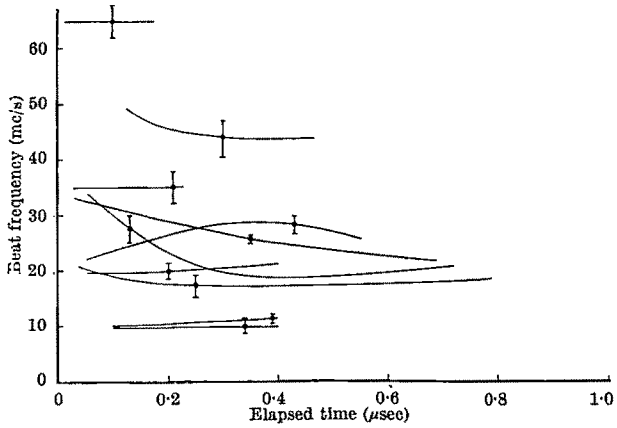


Fig. 4. Observed variation of beat frequencies with time. A single experimental point is shown for each curve merely to indicate the estimated accuracy of measurement from the photographs

dispersion are likely to be small[11], and in any event, should be nearly equal for both rubies emitting in coincidence. For the ~ 40 mm crystal lengths in this experiment adjacent axial modes are separated in frequency by 2·2 gc/s. The two lengths differ by 2 per cent, which assures that only beats between one axial mode of each ruby will fall within the detection band-width of the system. In fact, our detection band-width is about two orders of magnitude smaller than the possible range of beat notes. With this restriction we find in practice that slight variations from one maser flash to the next lead to one observable beat note in about 50 trials. The pumping conditions are so adjusted that on the average one spike coincidence occurs per maser flash, and this happens close to threshold for both rubies.

A photograph of a typical beat note is reproduced in Fig. 3, and shows a modulation on the lower trace. This beat note can be seen to persist for the full duration of the spike, drifting in frequency from 33 mc/s to about 21 mc/s in the course of 0·7 μsec. No systematic trend for the beat frequency to increase or decrease was evident from other similar photographs. This is shown by Fig. 4, which summarizes the results. It can be seen that the coherence time is of the order of ½ μsec, which is also the half-width of a typical spike.

Our results therefore show that transient coherence[1,22] can exist between independent ruby masers for periods of the same order as the spike width. This corresponds to a line-width for a single mode of a few mc/s and agrees with the results of McMurtry and Siegman[6].

We plan to apply a similar technique to measure the coherence time under conditions of quasi-continuous oscillation[23].

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ION EXCHANGE THEORY OF COAGULATION

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RECENTLY, S. Levine and E. Matijević have criticized[1] my ion exchange theory of colloid stability[2]. From this criticism it should follow that the thermodynamic deduction of the theory is incorrect, and that in order to confirm it the experimental results given were not correctly interpreted by me.

This article is restricted to point out the main errors on which the criticisms of Levine and Matijević are based.

(1) Their basic assumptions[1] concerning the ion exchange theory were summarized, and in my opinion an erroneous interpretation of this theory was given. This interpretation in connexion with the simplified notation used does not correctly explain the ion exchange theory neither is this note helpful in elucidating it to the readers.

The original version[2] of the interpretation of the ion exchange theory was based on chemical thermodynamics applied to the experimentally observed counter ion exchange on coagulated silver iodide. Later[3] electrical terms, namely, electrochemical potentials, were introduced into the theory, and on the basis of electrochemical thermodynamics and double-layer electrostatics, it was possible to extend the theory to the interpretation of electrokinetic phenomena. The theory of electrokinetic phenomena could be confirmed by available electrokinetic results. In this way the same theoretical postulates as in the original deductions in ref. 2 concerning counter ion exchange and coagulation could be obtained.

(2) The first point of the criticism, from which it should follow that the thermodynamic deduction of the theory is incorrect or impossible, is based on the opinion that the chemical potential of the adsorbed constituent ion $\mu_{A^-,ads}$ cannot be different from the chemical potential of the same ion in the solution phase μ_{A^-}. This would be an anomaly contradicting laws of thermodynamics. Consequently, $\mu_{A^-,ads}$ could not be independent of the activity of the same ions in the liquid phase as supposed in the ion exchange theory. A consequence of this statement would be that all particles of a two-phase system should be in distribution equilibrium and that all particles should follow the same thermodynamics. According to this requirement, the logarithm of the adsorbed quantity of constituent ions should be proportional to the logarithm of their activity. The existing experimental evidence on silver iodide, however, is that either the amount Γ of adsorbed constituent ions I^- (determined potentiometrically[4,5]) is approximately proportional to $p\mathrm{I}$ or decreasing with $p\mathrm{I}$, or that the amount γ (determined by the adsorbed

amount of labelled counter ions which cannot be decanted or washed out from the coagulated silver iodide) is constant with pI (refs. 6 and 7). Recently[18], the independency of the adsorbed bromide ions of their activity in the adsorption saturation region was observed. Thus none of the experimental methods of determination of the adsorbed constituent ions (I^-) confirms the thermodynamic postulate of Levine and Matijević.

On the other hand, it follows from experiments that the adsorbed quantity of two counter ions $x\gamma$ and $(1-x)\gamma$ varies as required by the equality of their (electro) chemical potentials in the adsorbed state and in the liquid phase. The total adsorbed amount of two counter ions γ, and the maximal adsorbed amount of one counter ion are equal and independent of the concentration of counter ions and of pI (refs. 6–8). Therefore the only possible logical conclusion is that these counter ions are in a reversible distribution equilibrium, while the constituent I^- ions in the amount which is equivalent to the adsorbed amount of counter ions are irreversibly adsorbed and are consequently not in a distribution equilibrium. The experimental evidence suggests that the quantity of adsorbed constituent ion γ which is constant with pI and which causes the selective adsorption of counter ions is determined by the specific surface of silver iodide and that the forces by which the constituent ions are fixed in the surface are great compared with the coulombic forces by which counter ions are adsorbed. Therefore the constituent ions are assumed as tightly bound or fixed at the surface (inner Helmholtz layer) while the counter ions form the outer (Helmholtz) layer. Experimental results cannot be in contradiction with thermodynamics and therefore theoretical explanations which explain the same experimental results cannot be in contradiction with thermodynamics either.

According to the second main point of the criticism of Levine and Matijević the standard distribution coefficients D'_0 and D''_0 of two different counter ions M' and M'' defined by:

$$\ln D'_0 = (\mu^0_{AM',\text{ads}} - \mu^0_{M'})/RT \text{ and } \ln D''_0 = (\mu^0_{AM'',\text{ads}} - \mu^0_{M''})/RT \quad (1)$$

cannot be equal as supposed by the ion exchange theory since standard chemical potentials of two different species should be different. (The notation of ref. 1 was adopted here.)

One of the basic suppositions of the theory of solutions is that different ideal solute particles exhibit the same thermodynamic action if present in systems of the same mole fraction (concentration). For example, the osmotic pressure, the freezing point depression, the liquid junction tension, Donnan equilibria, etc., depend on the number of present ideal particles only and not on their kind. The differences in thermodynamic action of real particles are accounted for by activity factors. The differences in valency of ions are accounted for by the number of ions taking part in the reactions and by the valency factors in the electrochemical potentials. For example, from the limiting law of the Debye–Hückel theory it follows that the activity factors depend on the valencies of ions only and not on their kind.

A proof for the correctness of this opinion can be given directly by the selectivity coefficient S of ion exchangers, which can be defined by:

$$\ln S = \ln\{F[c_{M''}x\gamma/c_{M'}(1-x)\gamma]\} = a(z'-z'') - [(\mu^0_{AM',\text{ads}} - \mu^0_{M'}) - (\mu^0_{AM'',\text{ads}} - \mu^0_{M''})]/RT = a(z'-z'') - \ln(D'_0/D''_0) \quad (2)$$

F is the product of all activity factors occurring in the expression. For ideal exchanging ions of equal valencies $F = 1$ and $z' = z''$. In the classical theory of ion exchange the opinion that for ideal exchanging ions $S = 1$ is generally accepted as valid. The experimental deviations of S from 1 with real ions are accounted for by activity factors in which case $F \neq 1$.

Therefore, according to the classical theory of ion exchangers as well as to the elementary theory of electrolyte solutions, it is correct to assume that:

$$D'_0 = D''_0,\ \mu^0_{M'} = \mu^0_{M''} \text{ and } \mu^0_{AM',\text{ads}} = \mu^0_{AM'',\text{ads}}$$

if the concentrations are measured in moles per litre. Therefore, Levine and Matijević erroneously claim that the terms D'_0 and D''_0, $\mu^0_{M'}$ and $\mu^0_{M''}$, and $\mu^0_{AM',\text{ads}}$ and $\mu^0_{AM'',\text{ads}}$ cannot be equal.

As experimental support of the latter argument, Levine and Matijević quote our own results on ion exchange on silver iodide for the ion pairs $Y^{3+} - La^{3+}$ and $Y^{3+} - Nd^{3+}$ (ref. 7, Fig. 8). The fact that for each ion pair two lines can be obtained (correctly by the last square method) they consider as the proof that standard chemical potentials of these ions are different. The maximal deviation in the logarithm concentration scale of the two points, from the drawn theoretical line having a slope of 45°, is 0·1. These deviations can apparently be attributed to an experimental error (eventually counting error in counting lower radioactivity in lower ion concentrations) and to necessary different activity factors of the two ions. This experimental (not reproduced) deviation in the slope is not significant and cannot certainly be accepted as a proof for the correctness of the assumption that $D'_0 \neq D''_0$ as claimed by Levine and Matijević. Eventually, if the deviation would be significant, it could be accepted as the proof that $f_{M''} = f(c)$ and that $f_{M'} \neq f_{M''}$, namely, that the activity factor is a function of concentration and that, in general, activity factors of different ions are different.

The maximal spread of the a values obtained in counter ion exchange, summarized in ref. 7, Tables 1 and 2, and reproduced in Table 2 here, is 1·5–1·7 for seven measurements of different ion pairs. For three ions (Th^{4+}, La^{3+}, Ba^{2+}) in combination with Nd^{3+} this value is constant and equals 1·2. The results with thorium (1·0) have to be discarded because the hydrolysis was not controlled. The spread between 1·5 to 1·7 can be attributed to activity factors and the lower values (1·3) to specific effects of some ions. This spread is equal or even smaller than the spread normally encountered in experiments with ion exchangers. Despite deviations of this kind the classical thermodynamics of ion exchangers is generally accepted and considered as valid. It follows that the scatter of the results on the value a is not significant enough and cannot be used as an argument against the ion exchange theory as it was proposed by Levine and Matijević.

(3) Equation (7) in ref. 1:

$$\ln(c''/c_0) = -z''a + \ln[x/(1-x)] \quad (3)$$

can be obtained for:

$$\ln c_0 = \ln c' + z'a \quad (4)$$

from equation (6) which reads:

$$\ln(c''/c') = (z'-z'')\,a + \ln[x/(1-x)] \quad (5)$$

and the latter was obtained from the equality of the sum of the values of equations (2) and (4).

In ion exchange experiments with two counter ions M' and M'' present in a total concentration greater than the coagulation value, the physical meaning of c_0 is that of a (calculated) concentration of the hypothetical non-existent counter ion of valency $z = 0$. This particle

Table 1 (ref. 9). A Comparison of Coagulation Values of Silver Halides

	$-\log c_{coag}$			a		
	K^+	Ba^{++}	La^{+++}	K–Ba	Ba–La	Mean
AgI	−0·83	−2·74	−4·51	1·91	1·77	1·84
AgBr	−1·24	−2·68	4·3	1·44	1·62	1·53
AgCl	−1·80	−2·68	−3·86	0·88	1·18	1·03

Table 2. SCHULZE–HARDY RULE CONSTANT a

AgI/I⁻	Counter ion valencies:		3–4	2–3	1–2	1–3
	Electro-kinetics	(Fig. 57, ref. 17, Fig. 3, ref. 3)	1·75	1·5	—	—
	Ion exchange, $c > c_{coag}$	(Tables 1 and 2, ref. 7)		1·6 1·6 1·7 1·5 1·5	1·7	1·3† 1·7
			1·0† 1·2†	1·2†	1·2†	
	Ion adsorption, $c < c_{coag}$	(Fig. 1)		1·78		
	Coagulation pI 1–pAg 3 Water Water–ethanol Water–acetone Water–glycine pI 1–pI 6	(Table 1, ref. 15) (Table 1, p. 307 ref. 12) (Fig. 11, p. 300 ref. 12) (Fig. 6, ref. 9)		 1·52(5)* 1·68(8) 1·48(9) 1·49(4) 1·56 (several) 1·50 1·77	 1·73(5)* 1·74(7) 1·91(9) 1·34(7) 1·76 (several) 1·65 1·91	
AgBr/Br⁻	Coagulation	(Fig. 6, ref. 9) (Table 1, ref. 16)	1·44	1·62 1·68	1·44 1·53	
AgBr/Ag⁺	Coagulation	(Table 2, ref. 16)	1·22	1·83	1·52	
AgCl/Cl⁻	Coagulation	(Fig. 6, ref. 9)		0·88	1·18	

* The figures in parenthesis are the numbers of measurements of which the result is the mean.
† Considered as specific cases.

was introduced to simplify the equation (only one z term!). In ion exchange experiments c' is an independent variable or a parameter of the experiment and therefore c_0 is an independently variable parameter too.

In coagulation experiments only one counter ion is present in a variable concentration c''. If we assume that the stabilizing particle M' is a constituent of the liquid (solvent) and therefore present in a constant activity c'_{stab}, which is independent of the variable concentration c'' of the coagulating counter ion M'':

$$\ln c_{0,\,coag} = \ln c'_{stab} + z'a \qquad (6)$$

is equal to the extrapolated constant coagulating concentration of the non-existent hypothetical particle of zero valency. According to experiments the coagulation concentration $c_{0,coag}$ is a constant and by the ion exchange theory it is explained as a constant because of the constant activity of the stabilizing particles c'_{stab}. This concentration was neither by Težak nor by the ion exchange theory assumed as constant because it is so much greater than the coagulation concentration of counter ions as incorrectly purported by Levine and Matijević.

The concentration c''_{coag} is in coagulation experiments a critical limiting concentration: in smaller concentrations the sols are stable or coagulate very slowly, in greater concentrations the coagulation is for orders of magnitude faster. Theoretically this coagulation concentration can be explained by a critical fraction $x = x_{coag}$ of adsorbed counter ions on stable sols and by a fraction $(1 - x_{coag})$ of adsorbed stabilizing particles which are the same for ions of different valencies. The coagulation concentration can then be defined for z = const. by:

$$c''_{coag} = c''_{(x\,=\,0\cdot5)}\,(1 - x_{coag})/x_{coag} = c''(1 - x)/x = \text{const.} \qquad (7)$$

This equation was obtained from the difference of two corresponding equations (6) or (7) in ref. 1.

Consequently no contradiction between equations (2), (4), (6) and (7) could be found and therefore the criticism of Levine and Matijević on this point is not justified.

(4) Table 1 shows results on coagulation values of different halides from ref. 9. From these it may be concluded that the coagulation values of Ba^{2+} are equal for silver chloride, bromide and iodide and that the Schulze–Hardy rule constant a is different for each halide. The same result was quoted in ref. 10, Fig. 6. The precision of the results is high enough and they may be summarized by the equation:

$$\log c''_{coag} = (2 - z'')a + \log c_{M^{z+},coag} \qquad (8)$$

($c_{M^{z+},coag}$ is the coagulation concentration of a bivalent cation).

On the basis of the hypothesis that the stabilizing particles M' are bivalent, the same equation can be obtained by the ionic exchange theory and the logarithm coagulation value of the bivalent ion can be defined by:

$$\ln c_{M^{2+},coag} = (\mu_{M'} - \overset{0}{\mu}_{M^{2+}})/RT \qquad (9)$$

($\mu_{M'}$ is the constant chemical potential of the stabilizing particle).

In pure water and in solutions of low concentrations of counter ions no other particles than water molecules are present, and only these can be assumed as bivalent. Therefore water molecules were accepted for the explanation of the stabilizing action. This conclusion is valid in the same extent in which the experimental result obtained by Matijević and Schulz[9] on which it is based is reliable. The conclusion is simple and logical and there is no reason to be called by Levine and Matijević a controversial conjecture.

The assumption that in the coagulation concentration $x = x_{coag} = 0{\cdot}5$ may be explained in the following way. The adsorption isotherm equations (6) or (7) in ref. 1 is a continuous function in which the only characteristic point is determined by $x = 1 - x = 0{\cdot}5$. For $x < x_{coag}$ the repulsing forces between the particles due to the stabilizing action are greater than the attracting Van der Waals forces. For $x > x_{coag}$ the electrostatic repulsing forces of the prevailing counter ions in the compressed outer layer are smaller than the Van der Waals forces. This assumption is equivalent to the basic assumption of the Derjaguin–Landau–Verwey–Overbeek theory except for the principle of the derivation of the electrostatic tension explained in ref. 3, which is a function of x, z'' and $c_{M''}$.

Fig. 1 shows the plots logarithm adsorbed quantity $x\gamma$ of Eu^{3+} and Co^{2+} against logarithm of their concentration in the region $(\log c''_{coag} - 1{\cdot}5)$ to $(\log c''_{coag} + 1{\cdot}0)$. (Experiments by M. J. Herak of this laboratory.) In low concentrations $c'' < c''_{coag}$ the silver iodide is present in a finely divided stable form (see electronmicrographs ref. 11, Fig. 12.1) and could be separated from the liquid by high-speed centrifugation only. In concentrations greater than the coagulation value the silver iodide is present in the coagulated form (electronmicrographs in ref. 11, Figs. 6, 7 and 8). Therefore the adsorption isotherm:

$$\log (c''/c_{0,coag}) = -z''a + \log [(x\gamma/(1 - x)\gamma] \qquad (10.$$

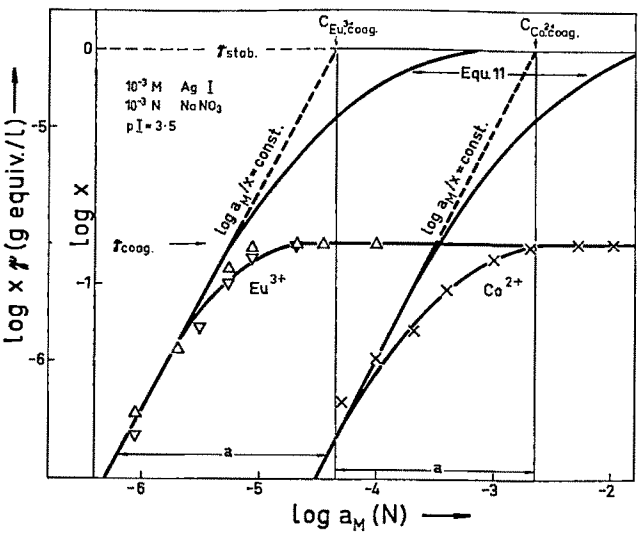


Fig. 1. The adsorbed quantity against the logarithm of Eu^{3+} and Co^{2+} concentration in the region of their coagulation values (10^{-3} M AgI, *p*I 3·5). Theoretical adsorption isotherms (equation (3) or (10)) for the stable state of AgI characterized by the value γ_{stab}. (Experiments by M. J. Herak, to be published.)

if measured on finely-divided stable silver iodide is different from that measured on the coagulated silver iodide. Therefore it should be expected that the measured plot will be a transition from the isotherm characterized by the value γ_{stab} valid in low concentrations of counter ions ($c'' < c''_{coag}$) to the horizontal part of the isotherm characterized by the directly measured value γ_{coag} in high concentrations.

Therefore it is not surprising, in fact it is necessary, that the measured plots of Fig. 1 do not fit the elementary isotherm equation (3) or (10) which would be valid if the solid would be present in the same state above and below the coagulation value. In low concentrations the plot asymptotically approaches the tangent with a 45° slope, while in high concentrations it becomes a horizontal line. However, the second important argument for the theory is that the plots are shifted for the constant $a - 1{\cdot}8$, which value is within the limits of the statistical scatter of this value (Table 2). Consequently the fact that the experimental isotherm does not fit the theoretical one is no argument against the ion exchange theory as purported by Levine and Matijević.

In Table 2 a collection of the values for the constant a obtained by different experimental techniques—electrokinetics, counter ion adsorption on stable silver iodide, counter ion exchange on coagulated AgI and coagulation—is presented. The maximal variation of this constant for the silver iodide system lies between 1·48 and 1·91 when some cases with effects which can be recognized as specific are not considered (for example, hydrolysis of Th^{4+}). This latter value (1·91) when corrected for the probable activity factor ($\log f = -0{\cdot}1$ to $-0{\cdot}2$ in 0·1 N solutions of monovalent electrolytes, in the corresponding solutions of bi- and tri-valent ions $\log f$ is $< 0{\cdot}05$) would be 1·8 to 1·7 or even less. This maximal variation includes the differences of the slope pointed out by Levine and Matijević in their discussion of ref. 7, Fig. 4. At present the number of available results is not great enough to make a statistical analysis which would eventually prove that the slopes of the lines 'valency-logarithm coagulation value' and 'valency-logarithm desorption concentration' are significantly different. Levine and Matijević claim, namely, that these experimental slopes ($= a$) cannot be considered as equal. The statistical variation of the different a value cannot certainly be used as an argument according to which the value a should not for theoretical interpretation be considered as a constant.

Levine and Matijević claim that there are examples which follow other formulations than the linear Schulze–Hardy rule, and quote the arsene sulphide sol. Presumably this statement is based on a work by Freundlich from 1910 cited in ref. 12, p. 307, Table 1. The result on arsene sulphide should be first reproduced by an up-to-date technique and the proof should be given that the logarithm activity factor correction does not cause the differences in the a values. The activity factors of 2–2 and 2–3 electrolytes ($S^{2-} - Ba^{2+}$, $S^{2-} - La^{3+}$) are irregular and as a rule smaller than those of monovalent electrolytes. If this result alone is accepted as sufficient to confirm the rule:

$$\log c''_{coag} = \text{const.} - 6 \log z \qquad (11)$$

then the evidence given in Table 2 for silver halides in the range up to about 11 *p*I units and the following values on different other sols should be accepted as confirming the linear rule for a larger number of different systems. Other examples which confirm the linear rule by the constancy of a are:

— Au (ref. 12, Table 1) 1·8 ($K^+ - Ba^{2+}$) and 1·8 ($Ba^{2+} - La^{3+}$)

— AgCNS (ref. 13, Fig. 15) 1·53 ($K^+ - Ba^{2+}$) and 1·63 ($Ba^{2+} - La^{3+}$)

— Cross-linked polystyrene (ref. 14, Fig. 5) 0·93 ($K^+ - Ca^{2+}$) and 1·06 ($Ca^{2+} - La^{3+}$)

The discussion of Levine and Bell[10], according to which the linear Schulze–Hardy rule is approximately true only in certain favourable cases at a discrete value for the surface potential (that is at a discrete *p*I value), cannot certainly be valid for the silver halide systems. Figs. 3, 4 and 5 and Table 1 of ref. 15 are an additional experimental argument that this rule may be valid over 9–11 *p*I units and that significant deviation from this rule occur only in the region $p\text{Ag}_{limit}$ to $p\text{Ag}_{limit} + 1$ and log c''_{coag} to log $c''_{coag} - 1$ ($p\text{Ag}_{limit} = p\text{Ag}$ at which the 'isoelectric' coagulation sets in) for ions of valency one and two.

Moreover, not even a qualitative similarity between these experimental plots and the theoretical plots obtained by Levine and Bell can be observed. Comparison made by ref. 10, Figs. 2 and 5, is meaningless since the plots in the equivalency region are poorly defined and because the abscissa values are incorrectly assigned. The maximum on the experimental plots of Fig. 3 is caused by a variation of the log c_{coag} value not greater than 0·2 for monovalent ions and can be attributed to irregularities in the activity factors in higher concentrations. The plots for bi- and tri-valent ions are constant with *p*I showing deviations smaller than 0·05. The corresponding theoretical plots of Levine and Bell are decreasing with decreasing *p*I in the greater part of the negative stability region.

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EIGHT CO-ORDINATION IN THE EARLY TRANSITION SERIES

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ALTHOUGH a few compounds containing an eight-co-ordinate metal atom have been known for some time, for example, the dodecahedral molybdenum and tungsten octacyanide[1] anions and the square antiprismatic octafluorotantalate[2] ion, it is only recently that the widespread occurrence of this co-ordination number in the early part of the three transition series has been recognized. Many elements display eight-co-ordination by forming polymers in three dimensions, for example, metals with a body-centred cubic lattice or salts having the cæsium chloride structure, but discrete eight-co-ordinate cations, anions or uncharged molecules were very uncommon. However, we reported recently a series of isomorphous monomeric compounds of the general type $MCl_4.2$diarsine[3] (Fig. 1) (where $M = Ti^{IV}$, V^{IV}, Zr^{IV} and Hf^{IV} and diarsine is as shown in Fig. 2) in which the metal atom is eight-co-ordinated.

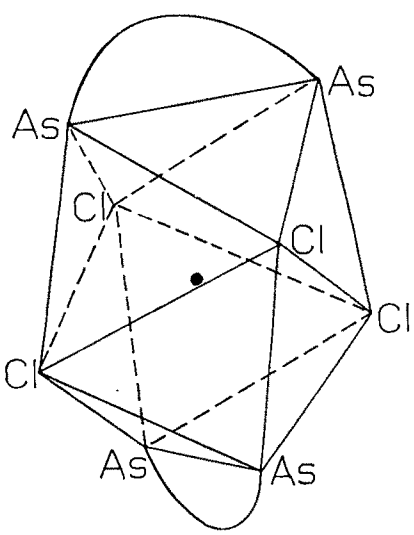


Fig. 1

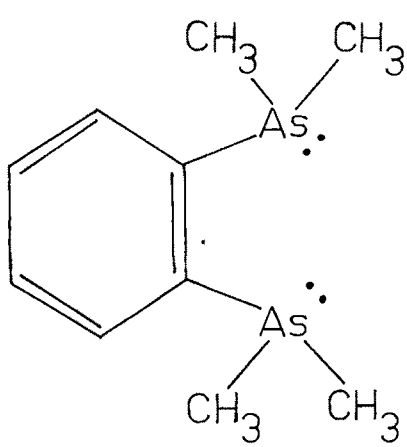


Fig. 2

Titanium[4], zirconium and hafnium tetrabromide[5,6] have since been found to form the iso-structural complexes $MBr_4.2$diarsine. In addition, we have now been able to obtain the corresponding eight-co-ordinate derivatives of niobium tetrachloride and niobium tetrabromide; these are the first magnetically dilute paramagnetic compounds of niobium to be reported.

Some general principles appear to be emerging concerning the formation of these compounds, and it seems timely for us to present a survey of the metals for which eight-co-ordination has been observed and to look for any relationship between the stereochemistry and the nature of the ligand employed.

High crystallographic co-ordination numbers are well known in metals and alloys, where for hexagonal and cubic close packing, there are twelve nearest neighbours and for body-centred cubic close packing, eight (Fig. 3). The co-ordination number is, however, somewhat ill-defined, for in the latter case there is a further set of six next nearest neighbours at a slightly greater distance (15 per cent) away from the metal. Alloys containing an eight-co-ordinated early transition metal include the isomorphous Th_3P_4, Th_3As_4, U_3As_4, U_3Bi_4, U_3Sb_4, and U_3Te_4 (ref. 15) and Th_7S_{12} and Th_7Se_{12} in which half the metal atoms are eight-co-ordinate and half nine-co-ordinate[16].

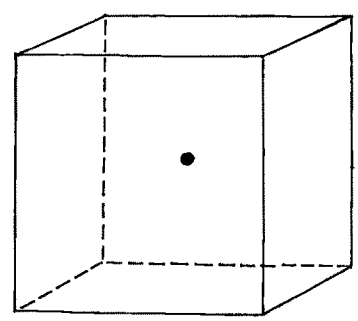


Fig. 3. Body-centred cube

The second general class of compounds exhibiting the crystallographic co-ordination number eight are the ionic crystalline solids. The simplest example is the body-centred cæsium chloride type of compound. The lattice is, of course, three-dimensional without discrete molecules. This structure is found also for CsCN, TlCN, CsSH, TlCl, and for all the rubidium halides at high pressures[18]; it also occurs in the compound ThTe[17]. The other common type of eight-co-ordinate crystalline lattice is that exhibited by fluorite, CaF_2. Those early transition metals having fluorite or distorted fluorite structures include TiH_2, ZrO_2, ZrH_2, HfO_2, $HfH_{1.5-1.75}$, ThO_2, ThH_2, PaO_2, UO_2, UC_2, UN_2 and NpO_2; in addition, the following have a similar structure with eight-co-ordination of both metals: K_2ThF_6, Na_2ThF_6, K_2UF_6, Na_2UF_6 and $CeUO_4$ (refs. 15, 18, 19).

The most extensive class of compounds exhibiting high co-ordination numbers may, broadly speaking, be termed "covalent", and subdivided according as the metal is part of a monomeric unit (Table 1), a finite polymeric unit, or polymerized in one, two or three dimensions (Table 2).

Table 1. Discrete Eight-Co-ordination

Ti	V	Cr	Mn
^{c} $Ti(CN)_8^{4-}$ (13)	^{a} $V(O_2)_4^{3-}$ (6)	^{a} $Cr(O_2)_4^{3-}$ (5)	—
Zr	**Nb**	**Mo**	**Tc**
^{b} $Zr(acac)_4$ (8)	^{a} $Nb(O_2)_4^{3-}$ (7)	^{ad} $Mo(CN)_8^{4-}$ (1)	^{c} TcH_8^{2-} (25)
^{a} $Zr(C_2O_4)_4^{4-}$ (10)		^{c} $Mo(CN)_8^{3-}$ (23)	
^{a} $Zr[N(OAc)_3]_2^{2-}$ (22)		^{c} $Mo(OH)_4(CN)_4^{4-}$ (14)	
		^{c} $Mo(CN)_4(MeCN)_2(H_2O)_2$ (23)	
		^{c} $MoCl_4.4Ph_3AsO$ (24)	
		^{c} MoF_8^{2-} (53) (55)	
		^{c} MoF_8^{2-} (55)	
Hf	**Ta**	**W**	**Re**
^{b} $Hf(acac)_4$ (8)	^{a} $Ta(O_2)_4^{3-}$ (7)	^{a} $W(CN)_8^{4-}$ (1)	^{c} $Re(CN)_8^{2-}$ (12)
^{a} $Hf(C_2O_4)_4^{4-}$ (10)	^{b} TaF_8^{2-} (2)	^{c} $W(CN)_8^{3-}$ (23)	^{c} $Re(CN)_8^{3-}$ (12)
^{a} $Hf[N(OAc)_3]_2^{2-}$ (22)		^{c} $W(CN)_4(OH)_4^{4-}$ (14)	^{c} $Re(CN)_7(NO)^{3-}$ (12)
		^{c} WF_8^{2-} (53) (54)	^{c} ReH_8^{2-} (25)
		^{c} WF_8^{3-} (54)	
Th	**Pa**	**U**	**Np**
^{b} α-$Th(acac)_4$ (9)	—	^{b} $U(acac)_4$ (9)	^{c} $NpO_2(OAc)_2^-$ (19)
^{b} β-$Th(acac)_4$ (8)		^{a} $U(Bz_2CH)_4$ (11)	
^{a} $Th(Bz_2CH)_4$ (11)		^{c} $U(C_2O_4)_4^{4-}$ (21)	
^{c} $Th(C_2O_4)_4^{4-}$ (21)		^{c} $UO_2(OAc)_3^-$ (26)	
		^{c} $UO_2(NO_3)_3^-$ (27)	

a, dodecahedron; *b*, square antiprism; *c*, hexagonal bipyramid; *d*, $Mo(CN)_8^{4-}$ appears to be square antiprism in solution (20); *e*, eight-co-ordination not definitely established; acacH, acetylacetone; Bz_2CH_2, dibenzoylmethane; O_2^{2-}, peroxide ion; $N(OAc)_3^{3-}$, nitrilotriacetate.

Several different arrangements of the ligands about the central metal atom have been reported for eight-co-ordinate compounds. These are: (1) The body-centred cube, O_h, $m3m$, point symmetry of the metal atom, as shown in Fig. 3. This arrangement is not favoured energetically in covalent compounds (the hybridization scheme necessary for this stereochemistry requires the use of higher-energy *f*-orbitals) but occurs in many metals, alloys and ionic compounds, for example, Th_3P_4 and CsCl, where packing considerations appear to be more important than covalent bond formation. (2) The dodecahedron (Fig. 4) with D_{2d}, $\bar{4}2m$ point symmetry of the metal atom, in which the eight vertices form two interpenetrating tetrahedra about the metal atom, one flattened (atoms *B*) and one elongated (atoms *A*) about the fourfold inversion axis. (3) The square antiprism (Fig. 5) with D_{4d}, $82m$ point symmetry of the metal atom is structurally similar to the dodecahedron. (4) The hexagonal bipyramid (Fig. 6) D_{6h}, $6/mmm$ symmetry; (5) The puckered hexagonal bipyramid (Fig. 7) D_{3d}, $\bar{3}m$ symmetry; (4) and (5) are commonly found in actinide chemistry, but not elsewhere. (6) The triangular prism with an additional group outside two of the square faces (Fig. 8), C_{2v}, mm point symmetry of the metal atom. It occurs in the compound Th_7S_{12}, and is also found in

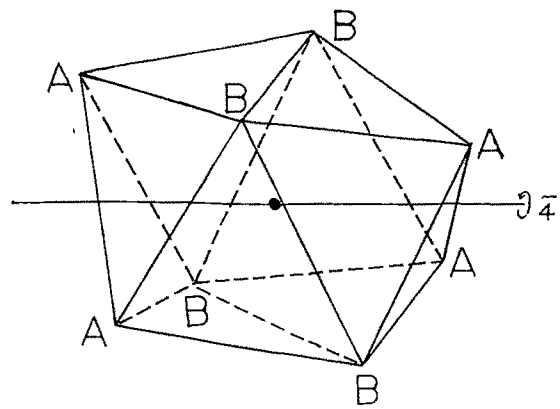


Fig. 4. Dodecahedron

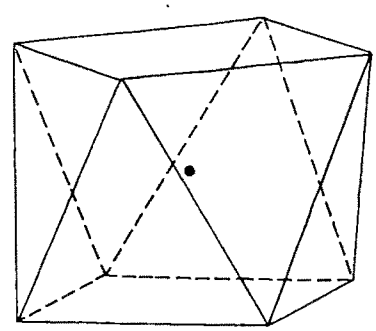


Fig. 5. Square antiprism

compounds having the $PuBr_3$ structure. It could be considered as midway between the dodecahedron and the square antiprism.

It should also be noticed that the puckered hexagonal bipyramid is midway in structure between the hexagonal bipyramid and the cube. Thus, as the puckering angle θ approaches 24°, the structure approaches a cube; in UO_2F_2 for example, it is 14°.

In addition to the foregoing, a number of *cyclo*pentadienyl compounds are known in which the metal can be regarded as being formally eight-co-ordinate if the assumption is made that the π-*cyclo*pentadienyl group (C_5H_5) occupies three co-ordination positions. Examples of this type include $(\pi\text{-}C_5H_5)_2MX_2$, where *M* is Ti, Zr, Hf, V, Mo, W and *X* is halogen, hydrogen, σ-alkyl, σ-aryl or carbon monoxide[46].

Table 2. Polymeric Eight-Co-ordination

Zr	Nb	Mo	Tc
^{b} $Zr_4(OH)_8.16H_2O^{8+}$ (condensed) (28)	^{b} $Nb_6Cl_{12}^{2+}$ (condensed) (37)	^{b} $Mo_6Cl_8^{4+}$ (condensed) (38)	—
^{a} K_2ZrF_6 (chain) (29)	^{b} $Nb_6Br_{12}^{2+}$ (condensed) (37)	^{b} $Mo_6Br_8^{4+}$ (condensed) (38)	
^{b} $Zr(SO_4)_2.4H_2O$ (chain) (30)			
^{b} $Zr(IO_3)_4$ (layer) (31)			
^{b} ZrF_4 (lattice) (32)			
^{a} $ZrSiO_4$ (lattice) (33)			
Hf	**Ta**	**W**	**Re**
^{a} K_2HfF_6 (chain) (29)	^{b} $Ta_6Cl_{12}^{2+}$ (condensed) (37)	—	—
^{b} HfF_4 (lattice) (32)			
Th	**Pa**	**U**	**Np**
^{b} $Th(OH)_2SO_4$ (chain) (34)	^{a} $PaCl_4$ (lattice) (18)	^{b} $U(OH)_2SO_4$ (chain) (39)	^{c} $NpBr_3$ (layer) (42)
^{b} $Th(OH)_2CrO_4H_2O$ (chain) (35)		^{b} $U(SO_4)_2.4H_2O$ (chain) (40)	^{c} NpI_3 (layer) (42)
^{b} ThF_4 (lattice) (32)		^{b} $U_2O_4(OH)_4(SO_4)_4$ (chain) (41)	^{d} NpO_2F_2 (layer) (19)
^{a} $ThCl_4$ (lattice) (36)		^{c} UI_3 (layer) (42)	^{a} $NpCl_4$ (lattice) (18)
^{a} $ThBr_4$ (lattice) (18)		^{c} UO_2CO_3 (layer) (43)	
		^{d} UO_2F_2 (layer) (44)	
		^{d} α-UO_3 (layer) (45)	
		^{d} $CaUO_4$ (layer) (45)	
		^{d} $SrUO_4$ (layer) (45)	
		^{a} UCl_4 (lattice) (36)	

a, dodecahedron; *b*, square antiprism; *c*, planar hexagonal bipyramid; *d*, puckered hexagonal bipyramid; *e*, $PuBr_3$ structure.

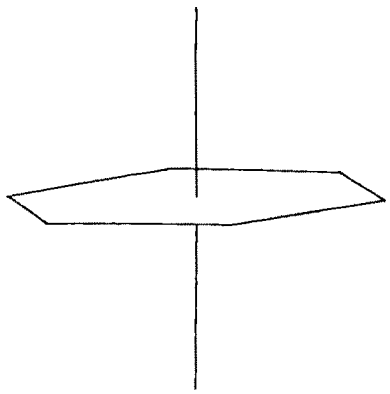


Fig. 6. Hexagonal bipyramid

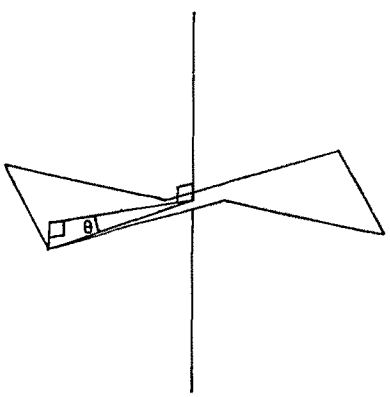


Fig. 7. Puckered hexagonal bipyramid

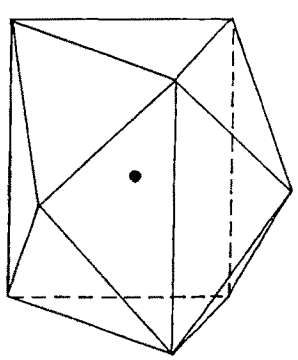


Fig. 8. Triangular prism with 2 extra ligands

In Table 3 are summarized the known complexes of the diarsine with the metal in eight-co-ordination. The niobium complexes are reported here for the first time. Those of the type MX_4,2diarsine (M = metal atom, and X = chlorine or bromine) are all isomorphous and dodecahedral, with the arsenic atoms forming the elongated tetrahedron (atoms A) (Fig. 4) while the halogen atoms form the squashed tetrahedron (atoms B). The niobium complexes of this type were prepared by treating the niobium tetrahalide or pentahalide with excess of the diarsine in sealed tubes at 100° (the pentabromide or tetrachloride) and 200° (the pentachloride). These are green compounds, insoluble in all solvents tried, and paramagnetic with a magnetic moment of about 2 B.M. Satisfactory analytical data have been obtained for these compounds and will be reported in full elsewhere, together with details of their diffuse reflectance spectra and magnetism.

Table 3. EIGHT-CO-ORDINATE DIARSINE COMPLEXES

Group IV	V	VI	VII
[a] $TiCl_4$.2diarsine (3)	[a] VCl_4.2diarsine (3)		
[a] $TiBr_4$.2diarsine (4)			
[a] $ZrCl_4$.2diarsine (3)	[a] $NbCl_4$.2diarsine		[b] $[TcCl_4.2diarsine]^+$ (50)
[a] $ZrBr_4$.2diarsine (51)	[a] $NbBr_4$.2diarsine		
[a] $HfCl_4$.2diarsine (3)	[b] NbI_4.2diarsine		[b] $[ReCl_4.2diarsine]^+$ (49)
[a] $HfBr_4$.2diarsine (51)	[b] WCl_4.diarsine		[b] $[ReBr_4.2diarsine]^+$ (50)

(*a*) Isomorphous dodecahedron; (*b*) Not definitely shown to be eight-co-ordinate, but probably dodecahedral.

For a molecule using d^4sp^3 hybrid bond orbitals, Racah[48] has calculated that the bonds divide into two sets of four (with the representation $2A_1+2B_2+2E$) yielding a dodecahedral stereochemistry. It is interesting to compare the angles predicted for this hybridization scheme, that is, θ_A, θ_B, the angles, respectively, between atoms A, B and the tetragonal axis, with those found experimentally for complexes in which the metal atom is eight-co-ordinated with D_{2d} point symmetry.

	θ_A	θ_B
Calc. for d^4sp^3 hybridization	34·6°	72·8°
$[Zr(C_2O_4)_4]^{4-}$	35·2°	73·5°
$TiCl_4$.2diarsine	36·3°	72·8°
$[ZrF_8]^{3-}$	34·7° and 35·9°	72·1° and 77·2°
$[CrO_8]^{3-}$	43·4°	86·8°
$[Mo(CN)_8]^{4-}$	36·0°	71·8°

Hence, except for the peroxychromate ion, in which the short O–O distance brings about a large distortion from the norm, all known compounds with this stereochemistry form their bonds at angles very close to that predicted for strictly covalent d^4sp^3 hydridization. For the purposes of this article, the peroxy compounds have been regarded as eight-co-ordinate, but the mode of bonding is not known. A distorted tetrahedron of four π-donor peroxy groups also seems possible[56]. For dodecahedral stereochemistry, it is the d_{xy} orbital (B_1 symmetry) which is necessarily excluded from this σ-bonding scheme. (This is the orbital which most effectively avoids the directions of the ligands.) It is thus the most stable orbital, and hence the one in which the single d electron of $[CrO_8]^{3-}$, VCl_4.2diarsine and NbX_4.2diarsine is presumed to lie, leading to magnetic moments close to the spin-only value of 1·73 B.M. In the d^2 complexes $[Mo(CN)_8]^{4-}$, $[W(CN)_8]^{4-}$ and $U(Bz_2CH)_4$, the two d electrons are presumed to pair off in the d_{xy} orbital leading to diamagnetism as observed. Thus the addition of one or two d electrons need not alter the hybridization scheme. Spectral assignments have been made for the octacyanides, but with evident disagreement between different authors[20].

In the square antiprism the representation of the hybrid bonds is $A_1+B_2+E_1+E_2+E_3$ and hence two schemes are possible: either d^4sp^3 hybridization with the d_{z^2} orbital (A_1 symmetry) not included, or d^5p^3 hybridization with the s orbital (A_1 symmetry) left out. For either scheme there is thus a stable orbital available for the accommodation of one or two d electrons, and it is perhaps surprising that the U(IV) compounds are the only known examples of d^1 or d^2 compounds with square antiprismatic stereochemistry.

Calculations by Hoard[22] suggest that there can be little if anything (energetically) to choose between the dodecahedral and antiprismatic structures.

The occurrence of eight-co-ordination in the early part of the transition series is considered to arise from one or more of several causes all acting uniquely in this part of the periodic table in favour of high co-ordination numbers. These are: (*a*) Since the number of non-bonding d electrons is small, there are many vacant d orbitals at least potentially available for covalent bond formation. Where the formation of a complex is dependent on utilizing all nine orbitals, as in simple metal carbonyls or substituted metal carbonyls (five(n-1)d, one ns and three np type), eight-co-ordination is specially favoured for the configurations d^1, for example, V^{IV}, Mo^V, W^V, Re^{VI}, etc., and d^2 (spin-paired), for example, Mo^{IV}, W^{IV}, Tc^V, Re^V. (*b*) The high formal charge on these ions arising from their high

oxidation states, usually +4 or +5, enables the attachment of a large number of ligands to acquire neutrality. (*c*) The relatively smaller overall energy separation between $(n\text{-}1)d$, *ns* and *np* orbitals, for a given oxidation state, at the beginning of the transition series is in marked contrast with that for the corresponding *ns*, *np* and *nd* separation for the metals in the early post transition series. The following figures illustrate this point.

Metal	Ti^{3+}	Zr^{3+}	Hf^{3+}	Si^{3+}
Electron transition	$3d' \rightarrow 4p'$	$4d' \rightarrow 5p'$	$5d' \rightarrow 6p'$	$3s' \rightarrow 3d'$
Promotion energy (eV)	15·9	10·2	8·4	19·8
4th ionization potential	43·3	34·4	33·3	45·3
Sum of first four ionization potentials	91·5	77·5	78·0	103·0

Metal	Ge^{3+}	Sn^{3+}	Pb^{3+}
Electron transition	$4s' \rightarrow 4d'$	$5s' \rightarrow 5d'$	$6s' \rightarrow 6d'$
Promotion energy (eV)	23·7	21·2	22·8
4th ionization potential	45·3	40·8	42·2
Sum of first four ionization potentials	103·5	93·5	96·5

These figures offer a reasonable explanation for the fact that the quadrivalent post transition halides, for example, $SnCl_4$, do not form eight-co-ordinate complexes readily as do the halides of Ti, Zr, and Hf.

It appears that for early transition metals having discrete eight-co-ordination the choice of stereochemistry lies mainly between the dodecahedron and square antiprism. The more polarizable ligands (for example, cyanide, diarsine and peroxide) appear to form the dodecahedral structure, whereas the less polarizable ligands (for example, fluorine and acetylacetone) seem to prefer the square antiprism arrangement. This correlation is purely tentative as it relates only to a very limited number of experimental data.

The ability of the diarsine to form eight-co-ordinate complexes with titanium and vanadium is remarkable, especially as the bidentate ligands $\alpha\alpha'$-dipyridyl, *o*-phenanthroline[52] and *o*-phenylene bisdiethylphosphine[47] have failed to do so. Whether the reason for this lies in the stereochemistry of the ligand, which seems unlikely, or in the nature of the donor arsenic atoms is a matter which cannot be decided at present.

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ENERGETICS OF ION MOLECULE REACTIONS IN ALKANES

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THE energetics of ion molecule reactions are of interest in the radiation chemistry of hydrocarbons since they may provide a guide to those ion molecule reactions which are most likely to occur. Williams[1] has calculated that in low-molecular-weight alkanes the parent ion radical may undergo energetically favourable dimerization and olefin formation reactions even in the liquid phase and concludes that such reactions may also be important in long chain alkanes. Libby[2] has considered the general principles of the chemistry of such ions and postulates dimer formation for both the parent ion radical and the parent carbonium ion in alkanes and polyethylene. However, Barker[3] presents calculations on alkanes which he interprets to mean that dimerization reactions of the carbonium ion are unlikely in molecules of more than three carbons.

This article presents a consistent set of heats of formation for alkane ions and shows that the heats of reaction of various ion molecule reactions reach a limiting value $\leqslant 0{\cdot}5$ eV. This suggests that these reactions are not energetically prohibited in long chain alkanes.

Heats of formation. The heats of formation of alkane ions are given in Tables 1 and 2. The values for the parent ions were obtained from the measured ionization potentials (IP) and the other experimental values were determined from the appearance potential (AP) of the ion as

measured up through the butyl radicals, but at th[illegible] in the series the radical ionization potentials conver[illegible] constant value. This rapid convergence was found [illegible] follows. The alkyl radical ionization potentials can be calculated by the group orbital method[9] as simplified by Franklin[10]. Each carbon atom is assumed to have a pseudo-π-orbital which contains two 'mobile' electrons except for the radical carbon, which has only one. The linear combination of atomic orbitals method is then used to obtain the secular equation where one atomic orbital is associated with each CH_2 group, and the necessary coulomb and exchange integrals are determined empirically. The energy of the highest filled energy level is identified with the ionization potential. Using the ionization potentials for methane, ethane and methyl radical[4] as parameters, the secular equations were solved on an IBM 7090 computer for the normal, iso, secondary and tertiary radicals having up to twelve carbons. It was found that the radical ionization potentials converged to a constant value for each structural type at the butyl radicals. Thus the values measured for the butyl radicals can be used for the higher alkyl radicals. A similar convergence is well known for the ionization potentials for the *n*-alkanes, but it does not occur until *n*-dodecane. Whenever these constant values are reached, the heats of formation of the ions in Tables 1 and 2 vary only with the heats of formation of the associated alkanes and therefore decrease by 5 kcal/mole for each successive carbon atom. The convergence of the ionization potentials means that the reactions discussed below will reach a limiting heat of reaction for a certain chain-length.

Table 1. Heats of Formation of Alkane Ions at 25° C in kcal/mole

n-Alkanes		iso-Alkanes		Protonated Alkanes	
CH_4^+	285			CH_5^+	180*
$C_2H_3^+$	249			$C_2H_7^+$	(175)
$C_3H_3^+$	234			$C_3H_9^+$	(170)
n-$C_4H_{10}^+$	219	iso-$C_4H_{10}^+$	212	$C_4H_{11}^+$	(165)
n-$C_5H_{12}^+$	208	iso-$C_5H_{12}^+$	196	$C_5H_{13}^+$	(160)
n-$C_6H_{14}^+$	202	iso-$C_6H_{14}^+$	188	$C_6H_{15}^+$	(155)
n-$C_7H_{16}^+$	194				
n-$C_8H_{18}^+$	186				
n-$C_9H_{20}^+$	180				
n-$C_{10}H_{22}^+$	175				

* Calculated from the proton affinity of $CH_4 = 7{\cdot}26$ eV (ref. 11). Extrapolated values in brackets; other values are experimental (ref. 4).

Table 2. Heats of Formation of Alkane Carbonium Ions at 25° C in kcal/mole

Ion formula	*n*-Alkyl ion		*sec*-Alkyl ion		iso-Alkyl ion		*t*-Alkyl ion	
	Exptl.	Calc.	Exptl.	Calc.	Exptl.	Calc.	Exptl.	Calc.
CH_3^+	259	261	–	–	–	–	–	–
$C_2H_5^+$	228	226	–	–	–	–	–	–
$C_3H_7^+$	222	220	199	198	–	–	–	–
$C_4H_9^+$	218	214	194	194	205	205	176	176
$C_5H_{11}^+$		209		188		200		171
$C_6H_{13}^+$		204		183		195		166

Experimental values from ref. 5; calculated values, see text.

	1	2	3	4	5	6	7	8	≥9
1	−18	3	3	7	12	13	13	13	13
2		−10	−6	3	6	4	6	9	9
3			−7	0	3	3	6	9	10
4				−3	2	3	6	9	10
5					2	3	6	9	10
6						3	6	9	10
7							6	9	10
8								9	10
≥9									10

If the product ion in reaction (1) isomerizes the heats of reaction are all decreased by about 10 kcal/mole. This statement also applies to those reactions tabulated later:

Condensation with molecular splitting:

$$n - C_nH_{2n+2}^+ + n - C_mH_{2m+2} = n - C_{n+m-p}H_{2(n+m-p)+2}^+ + n - C_pH_{2p+2} \quad (2)$$

Limiting value of $\Delta H_{\text{reaction}(2)} = -3$ kcal/mole for $p = 1$; 0 kcal/mole for $p \geqslant 2$

Olefin formation:

$$n - C_nH_{2n+2}^+ = 1 - C_nH_{2n}^+ + H_2 \quad (3)$$

Limiting value for $\Delta H_{\text{reaction}(3)} = 14$ kcal/mole

$$n - C_nH_{2n+2}^+ = \textit{trans} - 2 - C_nH_{2n} + H_2 \quad (4)$$

Limiting value for $\Delta H_{\text{reaction}(4)} = 3$ kcal/mole

Hydrogen atom transfer:

$$n - C_nH_{2n+2}^+ + n - C_mH_{2m+2} = C_nH_{2n+3}^+ + n - C_mH_{2m+1} \quad (5)$$

Limiting value for $\Delta H_{\text{reaction}(5)} = 5$ kcal/mole.

Parent carbonium ion reactions: dimerization:

$$n - C_nH_{2n+1}^+ + n - C_mH_{2m+2} = n - C_{n+m}H_{2(n+m)+1}^+ + H_2 \quad (6)$$

The various limiting values are given in Table 4.

For iso, secondary, or tertiary product ions in equation (6) the heats of reaction in Table 4 are lowered by 9, 21, and 38 kcal/mole respectively.

Condensation with molecular splitting:

$$n - C_nH_{2n+1}^+ + n - C_mH_{2m+2} = n - C_{n+m-p}H_{2,n+m-p)+1}^+ + n - C_pH_{2p+2} \quad (7)$$

Limiting value for $\Delta H_{\text{reaction}(7)} = -3$ kcal/mole for $p = 1$; 0 kcal/mole for $p \geqslant 2$

here are energetically important in systems of [illegible] radiation chemistry. If the product ion isomeriz[illegible] reactions for any chain length are thermoneutra[illegible] exothermic, and even if no isomerization occurs [illegible] maximum endothermicity of 10 kcal/mole may easily be overcome by the excitation energy of the ion. Stevenson and Schissler[12] have recently considered the average excitation energy of an ion formed in mass spectrometry and show that an average excitation energy of 3 eV (69 kcal/mole) is quite reasonable.

Examples of many of the foregoing reactions have been observed by mass spectrometry[13] for lower molecular

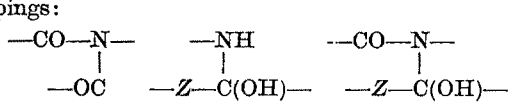
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RECENT ADVANCES IN CYCLOL CHEMISTRY

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ACCORDING to the amide hypothesis, the amino-acid components of peptides are united solely in acid amide groupings. According to the cyclol hypothesis[1,2], which depends essentially on the reactivity of amide groupings in peptides, the amino-acid components are united not only in the acid amide or one-bond peptide groupings, but also in the multiple peptide or cyclol groupings:



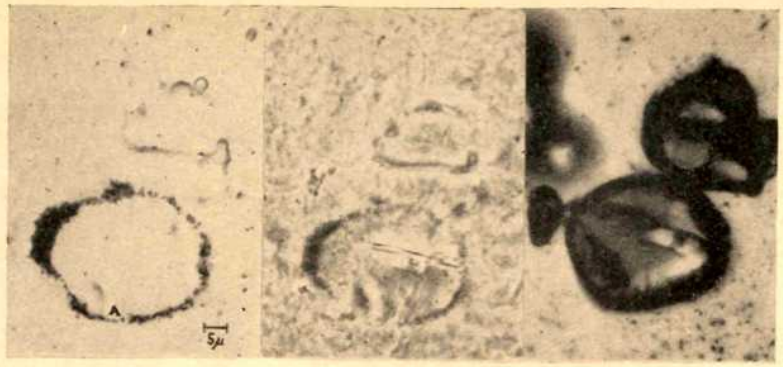
in which Z = NH, O, S. Generalizing the classical hypothesis in this way raises two crucial issues which, in the main, are expressions of the labile character of the cyclol groupings, a matter which has been under investigation ever since the original formulation of the cyclol hypothesis in 1936 [3–5]. (*A*) What are the types of atomic architecture within which cyclol groupings attain conditioned stability and to what structural features of these architectural types may their stabilizing influence be attributed ? (*B*) What types of labile bonds are expected in the cyclol system, and of what nature are the rearrangements, or syntheses and cleavages, of peptides which result from the formation of 'intermediates' containing such bonds ?

In this communication I am considering the cyclol syntheses recently announced. These are examined in the setting provided by the body of organic chemical findings which demonstrate the reactivity of the amide grouping in peptides when it is attacked at its nitrogen atom by an electrophilic reagent or at its carbonyl group by a nucleophilic reagent[6–12]. We conclude that a beginning can now be made in the systematic exploration of both these issues, making use of model molecules of simple kinds.

The researches of Stoll *et al.*[13], which prepared the way for most of the cyclol syntheses, showed that the ergot alkaloids, namely, the compounds (1) in Fig. 1, with *R*— = lysergyl—NH—, contain the three-bond cyclol grouping —(—O—)C(OH)—(—CO—)N— within their tripeptide moiety. Corresponding to the two degenerations by proton migration of this surely very labile grouping, to —OH, —CO—(—CO—)N— or to —O—CO—, —CO—NH—, the alkaloids would degenerate into the N-substituted diketopiperazine shown on the left of (1) or into the lactone peptide which had been adopted as the correct structure until 1951 shown on the right. Both these structures, related to the cyclol structure by proton migration and indicating alternative paths to its synthesis proved to be of significance in a recent discussion of the heat of formation of cyclol groupings (ref. 1, pp. 133–4, 179–81) in which stress was laid on the need to consider the non-bonded energy terms following the precedents set in various simpler cases[14].

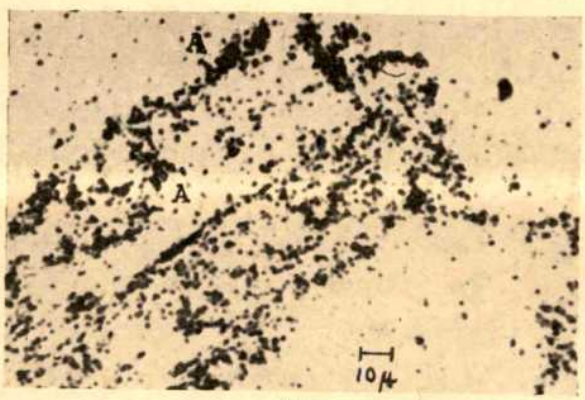
(*a*) In May 1961, Hofmann *et al.*[15] announced the synthesis of the ergotamine tripeptide, namely, the structure (1) with $R = NH_2$, R_1 = Me, $R_2 = CH_2Ph$. This, the first cyclol synthesis to be reported, proceeded by way of an *N*-acyl derivative of [L-phenylalanyl-L-prolyl-]diketopiperazine.

During 1962–63, Shemyakin *et al.*[16], Antonov *et al.*[17,20], Jones *et al.*[18], and Sheppard[19] announced cyclol syntheses which were confirmed by infra-red spectra, including the following: (*b*) Synthesis of 'ergotamine analogue'[16]

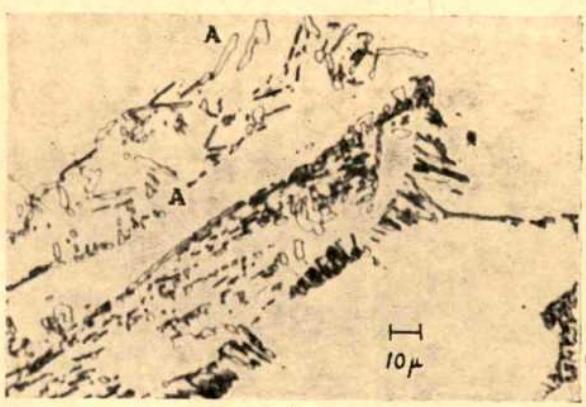


(a) (b) (c)

Fig. 1



(a)



(b)

Fig. 2

particles, resolution is limited to 3–5μ and is much worse for radioisotopes emitting particles of higher energy. A similar resolution can be obtained from very flat specimens using contact autoradiography and very dry conditions, but as resolution depends critically on the closeness of contact between the specimen and the emulsion the resolution achieved in a particular autoradiograph can be uncertain. The technique described in this article uses a film consisting of a single layer of sensitive silver bromide crystals at the surface of a plastic film. The film is softened in an organic solvent and applied to the specimen. As the solvent evaporates the film conforms to the surface and, when hard, forms a replica of the surface topography. After exposure the film is stripped from the specimen and developed. As the plastic is unaffected by water, the final autoradiograph retains a replica of the specimen surface. For use on the most highly reactive specimens a thin (about 0·3μ) supercoat of a second plastic is applied on top of the silver bromide. This is removed before development in a second non-polar solvent which does not affect the backing plastic. A supercoat of this thickness would not protect silver bromide in contact with a reactive specimen in the presence of water, for example, during *in situ* development.

To prepare a film a microscope slide is coated with silver by vacuum evaporation and converted to silver bromide in bromine vapour. The silver bromide grain size varies with the surface density of silver evaporated and with the conditions of bromination. A resolution of 1μ requires grains $< 0{\cdot}3\mu$ in diameter. As the effective sensitivity tends to increase with increasing grain size, conditions were selected which gave grains of approximately 0·3μ diameter. The silver bromide layer is backed with polyvinyl formal ('Formvar') applied from a 5–10 per cent dioxan solution. A backing about 10 microns thick has been found satisfactory. If the backing is too thin the film is difficult to handle; if it is too thick it does not soften satisfactorily and takes too long to harden on the specimen. The supercoat is prepared separately on a second slide. It is a 0·3μ thick layer of polyisobutylene prepared by dipping the slide in a solution of polyisobutylene in toluene and spinning horizontally until dry. The silver bromide/'Formvar' film is stripped from its slide, softened with methyl ethyl ketone, and applied to the supercoat. Controlled water treatment has been found to sensitize the silver bromide. The film can be sensitized by adding 80–90 per cent water to the methyl ethyl ketone. After drying the film is ready for use.

To take an autoradiograph, a portion of film is carefully stripped from the slide, dipped in pure methyl ethyl ketone, applied to the specimen and dried quickly. The exposure is made in a desiccator containing silica gel. After exposure the film is stripped from the specimen, washed in toluene or carbon tetrachloride to remove the supercoat, developed in 'Promicrol', and fixed in hypo.

Faint images can be located by examining the replica dry or mounted in glycol at low magnification. For examination at the highest resolution the autoradiograph is mounted in oil. The replica can then best be examined using phase contrast.

Fig. 1 shows an autoradiograph, replica, and micrograph of zinc sulphide crystals. The surfaces of some of the crystals had been coated with a tritium-containing polymer. The crystals were mounted in 'Araldite' and sectioned. The autoradiograph (*a*) shows that only one of the two crystals appearing in the micrograph (*c*) was tritium-coated. The replica (*b*), viewed using phase contrast, shows both crystals. Fig. 2 shows an autoradiograph (*a*) and micrograph (*b*) of a specimen of steel containing carbon-14. The steel was heat-treated to give a coarse pearlitic structure. As the specimen was a thick one carbon-14 within about 10μ of the surface contributed to the image and this reduced the resolution.

The plastic film technique described gives a resolution of better than 2μ with tritium *A* (Fig. 1) and 3–4μ with carbon-14 *A* (Fig. 2) and sulphur-35. Autoradiographs can be obtained free from chemical artefacts on surfaces as reactive as magnesium. The replica of the surface on the autoradiograph assists its interpretation. A number of autoradiographs can be taken from one specimen and a flat specimen surface is not required.

We thank D. E. Glanville and C. S. Lees for their help in this work.

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SOME UNUSUAL OBSERVATIONS IN A COMPARISON OF LIQUID AND SOLID PHASE AUTOXIDATION

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As a rule autoxidation research in the past has been confined to pure liquids being used as model substances such as methyl linoleate, tetralin, cyclohexene, etc., or composite solids such as lard, butter, rubber and a variety of polymers, but the information available concerning the autoxidation of well-defined pure solids is negligible. In an attempt to simulate polyethylene which, in practice, always has some residual double bonds, purified, peroxide-free docosane ($C_{22}H_{46}$; m.p. 44° C) and docos-1-ene ($C_{22}H_{44}$; m.p. 41° C) were selected as model substances. These hydrocarbons can be investigated at convenient temperatures both in the solid and in the liquid phase when autoxidation is induced by added metal catalysts or by ionizing radiations. Docos-1-ene has not only the additional advantage of readily measurable rates of autoxidation but has also, in common with all alk-1-enes, a single α-methylenic carbon atom with a weakened C . . . H bond. The preferential attack leads to a single product in the early stages of autoxidation thus facilitating a kinetic investigation. First it was established that the order of magnitude of the rates of autoxidation of alk-1-enes, as measured by oxygen uptake and hydroperoxide formation, did not vary with the chain length when the number of carbon atoms was $\geqslant 16$. Hexadec-1-ene (m.p. 4° C) and docos-1-ene were found to be particularly suitable for a comparison of liquid and solid phase autoxidation.

The following results indicate that *ceteris paribus* autoxidation proceeds more rapidly in the solid phase than in the liquid phase. Table 1 shows the results for γ-ray induced autoxidation with liquids, solidified melt and crystalline powders. The differences are best interpreted as due to a sharp reduction in the solid phase of the diffusion-controlled rate of termination by free radical recombination ($RO^{\bullet}_2 + RO^{\bullet}_2 \rightarrow$ products) in comparison with the rate-determining propagation reaction ($RO^{\bullet}_2 + RH \rightarrow ROOH + R^{\bullet}$), where $RO^{\bullet}_2$ reacts with the substrate itself. The latter reaction being largely dependent on the activation energy is expected to proceed at only a slightly reduced rate in the solid phase. The results presented in Table 1 made it apparent, however, that oxygen diffusion was also a significant superimposed factor under these conditions. Hence experiments were carried out with thin films of docos-1-ene on silica tubing and gold foil (Table 2). The reproducible rate of hydroperoxide formation was found to be independent of film thickness in the range of four to thirty microns. This suggests that oxygen diffusion was no longer a significant factor under these conditions. However, this rate, which in the early stages of autoxidation is proportional to dose, was found to be dependent on the nature of the surface on which the docos-1-ene film was deposited. Conceivably some surface-catalysed radical recombination occurs. From a quantitative point of view it is noteworthy that the rate of autoxidation of the solid docos-1-ene film on gold foil is about thirty times that of liquid hexadec-1-ene under the conditions of the experiment.

After finding that the recently discovered *N*-butyl salicylaldimino cobalt (II) complex[1,2] was an efficient autoxidation catalyst, use was made of this compound for a comparison of metal-catalysed liquid and solid phase autoxidation. The experimental findings recorded in Fig. 1 were similar, in principle, to those in γ-ray induced autoxidation. The catalyst was incorporated into the solid docos-1-ene by dissolving it in the molten hydrocarbon and grinding the solidified melt into a fine powder.

Table 1. Hydroperoxide Formation in γ-Ray Induced Autoxidation of Hydrocarbons at 25° C
(Dose rate = 0·21 megarad/h, dose = 4·7 megarad)

Hydrocarbon	P.V. *ROOH* formed (μmol/g)	G(*ROOH*) = P.V./0·96 g megarad
Hexadecane ($C_{16}H_{34}$) (liquid)	7·5	1·7
Hexadec-1-ene ($C_{16}H_{32}$) (liquid)	9·5	2·1
Docosane ($C_{22}H_{46}$) (crystalline powder)	19·2	4·3
Docos-1-ene ($C_{22}H_{44}$) (crystalline powder)	101	22·4
Docos-1-ene ($C_{22}H_{44}$) (crystalline mass of solidified melt)	41	9·1

Table 2. Gamma-ray Induced Autoxidation of Docos-1-ene Films

Film deposit on	Weight of film (mg)	Thickness of film (μ)	Time of irradiation (h)	Dose (megarads)	P.V. (μmoles *ROOH*/g)	G(*ROOH*) = P.V./0·96 g megarad
Gold foil	12·25	14	17·5	3·73	240	64·1
Silica tube	143·5	26			171	45·7
Gold foil	3·71	4·1	23·75	5·06	318	62·6
Silica tube	129·1	21·5			214	42·1

Furthermore, the apparently uncatalysed autoxidation, which according to one of us[3] should be regarded as trace-metal catalysed, exhibited similar features. While the rate of hydroperoxide formation with hexadec-1-ene drops by a factor of approximately ten between 50° C and 25° C, the rate with solid powdered crystalline docos-1-ene stored in air was found at 25° C to be almost identical with that measured at 50° C when the hydrocarbon is liquid (that is, 8 μmol hydroperoxide/g docos-1-ene/month). When peroxide-free docos-1-ene was stored at 7° C for three months the amount of peroxide formation was found to be 5 μmol/g, indicating a temperature dependence in the solid phase similar to that in the liquid phase. All peroxide values were measured iodometrically[4].

This anomalous aspect of autoxidation would explain the lower than expected stability of many solid materials.

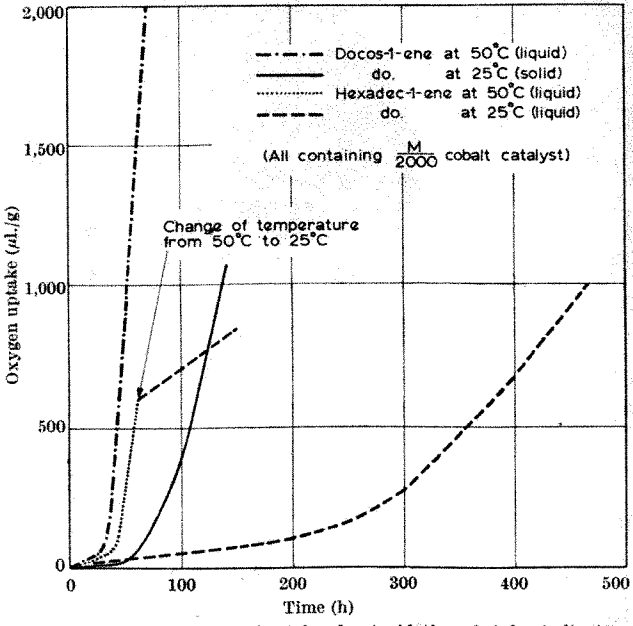


Fig. 1. Comparison of metal catalysed autoxidation of olefins in liquid and solid states

It also sheds some light on earlier observations[5,6] on the effect of ionizing radiations on butter-fat where a maximum rate of hydroperoxide formation was obtained at −20° C. Presumably at higher temperatures butter-fat is behaving more like a liquid and in this case, too, the most likely explanation of the anomalous temperature effect is not to be found in a changed rate of hydroperoxide decomposition but in a sharply reduced rate of $RO'_2 + RO'_2$ termination in the solid phase.

Work is also proceeding on the effect of crystal size and structure on the rate of autoxidation and it is hoped to publish a more detailed account elsewhere.

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A COMPARISON OF THE DISACCHARIDES OBTAINED FROM HUMAN BLOOD-GROUP A, B, H AND Le^a SUBSTANCES BY PARTIAL ACID HYDROLYSIS

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ATTEMPTS to correlate the chemical fine structures of the A, B, H and Le^a human blood-group substances with their serological specificities have been undertaken in a number of laboratories[1,2] and recently several di- and tri-saccharides have been obtained from these materials by partial acid hydrolysis[3–6]. The results of these investigations, together with the experimental findings now described, establish that four disaccharides are common to the partial acid hydrolysates of all four substances. It is also established that the serologically A-active disaccharide, 3-*O*-(*N*-acetyl-α-D-galactosaminopyranosyl)-D-galactose, already identified as a hydrolysis product of A substance[3], is not present in the acid hydrolysis products of B, H or Le^a substances, and that the B-active disaccharide, 3-*O*-α-D-galactopyranosyl-D-galactose, obtained earlier from B substance[4], is absent from the hydrolysis products of A, H and Le^a substances.

The specific substances examined were obtained from human ovarian cyst fluids[2,7], and appeared to be essentially homogeneous in the analytical ultracentrifuge and on free-boundary electrophoresis. The four substances (A, 4·5 g; B, 3·6 g; H, 2·7 g; and Le^a, 2·7 g) were hydrolysed separately with polystyrenesulphonic acid, as described earlier[8], and the yield of mono- and oligo-saccharides represented, in each experiment, about 80 per cent of the weight of the starting material, equivalent to about 95 per cent of the total carbohydrate.

The four hydrolysates were fractionated separately on columns (about 3 × 20 cm) of charcoal–Celite (1 : 1 w/w) by successive elution with water and 1·5, 2·5 and 5 per cent (v/v) aqueous ethanol respectively. When necessary, the materials obtained were further fractionated by preparative paper chromatography, or on small charcoal–Celite columns.

Each hydrolysate contained the same four monosaccharides, L-fucose, D-galactose, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine. Four disaccharides were also common to all four hydrolysates, and were identified as (1) 3-*O*-β-D-galactopyranosyl-*N*-acetyl-D-galactosamine, (2) 3-*O*-(*N*-acetyl-β-D-glucosaminopyranosyl)-D-galactose, (3) 3-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine and (4) 4-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine, respectively. In addition, the hydrolysates of A and B substances contained, respectively, the A-active disaccharide[3] and the B-active disaccharide[4] described earlier. The hydrolysates of H and Le^a substances did not appear to contain appreciable amounts of disaccharides other than the four (1–4) already named.

Disaccharide 1 was obtained crystalline from each blood-group substance, and was identified by comparison with the preparation of this disaccharide already isolated from A substance[5]. Disaccharides 3 and 4 were also obtained crystalline from each hydrolysate, and were identified by comparison with authentic specimens kindly provided by Prof. R. Kuhn and Prof. P. György, respectively.

A disaccharide, tentatively given the structure of disaccharide 2, was isolated from A substance by Côté and Morgan[3]. This structure has now been confirmed for a disaccharide which has been isolated from all four blood-group substances. Disaccharide 2 was obtained as a colourless syrup, having $[\alpha]_D^{22} = +33{\cdot}5°$ in water. Chromatography of acid hydrolysates of the disaccharide showed that glucosamine and galactose were present, and analysis by the Elson–Morgan[9] and phenol-sulphuric acid[10] methods indicated that these sugars occurred in approximately equimolar proportions. After reduction of the disaccharide with sodium borohydride[11], the galactose content was negligible, whereas the hexosamine content was essentially unchanged.

Disaccharide 2 (12 mg) was oxidized in 0·01 M sodium metaperiodate (50 ml.) at 22°. In the initial rapid phase of oxidation, which was complete in about 5 h, 2·2 moles/mole of periodate were consumed, and 0·4 mole/mole each of formic acid and formaldehyde were liberated. Oxidation under the same conditions, after reduction with borohydride[12], resulted in immediate liberation of 2·0 moles/mole of formaldehyde, followed by rapid over-oxidation.

Oxidation of disaccharide 2 to the corresponding aldobionic acid with bromine water, followed by mild oxidation with sodium metaperiodate under the conditions recommended by Clancy and Whelan[13], failed to liberate glyoxylic acid. The same behaviour was observed with authentic (1 → 3)-linked disaccharides of similar type, whereas authentic (1→4)-linked disaccharides readily afforded glyoxylic acid under the same conditions. That disaccharide 2 was (1→3)-linked was also suggested by the similarity of its rate of degradation by alkali, determined colorimetrically[14], to that of known (1→3)-linked disaccharides, and by its capacity to form a methyl glycoside under conditions leading to selective formation of methyl glycofuranosides (that is, in cold, 1 per cent w/w methanolic hydrogen chloride). On the basis of these observations, disaccharide 2 is considered to be 3-*O*-(*N*-acetyl-β-D-glucosaminopyranosyl)-D-galactose, a disaccharide first isolated by Kuhn and Baer[15] from human milk.

The relative amounts of the disaccharides (1–4) in the hydrolysates of the four group substances differed appreciably. The significance of this observation is uncertain, since it is not yet known whether the hydrolysis products obtained from different mucopolysaccharide preparations,

having the same specificity, would show a similar variation in composition.

Very small amounts of disaccharides, other than those just described, were present in the four hydrolysates, but it is doubtful at present whether significance should be attached to them, since these small amounts could have arisen as artefacts produced by the acid-reversion of monosaccharides. This possibility has already been suggested[16] for the disaccharide, 6-*O*-α-L-fucosyl-*N*-acetyl-D-glucosamine, obtained from the acid hydrolysis products of A substance[3].

The failure to isolate significant amounts of fucose-containing disaccharides in the present work reflects the extreme sensitivity to acid of the fucosidic linkages in the blood-group substances. The L-fucose residues, which are almost certainly attached as terminal, non-reducing branches to the main carbohydrate chains in the specific substances are, in our experience, split off rapidly and almost completely in the early stages of acid hydrolysis, before the galactosidic and hexosaminidic linkages are appreciably cleaved.

The disaccharides (1–4) had no detectable A, B or Le^a activity. Disaccharide 4 has already been shown to possess *Pneumococcus* type XIV activity[17], and disaccharide 2 inhibits the agglutination of O cells by *Cytisus sessilifolius* and *Laburnum alpinum* seed agglutinins[18], but does not inhibit[19] other anti-H reagents. However, since the results of enzymatic decomposition experiments[20] and enzymatic[21] and serological[18,22] inhibition studies indicate that L-fucose residues play an important part in H and Le^a specificity, the fucose-free fragments obtained by acid hydrolysis cannot be expected to provide evidence in support of this conclusion. An alternative method of degrading specific substances that gives good yields of fucose-containing fragments has now been developed, and the products will be described later.

In the present work, the demonstration that four disaccharides are common to the partial acid hydrolysates of A, B, H and Le^a specific substances strongly supports a view expressed earlier[2], that there is a close similarity in the general structural pattern of these serologically active mucopolysaccharides. The present findings are also consistent with a scheme for the biosynthesis of the blood-group substances proposed by Watkins[23] and later extended[24], in which it is envisaged that a precursor substance is common to all individuals, and that the specific blood-group characters arise from the addition, by genetically-determined enzymes, of single, non-reducing terminal sugar units to the carbohydrate chains in the precursor substance.

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A TUMOUR-INHIBITORY FACTOR IN THE POST-COITAL UTERINE FLUID

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IN previous investigations it has been shown that tumour cells transplanted into the uterine horn of rats grow well at that site irrespective of the phase of the cycle. However, I have already shown that the tumour cells fail to grow in a large percentage of animals if inoculated within 18 h after mating[1,2].

This article is an attempt to ascertain the factors responsible for this inhibition.

White Wistar rats weighing 150 g were obtained commercially and fed on 'Diet 41' and water *ad lib.* The tumour used was one induced by benzpyrene in the uterus. Suspensions were prepared by mixing one volume of tumour with 1·5 volumes of saline. 0·2 ml. of this suspension was injected into the uterine horn and a ligature placed above the injection site to prevent escape of the inoculum. Unfortunately, escape of some tumour cells on withdrawal of the needle could not always be prevented, with the result that extra-uterine growth sometimes developed.

To effect matings, female rats were placed in cages with males at 5 p.m. and removed the next day at 9 a.m. The following criteria were utilized to ascertain whether coitus had occurred: (1) the presence of a vaginal plug; (2) the presence of spermatozoa in uterus or vagina; (3) the presence of a hæmorrhagic discharge at the vulva (sign of evacuation of uterine contents); (4) the presence of embryos.

Several hours after mating there is an accumulation of purplish fluid in the uterus, varying from 0·1 to 1·0 ml. in amount. For the *in vitro* experiments this fluid was withdrawn and mixed with varying amounts of tumour suspension.

Spermatozoa were obtained by mincing testes and epididymes of a male rat in 1·0–5·0 ml. saline.

The viability of the treated tumour cells was assessed by intraperitoneal or subcutaneous injections into weanling rats as in Exps. 2 and 3.

Exp. 1. Tumour suspensions were inoculated into the uterine horns of young virgin rats (controls) and into the uterine horns of animals mated within the preceding 16 h. Preliminary investigations of the virgin controls had shown microscopic evidence of implantation of tumour

cells as early as 2·5 h after intra-uterine inoculation. Moreover, uterine lavage 3 h after tumour injection failed to inhibit tumour growth.

It seems likely that all mated rats have an accumulation of uterine fluid at some stage. Probably this is maximal 6–10 h after mating, and this fluid remains in the uterine horn for a short period, after which it is evacuated. The best result in respect of tumour inhibition is obtained if the tumour is inoculated into the horn during the period when it is full of fluid, as evidenced by the results of Table 1. The results with the remaining 45 rats, in which no fluid or little fluid was present at inoculation, are given in Table 1*A*.

Table 1. Intra-uterine Inoculation of Tumour Suspensions into Mated Rats with Copious Post-coital Fluid, and into Virgin Females

Experimental		Controls	
Mated females with copious post-coital fluid		Virgins	
T+	*T*−	*T*+	*T*−
2* (8%)	23 (92%)	38 (88%)	5 (12%)

* Tumours at site of inoculation only.
T+, tumour growth; *T*−, tumour inhibition, that is, no tumour growth.

Table 1*A*. Intra-uterine Inoculation of Tumour Suspensions into Mated Rats having Little or No Intra-uterine Post-coital Fluid

Condition of uterus				Other evidence of mating					
Little post-coital fluid		Fluid evacuated spontaneously or artificially		Mated 20 min previously		Plug but no fluid		No evidence of mating until embryos found 10 days later	
T+	*T*−	*T*+	*T*−	*T*+	*T*−	*T*+	*T*−	*T*+	*T*−
5	10	9	5	3	1	2	4	6	0

From a comparison of the Tables 1 and 1*A* it is clear that the presence of fluid is an important factor in inhibiting tumour growth.

Another factor affecting the inhibition was the length of time between mating and inoculation. In animals known to be mated within 20 min prior to tumour inoculation there was no apparent inhibition, whereas in those in which the interval was longer, inhibition was observed. It thus appears that tumour inhibition will only occur if the tumour is implanted more than 20 min after mating. Whether this inhibition is due to an alteration in the receptivity of the endometrium or to an oncolytic property of the post-coital fluids was tested by exposing tumour cells to uterine environments of various types.

Exp. 2. In this experiment the uterine contents of animals, treated with tumour as in Exp. 1, were evacuated and re-inoculated intraperitoneally or subcutaneously into weanling rats (Table 2) thus producing tumours at the site of inoculation. The tumours of the control recipients (that is, those receiving re-inoculations from virgin females) were larger than those of the experimental group. A minimal period of exposure of the tumour cells to the uterine environment was required before the cells lost their viability and transplantability.

Since the free tumour cells appeared to be inactivated by the post-coital uterine fluid *in vivo*, the possibility of an *in vitro* inactivation was then investigated.

Table 2. Aspiration of Uterine Contents from the Animals as in Tables 1 and 1*A* 6 h after Inoculation. Re-inoculation into Weanling Rats

Contents of donor uterus (mated rats)								
Copious fluid + tumour			Little or no fluid + tumour			Virgin + tumour		
D	*T*+	*T*−	*D*	*T*+	*T*−	*D*	*T*+	*T*−
10	1	25	3	15	16	2	25	4

D, died.

Exp. 3. In a series of preliminary experiments it has been established that tumour cells retain their viability and transplantability for up to 18 h *in vivo* and up to 24 h *in vitro*. Tumour suspensions were mixed with varying concentrations of post-coital fluid for varying periods of time (2–6 h). The results for 2- and 6-h exposures are shown in Table 3, which suggests that the post-coital uterine fluid retains its anti-tumour activity *in vitro*—but that it must remain in contact with the tumour cells for about 6 h.

Table 3. Exposure of Tumour to Post-coital Fluid *in vitro* with Subsequent Inoculation into Weanling Rats

Exposure (h)	Proportion of fluid to tumour						Control		
	2/1			1/1 or 1/2			Various prop. saline/tumour		
	D	*T*+	*T*−	*D*	*T*+	*T*−	*D*	*T*+	*T*−
2	0	3	1	0	1	0			
6	3	5*	33	0	5	0	0	33	2

* Tumours tiny.

Prof. G. A. H. Buttle of the School of Pharmacy, London, kindly agreed to carry out further *in vitro* tests, with the Walker carcinoma, the Crocker mouse sarcoma and the human *HS* 1 tumour, using the fluid from my mated rats and has confirmed my findings with these other tumours.

In Exps. 2 and 3, toxic effects were observed in some of the recipient weanling rats; such toxic effects were abscess formation and peritonitis. This toxicity proved to be due to an overwhelming infection by *Pasteurella* and *Streptococci*—both sensitive to penicillin. Intraperitoneal inoculation of uterine aspirates from virgin rats, or of tumour suspensions alone, or of epididymal sperm, did not produce any morbidity.

Exp. 4. When uterine fluid was injected directly into the tumour bed of animals with established tumours, no inhibition was observed: on the contrary, growth appeared to be enhanced.

The post-coital uterine fluid, which has an inhibitory effect on unattached tumour cells, consists of blood cells, capacitated sperm, œstrus fluid, seminal plasma and possibly also of substances secreted in reponse to insemination.

Exps. 5–8 were designed to identify the active factor.

Exp. 5. To test the effects of distension on the uterus, tumour suspensions (0·15, 0·1, 0·05 ml.) were injected into the uterine horns, which were then distended to capacity with saline. 6 h later the uterine contents from one horn were aspirated and re-inoculated into weanlings. Since the tumours grew well in both donors and recipients it may be concluded that their growth was unaffected by uterine distension or dilution of tumour suspension.

Exp. 6. Spermatozoa obtained from excised gonads were incubated with tumour suspension in proportion 2 : 1, and afterwards injected into weanling rats. In 11/12 recipients the tumour grew at the normal rate.

Exp. 7. Tumour suspensions were transplanted into uteri of animals in œstrus and the uterine contents evacuated 5–6 h later and re-inoculated into weanling rats. Œstrus fluid was also collected for *in vitro* assays. As shown in Table 4, the œstrus fluid appears to be devoid of tumour-inhibiting properties.

Table 4. Growth of Tumours Exposed to Œstrus Fluid

(1) Tumour inoculated into uteri of œstrus rats		Uterine contents of (1) inoculated into weanlings after 6 h		Tumour and œstrus fluid incubated *in vitro* 6 h inoculated into weanlings	
T+	*T*−	*T*+	*T*−	*T*+	*T*−
7	3	3	1	6	3

Exp. 8. Rats mated with vasectomized males were treated as in the previous experiment. The results suggest that post-coital fluid devoid of spermatozoa, or seminal plasma, had no antitumour activity.

Thus tumour inhibition could not be demonstrated with testicular or epididymal sperm, with œstrus fluid or with fluid obtained from uteri after mating with vasectomized males. It would appear therefore that tumour inhibition is associated with the presence of capacitated sperm.

The experiments described here suggest that the post-coital uterine fluid of the rat contains a factor—probably associated with capacitated sperm—which inhibits the viability and transplantability of free, unattached tumour cells *in vivo* and *in vitro*.

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PRODUCTION OF ERYTHROPOIETIN BY ANOXIC PERFUSION OF THE ISOLATED KIDNEY OF A DOG

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SINCE the publication of the communication by Jacobson *et al.*[1] many authors have directed their attention to the kidney as the site of the production of erythropoietin. Mirand *et al.*[2], who demonstrated the production in binephrectomized rats, consider the kidney an organ essential for complete erythropoietic response. Reisman *et al.*[3] were able to demonstrate erythropoietin on ureter ligated rats. But they obtained negative results with bilaterally nephrectomized or mercury–bichloride poisoned (kidney tubular necrosis) rats. Richet *et al.*[4], examining 21 patients with acute renal insufficiency, found no erythroblasts in the bone marrow at about the fifth day of illness.

The following experiments represent further attempts to examine the role of the kidney as the site of production of erythropoietin.

Mongrel dogs were anæsthetized with nembutal (30 mg/kg of body-weight) and heparinized (8 mg/kg).

900 ml. of blood were drawn from the jugular vein or the carotid artery. About 2/3 was filtered through gauze and used in the perfusion experiment; 1/3 (unfiltered) served as control.

After the bleeding a mid-line abdominal incision was made. The ureter of one kidney was cannulated and the kidney was removed. The organ was immediately transferred to a plastic container in a water bath controlled at 37° ± 1° C. The artery was cannulated by connexion with a Shuster–Dale pump and the apparatus previously described[5]. All tubes in contact with the blood had been siliconized. Perfusion was begun at once. The renal vein was allowed to drain directly into the plastic reservoir without cannulation, and the level of the blood was regulated to cover the organ. Perfusion pressure was 95–110 mm mercury. After perfusion without any oxygenation for 3 h, the blood was centrifuged and the plasma collected.

In another series of experiments, using the same technique, the kidneys were perfused with 10 per cent erythrocytes in plasma, prepared from the blood of the same animal. Full oxygenation (pO_2 350 mm mercury) with a gaseous mixture of 50 per cent oxygen, 5 per cent carbon dioxide and 45 per cent nitrogen was maintained throughout the perfusion.

The erythropoietic activity of the plasma was assayed either in the whole plasma or in a plasma extract prepared according to the technique of Borsook *et al.*[6] and further precipitation with 80 per cent ethanol described elsewhere[7].

Fasted (water *ad lib.*) male albino rats grown in our animal annex were used (strain from the Instituto de Biología y Medicina Experimental, Buenos Aires). When whole plasma was injected, only a single subcutaneous dose of 4 ml. was given, in order to avoid hæmolytic reactions to heterologous proteins. In the case of boiled plasma extract two abdominal injections were made on consecutive days.

The erythropoiesis was estimated by the 3-h iron-59 assay of Hodgson *et al.*[8,9] using 0·1–0·3 μc. injected into the saphena under light ether anæsthesia, 48 h after the whole plasma or 24 h after the second boiled plasma injection. 3 h after the injection of iron-59, 5 ml. of blood were removed through the abdominal aorta with a heparinized syringe.

Measurements of iron-59, plasma volume, blood volume, plasma iron and Biuret determination were made as usual[7].

Table 1. Anoxic Perfusion of Isolated Dog Kidney

Exp. No.	Material injected	No. of rats	Wt. of rats (g)	Percentage ^{59}Fe 3 h after injection			K (^{59}Fe) per cent (h^{-1})	Plasma iron (μg/ml.)	Total iron turnover (μg/h)	Starvation (h)
				Total plasma	Total erythrocytes	Liver				
68	4 ml. whole plasma after perfusion	5	181	8·8	20·1 $P < 0·02$	6·8	82	0·9	6·4	72
	Control 4 ml. whole plasma (non-perfused)	6	186	14·7	9·3	7·4	66	1·0	5·7	
74	4 ml. whole plasma after perfusion	6	211	7·2	10·6 $P < 0·01$	4·8	88	—	—	96
	Control 4 ml. whole plasma (non-perfused)	6	215	14·1	5·5	6·7	67	—	—	
B 110	5 ml. whole plasma after perfusion	5	172	16·9	21·3 $P < 0·05$	6·4	61	—	—	68
	Boiled and alcohol precipitated plasma extract 12·75 mg protein total dose	5	175	12·3	30·1 $P < 0·02$	6·9	74	0·9	4·4	
	Control Saline	5	174	43·8	8·1	11·7	28	2·3	4·4	

Table 2. PERFUSION OF ISOLATED DOG KIDNEY WITH 10 PER CENT ERYTHROCYTES IN FULLY OXYGENATED PLASMA

Exp. No.	Material injected	No. of rats	Wt. of rats (g)	Percentage ^{59}Fe 3 h after injection			K per cent (h)	Plasma iron (μg/ml.)	Turnover (μg/h)	Starvation (h)
				Total plasma	Total erythrocytes	Liver				
79	4 ml. renal perfusion plasma (3 h). Anæmic blood oxygenated with gas mixture 50 per cent oxygen	4	230	23·2	3·5	8·2	48	1·9	7·8	110
	Same as above. Another dog	4	224	18·7	5·2	6·5	58	1·8	7·8	
	Same as above. Another dog 4 h of perfusion	5	222	17·5	5·0	7·8	59	1·5	6·9	
	Control 4 ml. plasma before perfusion	4	224	19·6	3·2	6·2	54	1·9	7·5	

The iron-59 turnover constant (K; per cent/h) and the total iron turnover (μg/h) were calculated[8].

The mean uptake of iron-59 by erythrocytes was submitted to statistical analysis using the Fisher t test.

In the three experiments, both the whole plasma and the boiled extract from isolated perfused kidneys without any oxygenation had a significant stimulating erythropoietic effect compared with the same plasma without perfusion (Table 1).

The positive samples show higher erythrocyte iron-59 uptake (statistically significant), lower residual plasma and liver iron-59, as well as a higher iron-59 turnover constant, K. All these results indicate an erythropoietic stimulation of these groups when compared with the controls. The discrepancy between these results and the total plasma iron turnover is in agreement with the findings of Hodgson *et al.*[10], who showed that this parameter is an unsatisfactory index if the plasma iron is less than 2 μg/ml.

The positive results obtained with hypoxic perfusion are significant since an aliquot of the same plasma (non-perfused) was used for the controls. The erythropoietic stimulation observed is evidently due to a specific effect. The amount of protein injected was the same in the control groups as in the active ones. Furthermore, experiment 110 shows a high erythrocyte iron-59 uptake with a small amount of protein injected (12·8 mg total dose). The erythropoietic effect cannot be attributed to a hæmolytic action of the extract or of the active plasma because the hæmatocrits in the active (46·6 per cent) and in the control (47·9 per cent) groups were very similar.

The active substance seems to be 'erythropoietin': it is heat-stable at pH 5·5 (ref. 6) and is precipitated with 80 per cent ethanol[11] at pH 4·5.

These results agree with those of Kuratowska *et al.*[12] although we used a different animal and also a different bioassay.

The experiments described here provide direct evidence of the production of erythropoietin by the hypoxic kidney *in vitro*. Nevertheless, we cannot conclude that this is a normal mechanism in the whole animal. We cannot dismiss the possibility that autolysis of the renal tissue induced by the strong hypoxia liberates the active substance.

When we applied the same technique to the perfusion of isolated kidneys with 10 per cent erythrocytes in plasma prepared from the blood of the same animal but fully oxygenated (pO_2 350 mm mercury) the results were quite negative (Table 2).

These findings are compatible with the homœostatic mechanism theory of Jacobson and Goldwasser[13], which explains the production of erythropoietin as a function of the balance between oxygen supply and oxygen demand of the tissues rather than of either factor alone.

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SURVIVAL OF MOUSE ERYTHROCYTES IN HISTOINCOMPATIBLE RECIPIENTS

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THE strong histocompatibility-2 (H-2) locus in mice creates an efficient barrier against successful transplantation of normal tissues of various histological origin. The cellular H-2 isoantigens initiating the immunological rejection are at the same time blood group antigens and can be demonstrated on erythrocytes by agglutination hæmolysis, absorption and complement fixation[1–4]. The antigens on red blood cells and somatic tissue cells are determined by the same genes and have the same immunological specificity (for review see ref. 5). However, it has been demonstrated[6] that erythrocytes purified from contaminating white blood cells were unable to induce transplantation immunity against skin grafts. It was suggested, therefore, that red blood cells possessed a different kind of isoantigenic receptors, which could not stimulate the cell-bound transplantation immunity[6]. Contradictory results were reported, however, indicating that erythrocytes could efficiently immunize against tumour homografts[7].

The investigation recorded here represents an attempt to examine the transplantation behaviour of erythrocytes by following the elimination cells labelled with chromium-

51 transfused into compatible and H-2 incompatible recipients.

Fresh strain *A* red cells were used throughout as donor material. The cells were washed once in isotonic citrate solution (0·1 M) and twice in balanced salt solution, and thereafter re-suspended in an equal volume of balanced salt solution. To this was added 200–500 μc. chromium-51 per ml. of packed red cells in the form of $^{51}Na_2CrO_4$ and the suspension was incubated for 30 min at 37° C. The amount of chromium added never exceeded 10 μg per ml. packed red cells. After incubation the cells were washed three times in balanced salt solution. 0·2 ml. of a 10–30 per cent v/v suspension was injected intravenously into compatible strain *A* mice or into hybrids between *A* and other strains and into incompatible *A.CA* or *A.BY* recipients. Each recipient received between 2 and 20 μc. chromium-51 in different experiments.

The fate of the injected cells was followed by bleeding the recipients at 3–6 days' intervals. 0·05 ml. whole blood was taken and suspended in 3 ml. of water. Radioactive determinations were carried out in a well-type scintillation detector (Tracerlab). At the beginning of each experiment the samples had count rates 70–200 times higher than the background. All count rates were corrected for background and radioactive decay.

The elimination curves of strain *A* erythrocytes transfused into compatible recipients were linear when the count rates were plotted on a logarithmic scale. The survival times (arbitrarily taken as the day when 5 per cent of the original activity was still detectable) varied between 20 and 30 days (mean 26·3 days) in different individuals (Table 1). The same results were obtained after transfusion of *A* cells into *A.CA* or *A.BY* recipients: the elimination curves were linear without any evidence of immune elimination, and the survival times varied between 20 and 30 days (mean 24·6 days) (Table 1). There was no significant difference ($P > 2$) between the survival in compatible and incompatible recipients. The variation of survival times within one experiment was considerably smaller than between different experiments.

The persistence of chromium-51 in the circulation of the incompatible recipients was not due to transfer of chromium-51 to host erythrocytes. It could be demonstrated that all chromium-51 labelled cells could be lysed *in vitro* in the presence of complement with humoral isoantibodies directed specifically against the transfused *A* cells (*A.CA* anti-*A* serum). The lysate contained all the radioactive activity present in the total samples. Furthermore, injection of chromium-51 labelled cells which had been hæmolysed by the addition of water did not result in labelling of host cells.

Table 1. Survival Times* of Strain *A* Erythrocytes Labelled with Chromium-51 in Various Recipients

Recipients	No. of mice	Pretreated with:	50% survival times of transfused cells (days)	5% survival times of transfused cells (days)
A hybrids	14	—	5,5,6,6,6,7,7,7,7,7,8,9,9, Mean = 6·8	20,21,25,25,26,27,27,28,28,29,30,30, Mean = 26·3†
A.CA and *A.BY*	15	—	5,5,6,6,6,6,7,7,7,7,7,7,8,9, Mean = 6·6	20,21,22,22,23,23,24,25,25,26,27,27,28,29,30, Mean = 24·8†
A.CA and *A.BY*	24	A.*CA* or *A.BY* anti-*A* serum	4,5,6,6,6,6,7,7,7,7,7,8,8,8,8,8,8,8,8,8,9,9,9,9, Mean = 7·3	18,20,20,21,21,21,21,22,22,24,25,25,25,25,26,26,26,27,27,28,28,28,29,29, Mean = 24·2†

* The activity present at the first bleeding after transfusion (day 0 or 1) was taken as 100 per cent survival.

† There are no statistically significant differences ($P > 2$) between the mean survival times.

The absence of immune elimination in foreign recipients was not a characteristic for the intravenous route of injection. It was found that chromium-51 labelled strain *A* erythrocytes injected intraperitoneally into compatible and incompatible recipients were eliminated from the blood in a similar way and without evidence of immune elimination.

Incompatible *A.CA* recipients which received *A* cells developed humoral isoantibodies demonstrable as hæmagglutinins and hæmolysins. Detectable titres were observed 6 days after transfusion and reached a maximum after about 11 days. Antibodies were still detectable after 20 days. The antibody response was not strong, however, compared with that found after transfer of lymphoid cells, and the maximum titres varied between 1/32 and 1/256. Since no attempt was made to purify the red cells from contaminating white blood cells it cannot be decided whether the antibodies were actually induced by the erythrocytes.

Since erythrocytes can be agglutinated and hæmolysed *in vitro* by humoral antibodies it is noteworthy that no immune elimination occurred in spite of the fact that antibodies and target cells were circulating in the same hosts. A study was made, therefore, on the effect of humoral isoantibodies on the elimination of strain *A* red cells in *A.CA* recipients. Between 0·05 and 0·4 ml. *A.CA* anti-*A* serum was injected intraperitoneally into groups of *A.CA* hosts 30 min prior to the intravenous transfusion of red cells. It was found that antibodies could diminish the amount of circulating red blood cells from 10·0 to 88·8 per cent of the originally injected dose (Table 2). This elimination occurred very rapidly and was detected after only 1 h. However, antibodies had no effect on the subsequent elimination of the residual cells and elimination curves were strictly parallel in untreated and antibody treated groups (Table 1). Since the amount of antibody injected was sufficiently large to agglutinate and hæmolyse all the transfused red cells *in vitro*, it seemed less likely that the failure to accomplish complete elimination was caused by inadequate quantities of antibody. This was further emphasized by experiments in which the effect of various antibody doses were compared. In several experiments it was found that 8-fold differences in the antibody dose did not detectably change the extent of the initial elimination. The variation between different mice in the same experiment was sometimes large.

It seemed possible that insufficient complement-levels were responsible for the limited effect of antibodies *in vivo*. Therefore, 0·5 ml. fresh guinea pig serum as a source of complement was injected intraperitoneally together with antiserum and the effect compared with that of antiserum alone. In none of six mice did complement increase

Table 2. Effect of Antiserum* *in vivo* on the Removal of Transfused Strain *A* Erythrocytes

Recipients	No. of mice	Pretreatment of recipients	Mean destruction of transfused erythrocytes in % of the dose injected	Removal of transfused erythrocytes in % of the original dose. Distribution of mice				
				10–19·9	20–39·9	40–59·9	60–79·9	80–89·9
A.CA and *A.BY*	38	*A.CA* or *A.BY* anti-*A* serum	47·5	4	11	10	11	2
A.CA and *A.BY*	6	*A.CA* or *A.BY* anti-*A* serum and complement	19·5†	3	3			

* An intraperitoneal injection of 0·05 to 0·4 ml. antiserum was given to the recipients 30 min prior to the transfusion of the red cells.

† Seven controls which were injected with antiserum only showed a mean removal of transfused erythrocytes of 19·7 per cent of the originally injected dose.

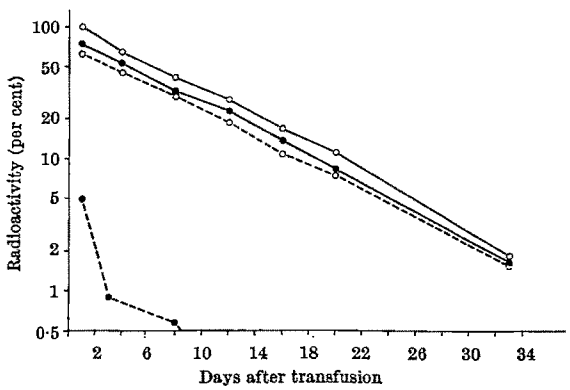


Fig. 1. Survival of strain *A* erythrocytes labelled with chromium-51 in compatible $(A \times A.CA)F_1$ hybrids (group, *A*, —○—), in incompatible *A.CA* recipients (group *B*, —●—), in *A.CA* pretreated with 0·2 ml. *A.CA* anti-*A* serum (group *C*, — —○— —) and in *A.CA* hosts repeatedly immunized against strain *A* normal tissue (group *D*, — —●— —). Each point represents the mean value of three mice

the initial elimination (Table 2). No effect was detectable on the slope of the elimination curves.

Although immune elimination of foreign red blood cells could not be achieved by passive immunization with humoral antibodies, it was found that active hyperimmunization of *A.CA* recipients with strain *A* normal tissue made them capable of immunological elimination of *A* erythrocytes (Fig. 1). However, repeated inoculations of the recipients with *A* tissue was a necessary prerequisite for the observed effect: a single subcutaneous or intraperitoneal inoculation did not make the *A.CA* recipients capable of immune elimination and the effect detectable was an initial elimination similar to that found with passive transfer of antiserum.

The absence of immune elimination of H-2 incompatible erythrocytes is in marked contrast to the results obtained with other cell types. It cannot be ascribed to absence of antigenic receptors on the blood cells, as indicated by various tests *in vitro*, nor to the absence of an immune response of the recipients, as shown by their production of humoral antibodies. Passive transfer of antibodies could accomplish partial removal of the red cells only, without significant effect on the survival time of the residual unaffected cells. This finding would be in agreement with that of Winn[4], who demonstrated that a fraction of red cells is often completely resistant to humoral antibodies *in vitro*. However, complete immune elimination was found in hyperimmunized recipients, indicating that the possible resistance of the red cells was not complete. The present findings cannot be adequately explained by the assumption that erythrocytes differ from somatic tissue cells with regard to the kind of isoantigenic receptors they carry, since complete elimination did not occur after passive transfer of humoral antibodies either, which are presumably specifically directed against the receptors of red blood cells[6]. Although the mechanism of this phenomenon is not known at present, it may be speculated that it is akin to the findings with certain tumours, capable of growing in foreign strains in spite of a homograft reaction. Such tumours have a low concentration of antigenic receptors but they are capable of inducing humoral antibody formation. As a result, the antigenic receptors are blocked and this subsequently leads to enhanced tumour growth[8,9].

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ULTRASTRUCTURE OF THE NODE OF RANVIER

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THE theory of saltatory conduction in peripheral myelinated nerve fibres of mammals is now generally accepted[1,2]. The myelin sheath is considered to act as an insulator confining the active generation of current to the nodes of Ranvier, the propagation of the impulse along the internode being effected by local electrotonic circuits. The active axonal membrane at the node will therefore be the site of relatively large ionic fluxes requiring the expenditure of energy. It would be expected that some provision for these energy requirements might be found in the ultrastructure of the cellular elements which contribute to the formation of the node.

In a previous communication[3] attention was directed to the deviation of the myelin sheath in the paranodal region from the cylindrical form generally held to characterize the internode. It was shown that in the rabbit, rat and mouse the myelin sheath becomes crenated as it approaches the node and that the external grooves are filled with Schwann cell cytoplasm containing numerous mitochondria. Mitochondria have been described near the node[4,5], but not in these large numbers, the concentration greatly exceeding that found in the Schwann cell cytoplasm immediately surrounding the nucleus, the region usually considered to contain the majority of Schwann cell organelles[6]. The Schwann cell columns end as a collar of cytoplasm over the zone of termination of the myelin lamellæ, and this collar has been shown to give rise to finger-like processes which surround the nodal axon[4,7–11]. These processes have been described in the frog as being about 500 Å in diameter, forming an irregular mesh around the nodal axon, and separated from it by a gap of 200–250 Å. In an investigation of the cochlear ganglion of the rat[11] a node is illustrated in which some processes abut against the nodal axon membrane, and appear to contain a single filament. The present study demonstrates the intimate relationship of the ends of these processes and the nodal axon membrane, and it is suggested that the close connexion thus established between the mitochondria rich paranodal Schwann cell cytoplasm and the nodal axolemma may be significant in energy exchanges across the latter, and will also affect the electrotonic current distribution through the nodal membrane.

The tissues examined have been the saphenous nerve of the rat and the sciatic nerve of the mouse. These have been fixed by immersion in ice-cold 1 per cent osmium tetroxide, buffered, for 1 h. The nerves were then dehydrated in ethanol, stained for 1 h in 1 per cent phosphotungstic acid, and embedded in 'Araldite'. Ultrathin sections were mounted on carbon-coated grids and examined in the R.C.A. *EMU 3E* electron microscope.

The appearance of a longitudinal section of the nodal region of an intermediate-sized myelinated nerve fibre is seen at low power in Fig. 1. The basement membrane, an



Fig. 1. Longitudinal section through node of a myelinated fibre of the mouse. *A*, axon: *M*, myelin sheath: *bm*, basement membrane; *c*, collagen of sheath of Plenk and Laidlaw: *d*, dense bodies: *g*, gap substance: *mi*, mitochondria: *np*, nodal processes of Schwann cells: *sc*, Schwann cell cytoplasm. (scale = 1μ)

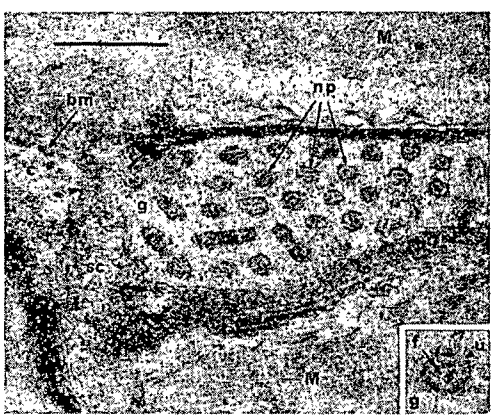


Fig. 2. Paraxial longitudinal section of the node of a myelinated fibre of the rat. Note regular array and internal structure of nodal processes. *bm*, Basement membrane: *c*, collagen: *g*, gap substance: *M*, myelin sheath: *np*, nodal processes of Schwann cells: *sc*, Schwann cell cytoplasm. Inset: *f*, filament within nodal process: *g*, surrounding gap substance: *u*, unit membrane. (scale = 0·5μ, inset × 2½)

amorphous dense layer about 250 Å thick, separated from the underlying Schwann cell unit membrane by a gap of approximately 300 Å, is continuous across the nodal gap. External to it are the small endoneurial collagen fibres of the sheath of Plenk and Laidlaw; these also dip in at the node. Between the basement membrane and the nodal axon is the 'gap substance', apparently structureless material of noticeably greater electron density than the endoneurial ground substance external to the basement membrane. Embedded in this 'gap substance' are the finger-like Schwann cell processes.

The internal ultrastructure of the axon at the node has been well described[7]; but less attention has been paid to its overall dimensions. Through the juxtanodal region of termination of the myelin lamellæ, in larger fibres, the axon is reduced to about one-third of its mean internodal diameter, expanding to about half the internodal diameter at the node itself. The axolemma at the node is densely osmophilic and presents an irregular biconical profile in longitudinal sections; the juxtanodal axolemma is also osmophilic and is very closely applied to the Schwann unit membrane loops at the ends of the myelin lamellæ[7]. The nodal axoplasm is traversed by neurofilaments, more densely packed than in the internodal axon, and these occasionally appear to make contact with the nodal axolemma. It contains mitochondria, one or two being a constant feature of the expanded portion of the nodal axon, often closely related to the axolemma, and also large and small vesicular profiles sometimes identified as endoplasmic reticulum[7].

The collar of Schwann cell cytoplasm over the end of the myelin sheath is continuous with the columns of cytoplasm in the crenated outer aspect of the paranodal myelin. Like these columns it contains numerous small mitochondria and 200 Å cytoplasmic filaments, and, in addition, small densely osmophilic bodies of about 1000 Å diameter. This collar of cytoplasm gives rise to the finger-like process which surround the nodal axoplasm (Fig. 1). These are slender cytoplasmic processes 700–800 Å in diameter, limited externally by a continuation of the Schwann cell unit membrane, and containing longitudinal filaments of approximately 100 Å diameter. There are usually five filaments, occasionally four or six, arranged in a ring within the process and without any evident basal apparatus in the Schwann cell collar. In small myelinated fibres, where the length of exposed axon at the node is larger, the processes are irregularly arranged, running



Fig. 3. Transverse section of the node of a myelinated fibre of the rat. Note close apposition of ends of nodal processes to axolemma and dense osmophia at point of contact. *A*, axon: *bm*, basement membrane: *c*, collagen of inner endoneurium: *mi*, mitochondria in axoplasm: *np*, nodal processes of Schwann cells. (scale = 0·5μ)



Fig. 4. Higher power view of transverse section at the node. Note internal structure of nodal processes and the appearance of the axolemma at their point of contact. *af*, Axon filaments; *bm*, basement membrane; *c*, endoneural collagen, *g*, gap substance; *mi*, axonal mitochondria; *np*, nodal processes; *v*, vesicles in axoplasm. (scale = 0·5μ)

the greater part of their length parallel to the axon surface. In large myelinated fibres the processes are radially disposed in a regular hexagonal array (Fig. 2). They are embedded in the amorphous 'gap substance' and have a minimum separation of about 800 Å. A transverse section at the node (Fig. 3) shows the regular palisade of Schwann cell processes (Fig. 4) and the very close apposition of their distal ends to the axon unit membrane. It has not been possible to resolve any gap between the two and it may be that it is of the same order as the gap between the loops of termination of the myelin lamellæ and the axolemma in the juxtanodal region, about 20 Å (ref. 7). At the point of contact the axolemma shows greatly increased electron density with diffuse osmophilia in the immediately underlying axoplasm. On occasions axon filaments appear to enter this region. There is no evidence of a syncytial connexion between adjacent Schwann cells.

Previous investigators of the dimensions of the axon membrane at the node of Ranvier in vertebrate myelinated nerve fibres, in relation to its electrical properties, have assumed that the exposed axon is in the form of a short cylinder, of homogeneous external surface, and of length equal to the optically visible nodal gap[12,13]. The observations reported suggest, however, that the nodal axon membrane is a non-cylindrical mosaic, some areas being related to the ends of the Schwann cell processes, others to the 'gap substance'. The potential difference across this membrane is dependent on the presence of ionic gradients, and the process of maintaining these gradients requires metabolic activity. Although one or two mitochondria are usually visible in the nodal axoplasm they are not impressive as an energy source for so active a region. On the other hand, the mitochondria rich Schwann cell cytoplasm of the paranodal furrows is directly continuous with the nodal processes, so closely applied at their distal ends to the axon membrane. It is therefore possible that one of the functions of the Schwann cell, as the axon's satellite, is to provide the energy mechanism necessary for the ionic pump at the node. Investigations into the concentration and distribution of energy-releasing enzymes in the nodal region are in progress.

A further problem concerns what part of the axolemma at the node is involved in the active generation of current. It is suggested that the point of contact of the loop of termination of the external myelin lamella with the axon provides the longitudinal limit of the active nodal membrane. Within these limits the mosaic structure of the membrane allows two anatomically distinct sites for ionic flux. Interchange may occur either between axoplasm and 'gap substance', or between the axoplasm and the cytoplasm of the Schwann cell processes at their point of contact. At the first site only one unit membrane is concerned, but movement of ions across it may well be affected by the nature of the 'gap substance'. That this may possibly be an unsulphonated hyaluronic acid–protein complex is suggested by histochemical evidence of hyaluronic acid in the axon and 'neurilemma' of mammalian myelinated nerves, noted to be especially dense around the nodes[14]. Such a complex has been shown to be a cation acceptor, binding potassium in preference to sodium. At the second site the unit membranes of both axon and Schwann cell are involved, together with the small intervening gap. A similar exchange between axon and satellite cell cytoplasm has been suggested for the route of ionic flux in unmyelinated mammalian and invertebrate nerves[15,16].

A commonly held view of the external part of the local electrotonic circuit, by which activation of neighbouring nodes occurs, is that it traverses the tissue fluid which bathes the external surface of the axolemma at the node. It has been shown that the axon is separated from the endoneurial tissue fluid spaces by the relatively more dense 'gap substance' and the satellite cell basement membrane, and these therefore must necessarily be included in such a circuit. This view has been questioned by Tasaki, who has suggested a significant role for the Schwann cell cytoplasm[12]. Such an external Schwann cell cytoplasmic route would be provided in series by the nodal processes, the perinodal collar, the longitudinal paranodal cytoplasmic columns, and the more attenuated external internodal Schwann cell cytoplasm with its small perinuclear concentration.

Finally, it is possible that the Schwann cell processes and the 'gap substance' may have a mechanical function in protecting the ostensibly weakest portion of the peripheral myelinated nerve fibre from damage when stretched. In this connexion it is of interest that fresh teased nerve fibres when pulled, stretch at the internode and not at the node[17]; moreover, glial cell processes and 'gap substance' have not yet been reported at the nodes of myelinated fibres in the central nervous system[10].

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THE PERINEURAL EPITHELIUM: NATURE AND SIGNIFICANCE

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NATURE of perineural epithelium. We have shown a 2–5 layered flat squamous-celled epithelium surrounding the nerve trunks and nerve fasciculi of peripheral nerves, found immediately under the perineurium[1–3] (Fig. 3). Such a structure has been indicated by other authors[4–7]. Further investigations have demonstrated[1,2] that this membrane represents an extension of the pia arachnoid mater (Figs. 1 and 2), which has been traced on to the spinal roots and then on to the peripheral nerve fasciculi, in serial isolations. It has the same number of cellular layers and the same types of cells with identical nuclei (Figs. 1, 2 and 3) as the pia arachnoid mater. The nuclei show well-defined sex chromatin in the female cat and rat in both perineural epithelial cells and those of the pia arachnoid. We have also shown that small blood vessels ramify between the layers of the perineural epithelium in the same way as they do in the pia arachnoid mater.

The perineural epithelium also covers the spinal ganglia (it has been described by histologists to date, as the 'capsule' of the ganglion). An identical membrane covers the optic nerve and the whole of the autonomic system: the vagus nerves[8], the sympathetic ganglia and the rami communicantes[9].

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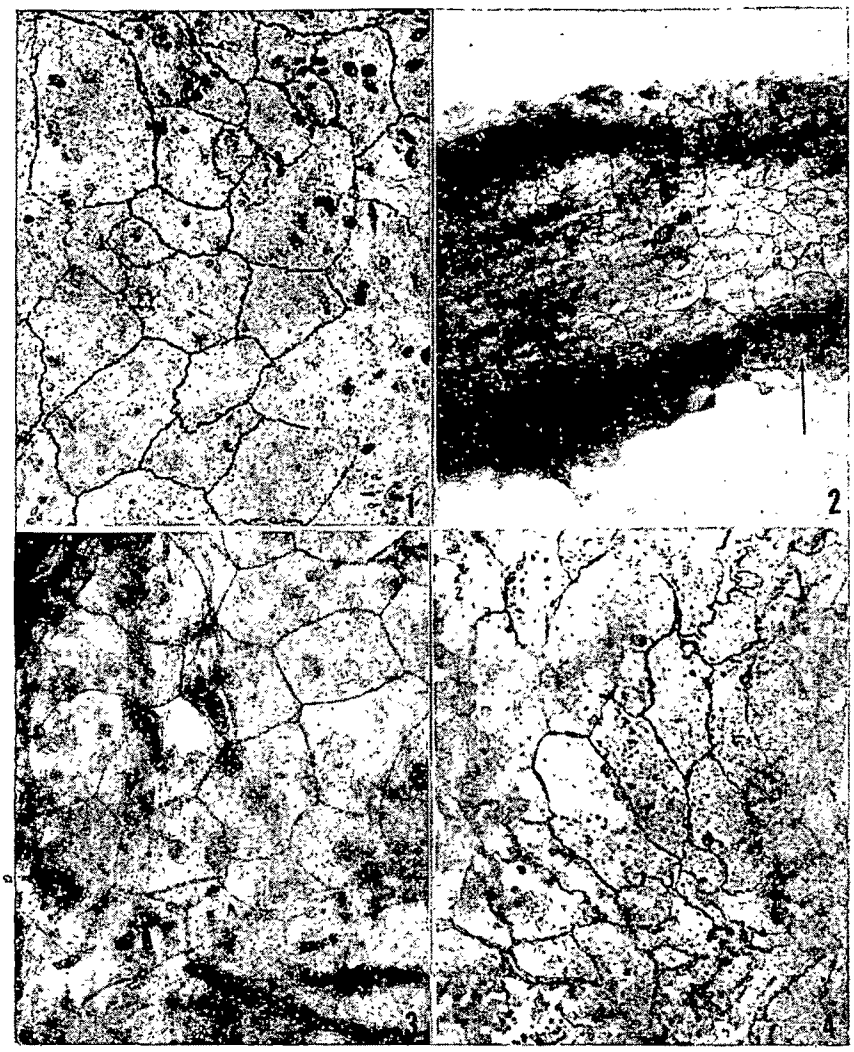


Fig. 1. Rat leptomeninges isolated under binocular dissecting microscope. Stained to show cellular borders. Note squamous epithelial cells in the leptomeninges. (× *c.* 200)
Fig. 2. Rat spinal root covered with its membrane. Isolated under dissecting microscope and stained to demonstrate cellular borders. Note the spinal root (arrow) is covered by leptomeningeal squamous epithelial cells. (× *c.* 85)
Fig. 3. Cat sciatic nerve. Isolated perineural epithelium from peripheral nerve fasciculus under dissecting microscope. Stained for cellular borders and nuclei. Note clear flat squamous epithelial sheet with nuclei. (× *c.* 200)
Fig. 4. Choroid from the rat eye, isolated under dissecting microscope and stained for cellular borders. Note the whole choroid is made up of flat squamous epithelial cells. By altering the fine adjustment of the photomicroscope we can see blood vessels and pigment cells in between these layers of cells. (× *c.* 200)

As the individual nerve bundles divide and become smaller, the epithelium divides with them until it finally surrounds each terminal nerve fibre. In motor nerves the epithelium ends as a funnel surrounding and covering the motor end-plate and makes direct contact with the sarcolemma (the bell mouth of Henle). In sensory nerves the epithelium is shown to be associated with the formation of the end organs. This is especially true of the Pacinian corpuscle, the lamellæ of which are formed by flat squamous epithelial cells and not by fibroblasts as was previously thought (Fig. 5) (ref. 10). The lamellæ of the Herbst corpuscle of the duck[11] and the capsule of the muscle spindle[12] are also made up of a squamous epithelial cell layer which is an extension of the perineural epithelium.

Its relation to the membranes of the eye. The perineural epithelium of the eye appears to extend under the sclera (Fig. 4); it develops pigment and becomes highly vascularized, too, and, in fact, becomes the choroid membrane. The squamous cells (Fig. 4) of the choroid are continuous anteriorly with a layer of squamous cells at the back of the cornea and on the front of the iris and are probably further continuous with the internal limiting membrane of the retina (Fig. 6). The internal limiting membrane has been thought to be a structureless homogeneous mass, but in fact it appears to be made up of squamous epithelial cells together with the expanded ends of Muller's fibres (Fig. 6). Further details of the relation of the perineural epithelium to the structures of the eye will be published elsewhere. However, it might be noted that like the terminations of other peripheral nerves the retina is physiologically isolated by a continuous cellular membrane composed of the choroid and the internal limiting membrane.

Its physiological significance. In the light of all these results we have to envisage the whole of the peripheral nervous system as being surrounded by a membrane similar in structure to, and continuous with, the pia arachnoid mater. Furthermore, this membrane, like the pia arachnoid, is rich in dephosphorylating enzymes, for example, ATPase, 5-nucleotidase, creatine phosphatase, glycerophosphatase, etc.[2], indicating that it represents a metabolically active diffusion barrier. In addition, it becomes obvious that the space between the perineural epithelium and the nerve fibres is continuous with the sub-pial space of the brain and spinal cord and the fluid in the latter space must therefore be able to circulate to the furthermost reaches of the peripheral nervous system. The peripheral nervous system is thus encased in an environment which can be kept independent of that of the surrounding tissue fluids, and more or less identical with that of the central nervous system. One of the functions, presumably, of this membrane is to maintain a certain concentration of Na^+ and K^+ ions and glucose in the vicinity of the nerve fibres. Removal of fluid from this sub-perineural space and analysis for these and other constituents is obviously an important future step.

It is of interest that Vorontsov[13] has shown that in the genesis of physical electrotonus the fast part of the electrotonus is for the most part due to what he calls "the connective tissue sheath of the nerve fibre and especially to the perineurium". If a nerve fasciculus is denuded of its perineurium the nerve fails almost completely to exhibit the fast part of the electrotonus. Vorontsov concludes that this effect can only be brought about if the perineurium acts as an obstacle to the passage of ions and to the same extent for anions and cations. It appears that this property of the perineurium is most likely due to the presence of the perineural epithelium, which as an intact protoplasmic membrane is the most likely structure in the perineurium to have such properties. This is supported by the fact that the epineurium, which has no such membrane, does not have the same properties as the perineurium. This difference between the two sheaths also provides the solution to the controversy between Lorente de No[14] and Feng and Liu[15] concerning the permeability of nerve membranes. The former, who found the membranes acted as a diffusion barrier,

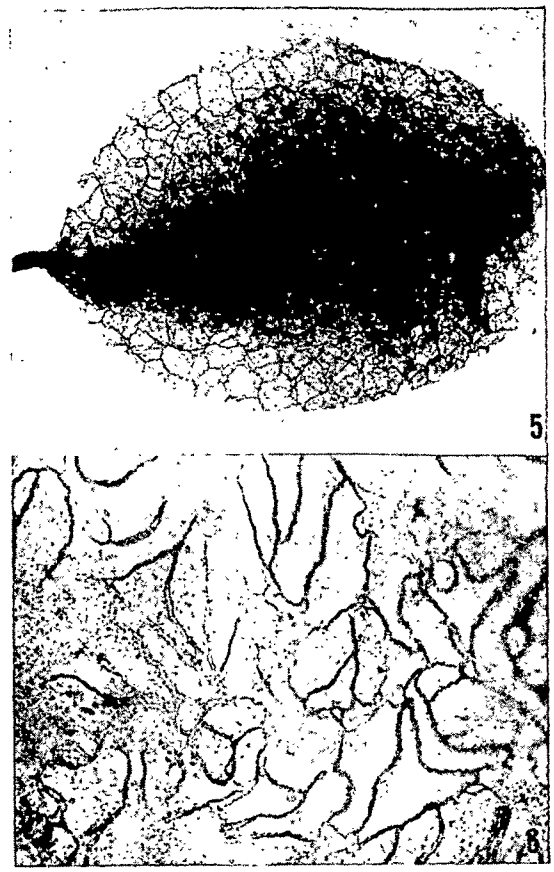


Fig. 5. Whole mount of Pacinian corpuscle, isolated from rat mesentery and stained for cellular borders. Note that the whole Pacinian corpuscle is made up of continuous sheets of flat squamous epithelial cell lamellæ. (× *c.* 90)

Fig. 6. Internal limiting membrane from the rat retina, isolated under the dissecting microscope. Stained to demonstrate cellular borders. It appears that the whole internal limiting membrane is made up of the expanded ends of Muller fibres along with flat squamous cells. (× *c.* 665)

used perineurium, and the latter, who did not, used epineurium.

When a nerve is damaged and degenerates the perineural epithelium does not degenerate but remains as an intact tube which helps to guide the regeneration sprouts of the nerve fibre into the proper region where they can find their way through the neurilemmal tubes leading them to their peripheral destination. There is no evidence of proliferation of the cells of this epithelium after injury[16]. It is of interest that the 'nuclear mass' which is said to remain at the motor end-plate area after severance and degeneration of the nerve is simply composed of the nuclei of the perineural epithelium, which remains as a bell-shaped structure covering the end-plate region, through which the regenerating nerve fibre reaches the proper locus on the muscle fibre.

The presence of the perineural epithelium has a number of implications in neurological diseases as noted below.

Peripheral neuritis. It is of interest that as a nerve extends distally the number of squamous cell lamellæ in the perineural epithelium becomes decreased. It seems likely that one of the functions of the perineural epithelium is protection of the nerves from infection, as well as protection from surrounding body fluids. The degree of protection should be directly related to the number of epithelial layers. Around the very small nerve bundles and in the terminal nerves the number of layers is reduced to one or two. Oedema fluid and toxic substances could penetrate more easily between the cells of such thin layers and reach and damage the nerve fibres. It is possible that the distal distribution of peripheral neuritis develops because of the greater vulnerability of the smaller more peripheral nerves. The presence of a considerable number of mast cells associated with the perineural epithelium is undoubtedly part of its defensive mechanism.

Neurotropic virus infections. The small fibres of the olfactory and gustatory nerves are also surrounded by perineural epithelium[17] so it is possible that once the olfactory and gustatory perineural epithelium has been penetrated by an infective agent (for example, encephalitis virus, meningococcus) it has a ready-made path straight to the central nervous system underneath this perineural epithelium. We feel that it is much more likely that neurotropic viruses and bacteria are transported in this way than that they are transported within the nerve fibre itself.

Sympathetic ophthalmia. The transfer of an affection from one eye to another in sympathetic ophthalmia is probably a good example of the role of the perineural epithelium in the transport of infective or affective agents.

Sympathetic ophthalmia usually commences with traumatic injury to the uveal system of one eye; after a lapse of 2 weeks to 2 months the other eye (the sympathizing eye) gives indications of the development of uveitis. Damage to a single member of other paired organs in the body, for example, parotid glands, testes and kidneys does not usually result in involvement of the other member.

We have noted earlier that the pia arachnoid mater of the optic nerve is continued into the eye as the choroid (perineural epithelium). The pia arachnoid of the optic nerve on one side is continuous with that of the optic nerve on the other side through the optic chiasma. The causative agent of sympathetic ophthalmia, whether bacterial, rickettsial, viral or homologous uveal antigen in nature, thus could travel from the eye originally damaged directly to the other eye through this route.

Neurofibromatosis. The work recorded here suggests that the small tumours of neurofibromatosis may be derived from the perineural epithelium. The perineural epithelium is derived embryologically from the ectoderm and it is possible that small masses of undifferentiated embryological cells may be scattered along the peripheral nerves providing areas capable of spasmodic growth. It is possible, too, that the perineural epithelial cells themselves may retain the ability of rapid growth under stimulation by some activating agent.

Conclusions. The examples we have given suggest that we need to think of a number of neurological diseases in terms of the presence of the perineural epithelium; the normal functioning of the peripheral nervous system must also be thought of in terms of an enclosed system with an environment probably identical with that of the central nervous system.

In Figs. 1–6 note similarity of cells which form the leptomeninges, and which cover the spinal roots, peripheral nerve fasciculi, Pacinian corpuscles, choroid of the eye and form the internal limiting membrane of the eye.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Interplanetary Magnetic Fields as a Cause of Comet Tails

It has been generally recognized recently that the historic explanation of comet tails in terms of solar radiation pressure by either light or particles is impossible. Solar light pressure has been found to be insufficient by several orders of magnitude. Coulomb collisions or acceleration coupled with charge transfer by protons in the solar wind have also been shown by Biermann and Treffitz[1], in particular, to be grossly insufficient processes to account for comet tails streaming within a cylinder of small diameter and great length away from the Sun. It is the purpose of this communication to point out that the gases in cometary comas will be efficiently ionized by the solar wind of ionized hydrogen embedded in a magnetic field[2-4] and that an interplanetary magnetic field will couple the cometary gas to the solar wind.

The neutral gases evaporated from comet nuclei will be ionized both by thermal electrons[4] in the solar wind which have energies of several tens of electron volts and, as Heubner[5] has emphasized, by Alfvén's[6] process of ionization of a neutral gas by a fast moving plasma embedded in a magnetic field.

A comet head may be regarded as a densely ionized plasma essentially stationary in a high-pressure solar wind containing a low-pressure magnetic field moving with the wind. Recent satellite measurements[4] have established that the solar wind consists of plasma the protons of which have an isotropic thermal energy of a few electron volts and a kinetic energy due to their stream velocity of a kilovolt or so. The interplanetary magnetic field observed to be embedded in the plasma is about 5γ (1γ is 10^{-5} gauss). Thus the magnetic pressure, $H^2/8\pi$, is about equal to the thermal (isotropic) pressure of the wind in the moving co-ordinate frame of the wind.

The stream pressure of the solar wind, however, is the overwhelmingly dominant pressure in space, being two orders of magnitude larger than the free space magnetic pressure. Hence, the interplanetary magnetic field is compressed against any obstacle such as the stationary plasma provided by the comet coma or the stationary magnetic field provided by the geomagnetic dipole. Just as in the case of the example furnished by the geomagnetic dipole[7], the interplanetary field is compressed against the obstacle offered by the comet coma so that the magnetic pressure increases to approximate equality with the stream pressure. If the interplanetary field is parallel to the solar wind velocity, the compressed field tails off parallel to the solar wind in a cylindrical shape for a large distance downstream from the comet coma confining the comet plasma within this volume. If the interplanetary field is perpendicular to the wind velocity, the compressed field lines on the solar side of the comet slip around the edge of the comet and then continue moving with the solar wind[7]. The comet plasma will become embedded in the moving field lines and will be carried radially away from the Sun, acquiring the velocity of the magnetic field as Heubner[5] first proposed.

Quite simply the magnetic field may be regarded as the mechanism by which the solar wind is efficiently coupled to the comet plasma which Coulomb collisions are not able to provide. Fluctuations in solar wind pressure, or the magnetohydrodynamic waves the fluctuations generate, will also create the accelerations in comet tails which first led Biermann[2] to deduce the presence of the solar wind.

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Model Atmospheres for Central Stars of Planetary Nebulæ

The central stars of planetary nebulæ have effective temperatures[1-3], T_s, ranging from 3×10^4 ° K to about $2{\cdot}5\times10^5$ ° K. Such stars may therefore be much hotter than the hottest main sequence stars which have temperatures of about 4×10^4 ° K. To obtain a better understanding of the nature of very hot stars, a number of non-grey model atmospheres have been computed.

The three models of Table 1 have been calculated assuming a grey temperature distribution and a grey radiation pressure gradient and using a Rosseland mean absorption coefficient, $\overline{\varkappa+\sigma}$. Model I was calculated for comparison with earlier work[4,5], and good agreement was obtained. It is believed that models II and III are the first to be calculated for stars of effective temperature much greater than 4×10^4 ° K: in these models, the surface gravity, g, has been taken to be only slightly greater than the minimum value required for mechanically stable atmospheres. All three models have the same chemical composition, hydrogen : helium = 85 : 15 by numbers of atoms.

Table 1

	T_s (10^5 °K)	Log g
Model I	0·417	4·2
Model II	1·000	4·8
Model III	2·000	6·0

An important feature of these models is the large contribution of electron scattering, σ, to the opacity of the atmosphere, the ratio, $\sigma/\overline{(\varkappa+\sigma)}$, being about 0·95 for model II and about 0·99 for model III. The remaining contribution comes from continuous absorption by neutral hydrogen and singly ionized helium, the relative abundances of the ions being of the order $N_H{}^0/N_H \sim 10^{-8}$, $N_{He}{}^0/N_{He} \sim 10^{-15}$, and $N_{He}{}^+/N_{He} \sim 10^{-8}$.

The monochromatic source function for an atmosphere in which there is both continuous absorption and continuous scattering is given by the integral equation:

$$S_\nu(\tau_\nu) = \frac{\varkappa_\nu}{\varkappa_\nu+\sigma} B_\nu(T) + \frac{\sigma}{\varkappa_\nu+\sigma} J_\nu(\tau_\nu) \qquad (1)$$

where:

$$J_\nu(\tau_\nu) = \tfrac{1}{2}\int_0^\infty E_1(|\tau_\nu - t_\nu|) S_\nu(t_\nu)\,dt_\nu \qquad (2)$$

is the mean intensity. The condition of flux conservation may be written as :

$$\int_0^\infty \kappa_\nu J_\nu d\nu = \int_0^\infty \kappa_\nu B_\nu d\nu \quad (3)$$

or as

$$\int_0^\infty \kappa_\nu (\kappa_\nu + \sigma) S_\nu d\nu = \int_0^\infty \kappa_\nu (\kappa_\nu + \sigma) B_\nu d\nu \quad (4)$$

The expression for the emergent flux is

$$F_\nu(0) = 2\int_0^\infty E_2(t_\nu) S_\nu(t_\nu) dt_\nu \quad (5)$$

The usual techniques for solving equation (1) by iterative procedures in which S_ν is initially replaced by B_ν are no longer practicable when σ is large compared with κ_ν. In our method, therefore, the integral equation is replaced by a set of linear inhomogeneous equations and solved by matrix inversion. The integrated flux was found to be constant to within about 2 per cent.

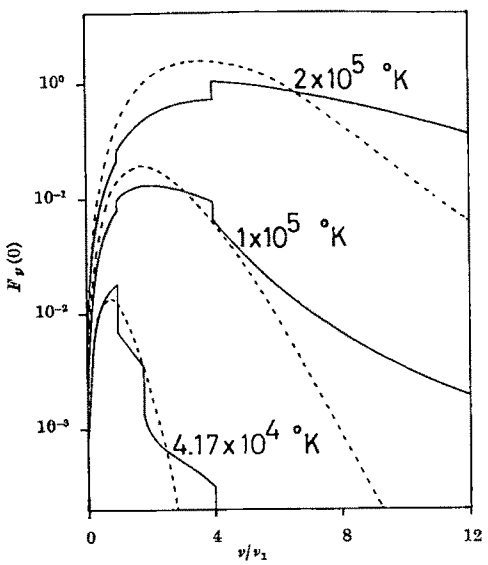


Fig. 1. The frequency distribution of the emergent flux for model atmospheres of effective temperatures, T_s = 4·17 × 10⁴ °K, 1·00 × 10⁵ °K and 2·00 × 10⁵ °K. The abscissa measures the frequency (in units of ν/ν_1, where ν_1 is the frequency of the H Lyman limit), and the ordinate measures the flux, F_ν (πF_ν in units of ergs cm^{-2} sec^{-1} per unit frequency interval where the unit of frequency is the cycle sec^{-1}). The black-body frequency distribution, B_ν (in the same units), for the same temperatures is given by the dashed curve

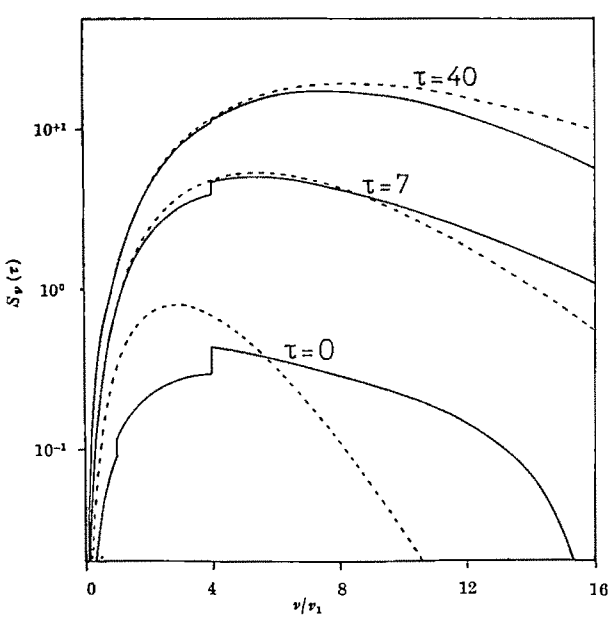


Fig. 2. The frequency distribution of the source function at optical depths, τ = 0, 7 and 40, for a model atmosphere of effective temperature 2·00 × 10⁵ °K. The abscissa measures the frequency (in units of ν/ν_1, where ν_1 is the frequency of the H Lyman limit), and the ordinate measures the source function, S_ν (in units of ergs cm^{-2} sec^{-1} sterad^{-1} per unit frequency interval where the unit of frequency is the cycle sec^{-1}). The black-body frequency distribution, B_ν (in the same units), for the corresponding temperatures is given by the dashed curve

Fig. 1 shows the distribution of emergent flux with frequency for each of the three models with the black-body distribution $B_\nu(T_s)$ plotted for comparison. For the lower temperature of model I, it will be noted that in regions of low opacity the stellar energy exceeds that of a black-body at the same effective temperature but that it drops sharply at the ionization limits of H, He, and He$^+$. For the hotter models, where the opacity is dominated by electron scattering, the behaviour is quite different. In model II, the stellar flux in the vicinity of the H Lyman limit is less than that given by the black-body curve and actually increases at the limit itself. The discontinuity being in emission, the series lines in this region will also be in emission. The discontinuity at the He I ionization limit has now disappeared, the ionization of He$^+$ being virtually complete. At the He II Lyman limit, the flux again drops as for the cooler star. For the still higher temperature of model III, the flux increases at both the H and He II limits.

In Fig. 2, the source function, together with the black-body curves, $B_\nu[T(\tau)]$, is plotted at three optical depths in model III. At high frequencies, S_ν is seen to be larger than B_ν due to the contribution to J_ν from hotter layers at greater depths. But to satisfy the conservation condition (equation 4), S_ν must then be less than B_ν at lower frequencies. At these frequencies, an increase in k_ν causes S_ν to increase towards B_ν and tends to produce an emission feature.

Whether an increase in κ_ν produces an absorption or an emission feature depends on two competing factors in equation (5). On the one hand, the exponential integral, E_2, will decrease rapidly with increasing depth so that the main contribution to the integral comes from the higher, cooler layers of the atmosphere where S_ν is relatively small: this will tend to reduce $F_\nu(0)$. On the other hand, at any given layer, S_ν itself may increase with increasing opacity, thus tending to increase $F_\nu(0)$. A related topic has been discussed in articles by Schuster[6] and Underhill[7] on the formation of emission lines in media with continuous absorption and continuous scattering.

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RADIOPHYSICS

Extremely Low-frequency Radio Propagation in an Inhomogeneous Ionosphere

Experimental results[1] show that the attenuation rate of extremely-low frequency radio waves decreases with decreasing-frequency in the range from a few kc/s to about 70 c/s, below which it appears to increase again. The experimental results, however, are not yet fully confirmed. Moreover, theoretical work to date has given no indication that this behaviour would be expected. It has been suggested[1], however, that it might be possible to explain the observations by considering a stratified ionosphere consisting of three homogeneous layers having different conductivities. Although a two-layer model was

considered, no calculations have so far been given for the more complicated case.

It is the purpose of the present communication to show that the observed increase of attenuation rates as the frequency tends to sufficiently low values can be predicted on theoretical grounds using a relatively simple ionospheric model.

The propagation of vertically polarized waves in an isotropic, horizontally stratified medium can be represented by a Hertz vector which has a vertical, or z-component, only[2]. The z-wise variation of the Hertz vector is given by a factor $f(z)$ which satisfies a certain well-known differential equation[2]. It has recently been pointed out[3] that the equation has exact solutions for certain simple profiles of the refractive index. One of these cases, to be considered here, is that of an ionosphere having a linear variation of refractive index beginning at a height $z=h$ above a perfectly conducting earth at $z=0$.

The wave impedance, Z, is given in terms of f by the equation:

$$Z = -\frac{i\omega\mu_0}{k^2} \cdot \frac{1}{kf} \frac{\mathrm{d}(kf)}{\mathrm{d}z} . \qquad (1)$$

In this equation ω is the pulsatance, μ_0 is the permeability which is taken to have the free space value everywhere, and k is equal to the product of the plane wave propagation constant k_0 with the refractive index.

The boundary conditions require the surface impedance $Z_s = Z_{z=h}$ to be matched at the interface between two media. At the surface of a perfect conductor Z must vanish, so that in the air space:

$$f(z) = C\cos(gz),\ g^2 = k_0^2 - \gamma^2 \qquad (2)$$

where C is a constant and $\gamma = k_0 + iD$ is the propagation constant of the waves. The boundary condition at $z=h$ gives:

$$Z_s = \frac{i\omega\mu_0}{k_0^2}\, g\tan(gh) \qquad (3)$$

When $f(z)$ is known throughout the ionosphere equation (3), together with the condition on the fields at infinity, determines the eigen-values of g and hence of γ.

The conductivity parameter, ω_r, is determined by the ratio of the conductivity σ to the free space permittivity ε_0. It is much larger than ω for extremely low frequencies and in this case the ionosphere behaves like a good conductor. Attention will be restricted to the zero order mode which is dominant for frequencies in the ELF range. For this mode, with $\omega \ll \omega_r$, $|gh|$ is small. If also $|D| \ll 2k_0$ then equation (3) gives:

$$D = \frac{k_0}{2\,\omega\mu_0 h} Z_s \qquad (4)$$

For an ionosphere with a linear variation of refractive index[3]:

$$k(z) = \bar{k}(1+bz_1),\ z_1 = z - h \qquad (5)$$

and:

$$f(z) = \{\bar{k}(1+bz_1)\}^{-\frac{1}{2}} W_{\pm p,\,3/4}[\pm i\bar{k}(1+bz_1)^2/b] \qquad (6)$$

where $p = i\gamma^2/4\bar{k}b$ and $W_{p,m}$ is a Whittaker function. The bar above a symbol is used to denote its value at the lower face of the ionosphere.

Using equation (6) it can be shown that:

$$Z_s = \frac{\omega\mu_0}{\bar{k}}\left[-\frac{ib}{2\bar{k}} \pm \frac{2W'_{\pm p,3/4}(\pm i\bar{k}/b)}{W_{\pm p,3/4}(\pm i\bar{k}/b)}\right] \qquad (7)$$

where the dash on the Whittaker function denotes differentiation with respect to its argument. Provided $|b|$ is not too large the Whittaker functions may be replaced by their asymptotic expansions. Writing $\bar{k}^2 \doteqdot i\omega\,\mu_0\bar{\sigma}$ and neglecting $\gamma^2/\bar{k}^2$, a series for Z_s is obtained which, when combined with equation (4), gives:

$$D = \mp\frac{(1-i)}{2h}\left(\frac{\omega\varepsilon_0}{2\bar{\sigma}}\right)^{\frac{1}{2}} - \frac{b\varepsilon_0^{\frac{1}{2}}}{4h\mu_0^{\frac{1}{2}}\bar{\sigma}} \mp \frac{(1+i)5b^2\,\varepsilon_0^{\frac{1}{2}}}{8h\mu_0\omega^{\frac{1}{2}}(2\bar{\sigma})^{3/2}} + \qquad (8)$$

The real part of this formula gives the attenuation coefficient. The requirement that the wave amplitude decreases as the wave propagates resolves the ambiguity of sign in equation (8), the lower signs being taken.

When $b=0$ the ionosphere is homogeneous and only the first term remains, giving the result which is often quoted in this case[4]. When $b \neq 0$ the second term in (8) gives a constant shift, up or down, in the attenuation versus frequency curve. The third term varies as $\omega^{-\frac{1}{2}}$ and it is this and later terms which give rise to increasing attenuation as the frequency decreases to very low values. A similar behaviour results in the case of an exponential variation of refractive index when the exact solutions[3] are applied.

A much more complete investigation of these novel expansions for this and other profiles is at present being carried out and it is hoped to submit this work for publication at a later date.

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Sudden Changes in Amplitude and Phase of 16 kc/s Very Low-frequency Signal received in New Zealand

In *Nature* of February 23, 1963, appears a report by P. Rohan, L. L. Anderson and D. J. Cooke on the anomalous behaviour of the radio signal received on 16 kc/s in South Australia.

We wish to confirm that very similar results, both in phase and amplitude, were obtained at Lower Hutt, New Zealand, on May 31 and June 25, 1962, at the times indicated in the report. Our records of *GBR* for June 11 and 12 may be among some sent overseas for investigation, so we are unable to examine them at present. However, a *GBR* receiver which we had operating in Wellington at the Time Service Observatory was observed by Mr. B. R. Gibson to give anomalous records on June 11 and 12. Our aerial was a bi-directional loop oriented to receive the maximum signal from *GBR*.

At the corresponding times on May 31, June 11 and June 12, the records of *NBA* 18 kc/s show nothing unusual. We do not have a record for June 25 of *NBA*.

From the Australian results and our own, we believe that it is likely that the anomalous signal on May 31 had a frequency of 4·5 ± 1 parts in 10^8 slow in respect to the 16 kc/s transmitted by *GBR*. On June 25 we have a record of 16 kc/s giving the sign and rate of change of phase and amplitude separately. The phase record during the disturbance is coherent although short, and indicates a frequency of 13 ± 3 parts in 10^8 fast with respect to the 16 kc/s transmitted by *GBR*.

The integrated amplitude of the anomalous signal received on May 31 and June 25 was about 4–5 times greater than the signal received from *GBR* when transmitting its normal duty cycle.

As no disturbance was observed on 18 kc/s there does not appear to have been a general disturbance of the ionosphere at the times in question. The unifo rm phase rate and the sudden cessation of the anomalous signal does not resemble a record obtained from a solar flare for instance. These disturbances were not observed by the British Post Office at Dollis Hill, so the signal transmitted

by *GBR* was normal. We believe that the National Bureau of Standards records also show no unusual occurrences.

As we are separated from Salisbury by about 3,200 km and as the National Bureau of Standards, Boulder, records were not affected, then presumably there is a powerful transmitter besides *GBR* able to operate on 16 kc/s relatively close to Australia and New Zealand.

This conclusion could be modified if the receiving aerials at the National Bureau of Standards are directional.

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GEOPHYSICS

Radiogenic and Atmospheric Argon Content of Tektites

In previous publications it has been shown that tektites can be dated by the potassium/argon method[1-3]. The potassium/argon ages give the time of their formation and also show which tektite localities belong together. It has been found that tektites from North America, the moldavites and tektites from Indo-China, Indonesia and from Australia form three independent strewn fields. The size of the strewn fields is of crucial interest to all hypotheses concerned with the origin of tektites. Therefore, these experiments have been extended with improved techniques and with a larger variety of samples. The results are given in Table 1.

Table 1. Average Potassium/Argon Ages of the 4 Different Tektite Strewn Fields

Locality	Age (10^6y)
Georgia	34·0
Texas	34·2
Martha's Vineyard	33·7
Moldavites (results of 8 different localities)	14·6
Indo-China, Billiton, Java, Borneo and the Philippines (results of 12 specimens)	0·71
Australites (6 widespread localities)	0·72
Ivory Coast	1·3

Six specimens from different localities of Texas, two specimens from Georgia, and a fragment of the recently discovered Martha's Vineyard tektite gave the same age of $34{\cdot}2 \pm 2 \times 10^6$ y. The Texas tektites are found in the Jackson formation, which is late Eocene. The agreement with the potassium/argon age is quite good. The stratigraphy of the Georgia and Martha's Vineyard tektites is not well established. From the close agreement of the potassium/argon ages, one has to conclude that all North American tektites belong to one event.

Eight moldavites from different localities gave a potassium/argon age of $14{\cdot}6 \pm 0{\cdot}9 \times 10^6$ y. They form a distinctly different group. Their potassium/argon age is in reasonable agreement with their stratigraphic age, which is thought to be late Miocene.

Twelve samples from Indo-China and Indonesia, and six samples from Australia have been investigated. Although previously only one australite has been analysed, it was already indicated that all these tektites belong to one strewn field. For these reasons, more australites from widely spread locations throughout Australia have been selected. For all these specimens an age $0{\cdot}72 \pm 0{\cdot}6 \times 10^6$ y has been found, and, within the errors, no difference in the potassium/argon age of Indo-China, Indonesia, and Australia can be observed. This result has important consequences. While the tektite sites from Indo-China and Indonesia are thought to be of Middle Pleistocene age and agree within the uncertainties of the time scale with the potassium/argon ages, there seems to exist a discrepancy for the Australian tektites. Although no definite stratigraphic age exists for these finds, some authors believe that they can be only a few thousand years old[4,5]. Some of them were found 5 m deep in the clay drift of an old river course; others were lying at the surface and in recent soils. The fact that older material can always be transported in younger deposits makes an estimate of the age difficult. An underestimation of the age seems to be likely. It is suggested, instead, that the potassium/argon measurements be considered as a basis for the age discussion, and that confirming geological evidence be sought. There are also relations in the chemical composition among the whole group of these tektites. The aerodynamic structures of australites are no longer an outstanding feature since these structures are also found in samples from Flores, Java, and Billiton.

If the australites were really younger the potassium/argon values could only be understood if the australites were incompletely degassed. It is, however, extremely unlikely that the amount of inherited argon in the australites is exactly the same as the radiogenic argon content of the tektites from Indo-China and Indonesia.

From these results, it may be concluded, therefore, that all tektites from this area belong to a single event. The strewn field is of tremendous dimensions, and reaches 8,000 km from Indo-China to Australia. This fact has to be faced by all hypotheses about the origin of tektites.

Three tektite specimens from the Ivory Coast have also been investigated, and their age has been determined to be $1{\cdot}3 \times 10^6$ y. These tektite finds have to be considered as a fourth independent strewn field. A comparison with the stratigraphic age is, however, not possible, since more should be known about the geology of their sites.

Table 2. Argon Excess and Gas Pressure in Bubbles of some Gas Rich Specimens. The Isotopic Composition of Argon is the same as in Air Argon

Locality	Argon excess ($\times 10^{-6}$ c.c./g)	Gas pressure in bubbles (mm Hg)	$^{40}A/^{36}A$ ratio
Muong Nong	1,040	40	298
Hai Nan	380	—	305
Philippinite	83	1·3	294
Ivory Coast	7·8	—	295
Libyan Desert Glass	290	24	300
Darwin Glass	440	—	305
Atmospheric argon	—	—	296

In addition to the age determinations, bubble-rich specimens have been investigated for excess gases of other than radiogenic origin[6]. These have been found in the Muong Nong tektites, in the Darwin Glass and the Libyan Desert Glass, and also in the Ivory Coast tektites. In these samples the gases are homogeneously distributed. Excess gases have also been found in some philippinites from the Santa Mesa district, which had visible macroscopic bubbles. Nitrogen, argon, krypton and xenon have been measured; their abundance and the isotopic composition of argon are the same as in air. By cracking the samples in the ultra-high vacuum it was found that these gases are mainly included in the bubbles. The pressure in the bubbles, calculated from the amount of gases released and from the deviation of the densities, reaches values up to 40 mm mercury (Table 2). The characteristic value of the $^{40}A/^{36}A$ ratio in air is a conclusive criterion that these gases are of terrestrial origin. Since the gases in most of these samples are homogeneously distributed and the weight of the Muong Nong tektites amounts to kg, the inclusion of the air cannot have occurred through ablation in the atmosphere. The high pressure found in some bubbles also indicates that tektites were molten close to the surface of the Earth. These investigations, therefore, are considered as further support to the hypothesis that the tektite material itself is of terrestrial origin.

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PHYSICS

Radioactivity of Air Dust, Rain Water and Turf Grass in Bandung, Java

During the period November 1961–December 1962 the radioactivity found in air dust, rain water and turf grass was determined in one single place in Bandung.

The air dust was collected once a month on a sheet of tracing paper, about 0·19 m², covered with a layer of 'Vaseline' (about 2 g per sheet). The paper sheet was fixed at a height of 1·5 m on the internal wall of an enclosed yard and protected from the rain by a concrete horizontal shade. (As comparison with paper sheets exposed in the open shows, the deposition of dust on the paper placed in the yard was independent of the direction of wind but dependent on the degree of turbulence of the air produced in the yard.) The paper sheets were exposed during the last 10 days of every month.

The weight of ash obtained after burning the paper sheets varied usually between 200 and 350 mg per sheet, probably depending on the variety of paper and on the slight variation of the conditions under which burning was carried out. Total error of the determination of the weight of obtained ash was + 13 ± 10 mg. Specific radioactivity of the ash varied between 20 and 90 pc./g with an error of measurement between 300 and 25 per cent.

The rain water was collected in enamelled wash-basins placed in the yard on a table (0·5 m high) under open sky. The basins were normally put out when the rain had already begun and removed after the rain had stopped. The volume of collected and evaporated rain water varied between 1 and 7 l. ± 1 cm³, depending on the intensity of rain.

The weight of sediment obtained after evaporation of the rain water varied, usually between 8 and 35 mg (in one exceptional ease, 62 mg) depending on the quantity of evaporated water and its dust content, which varied usually between 4 and 25 mg/l. The error of weighing of the sediment was ± 0·5 mg due to unavoidable handling of evaporation dishes. The error due to precipitation of the dust from the rain water while it was in the storage before evaporation was usually about + 5 ± 2 mg, depending upon the kind of dust contained in the rain water. The error of sedimentation on the walls of the beaker during the first stage of evaporation was about + 4 ± 1·5 mg. Both of these positive errors were taken into account. Specific radioactivity of the sediment varied between 416 and 11,400 pc/g with an error of measurement between 300 and 5 per cent.

The grass was collected at the end of every month from the same place. The quantity of grass put on the drying shelf varied between 720 ± 1 and 120 ± 1 g. The quantity of dry grass obtained varied correspondingly between 138 ± 1 and 37 ± 1 g. The quantity of dry grass taken for burning was always 30 ± 1 mg.

The quantity of ash obtained after burning of the dry grass varied usually between about 4 and 3 g. The total error in the determination of the weight of ash was + 13 ± 10 mg. Specific radioactivity of ash varied between 120 and 300 pc/g with an error of measurement between 33 and 4 per cent.

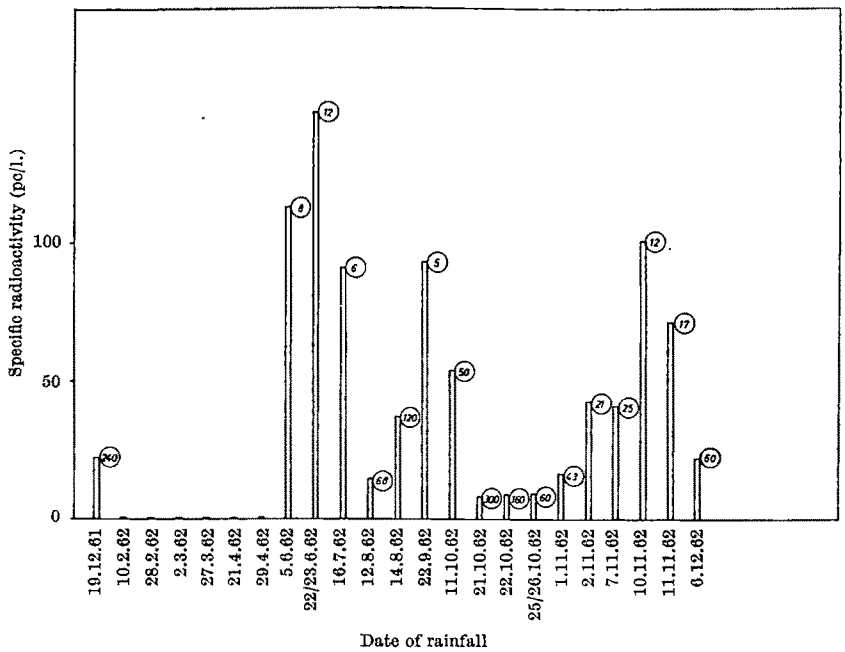


Fig. 1. Specific radioactivity of rainwater. Figures in circles indicate the relative errors of measurement (per cent)

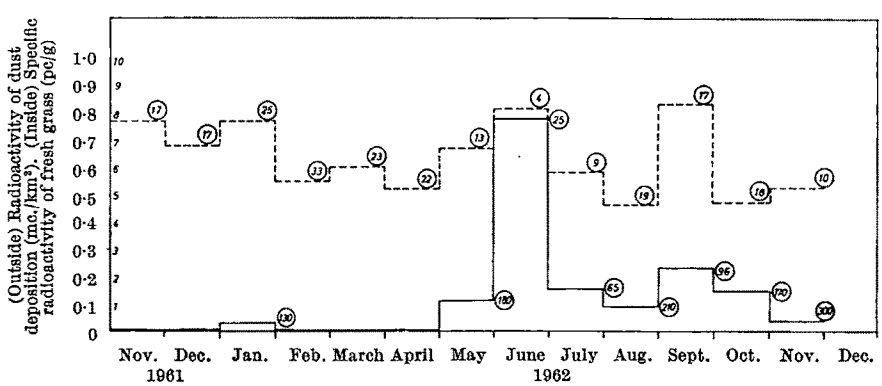


Fig. 2. —, Radioactivity of monthly dust deposition; – – –, radioactivity of monthly grass growth. Figures in circles indicate the relative errors of measurement (per cent)

The radioactivity of prepared ash probes was measured in the First Institute of Physics, University of Munich. The sensitivity of measuring equipment was 1 c.p.m. = 4·12 pc. The instrumental error was ± 3 c.p.m. (12·36 pc) and statistical error ± 0·4–0·6 c.p.m. Decrease of radioactivity due to decay during the delay of measurements was in all cases within error of measurement.

The radioactivity of air dust deposited per km², specific radioactivity of rain water and of fresh grass calculated from the obtained results of measurements are given in Figs. 1 and 2.

I thank Dr. Klaus Stierstadt of the First Institute of Physics, University of Munich, for measuring the radioactivity of ash probes.

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Dislocations and Catalysis

CAUSAL connexions between dislocations and catalysis have been suggested by a number of workers over the past twenty years[1–6]. Conclusive evidence, however, has often been lacking because of surface contamination or other effects which could not be implicated as factors in catalysis. Recently an unusual amount of valuable results have been obtained on dislocations and mechanical properties of lithium fluoride crystals. Gilman[7–16], Forty[17] and Benson *et al.*[18] have determined the role of cleavage in nucleating dislocations in lithium fluoride and described the character of dislocations and stresses needed to produce them. This wealth of experimental results offers a fertile ground for exploring the role of dislocations in catalysis on this simple and well-documented crystalline substance. By using Gilman's[8,10,11,15] techniques for cleaving, etching, and stressing crystals it is possible to control the introduction of dislocations to a satisfactory degree and to identify them without spending a major effort on what could be a most illusory problem.

We are, therefore, examining dislocations and catalysis using this background on lithium fluoride and utilizing the reaction of dehydrogenation of ethyl alcohol. A precise reactor system similar to that described by Pozzi and Rase[19], but now made automatic and reduced greatly in size, is being used. Very pure (less than 1 p.p.m. impurities) grade lithium fluoride crystals obtained from the Harshaw Chemical Co. are cleaved into pieces approximately (1 mm × 2 mm × 2 mm) and tested in various ways.

A recent series of experiments has proved interesting as shown by the results in Fig. 1. Crystals of lithium fluoride were cleaved and treated in various ways in order to produce dislocation densities of different orders of magnitude (Table 1 outlines the history of each sample). The samples were then tested in the reactor at 260° C for acetaldehyde production. The differential-type reactor results enabled direct calculation of rates. These results represent the first quantitative representation of a correlation between catalytic activity and dislocation density.

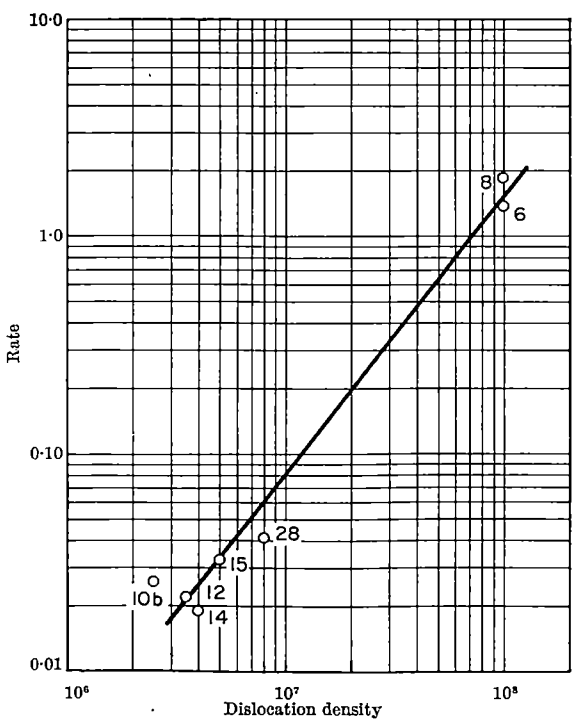


Fig. 1. Effect of dislocation density on catalytic activity of lithium fluoride crystals.

$$\text{Rate} = \frac{\left(\frac{\text{moles converted}}{\text{mole charged}}\right)\left(\frac{\text{c.c. ethanol}}{\text{h}}\right)}{\text{cm}^2 \text{ of surface.}}$$

Dislocation density = No. of dislocations per cm².

Table 1

Run No.	Treatment	Comments
6	As-cleaved	These samples exhibited unusually large numbers of dislocations when cleaved slowly
8	As-cleaved	
106	Cleaved, annealed and cooled rapidly	These samples showed moderate dislocation densities upon cleaving as shown in Fig. 1. Activity and dislocation density remained relatively constant before and after treatment
12	Cleaved, annealed and cooled rapidly	
14	Cleaved, annealed and cooled rapidly	
15	Cleaved, annealed and cooled rapidly and then each piece stressed	Prior to stressing no activity was observed
28	Annealed and cooled rapidly and then stressed prior to leaving	Samples of this material which had not been stressed showed no activity upon cleaving and testing. Dislocation densities in unstressed material were ~ 10^5

Equally as significant, however, is the rather dramatic production of activity in inactive and relatively dislocation-free crystals (for example, runs 15 and 28) by stressing annealed and rapidly cooled crystals. Gilman has shown that annealed and rapidly cooled lithium fluoride crystals are soft and moderate stresses can be applied to multiply dislocations profusely. We have observed this same phenomenon and seen the vastly increased numbers of dislocations on the etched crystals and the corresponding appearance of catalytic activity. Annealing and rapid cooling without stressing do not produce changes in dislocation density or catalytic activity.

Work is continuing to define these effects more precisely; but the value of the known techniques with lithium fluoride when applied to reaction studies has already been demonstrated, and the role of dislocations on catalytic activity of lithium fluoride has been demonstrated quantitatively.

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METALLURGY

Growth and Nucleation of the β→α Transformation in Uranium 0·45 per cent Chromium Alloy

THIS communication describes some experiments with uranium 0·45 per cent chromium, the purpose of which was to measure the growth and nucleation rates of α-uranium during the β → α transformation. To the best of our knowledge, these results are the first quantitative measurements of growth-rate of the β → α transformation.

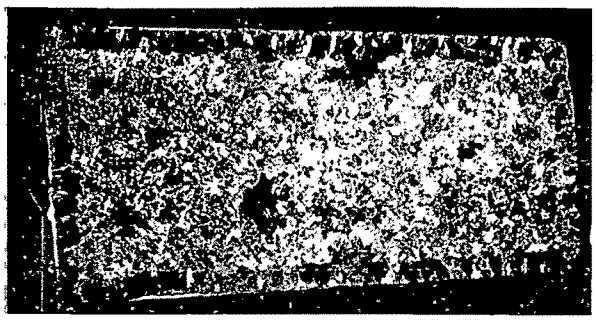


Fig. 1. Columnar growth in a uranium 0·45 per cent chromium specimen, solution treated at 720° C for 15 min followed by isothermal transformation at 625° C for 8 min (× *c.* 3·9)

The growth-rate G was established at four different temperatures by measuring the change in length of columnar grains after various transformation periods. In Fig. 1 the columnar grains formed isothermally at 625° C after 8 min can be seen clearly. The results of G are given in Table 1.

To calculate the rate of nucleation, $\dot{N}$, the relation $\dot{N} = G/D^4$ was used, where D is the mean macro grain size, measured after the completion of the transformation. The results of calculated $\dot{N}$ values are given in Table 2.

Table 1. Growth-rate as Function of Temperature

T (° C)	G cm/sec × 10^{+4}
630	0·47
625	1·00
615	1·30
605	1·78

Probable error ± 20 per cent.

Table 2. Rate of Nucleation as Function of Temperature

T (° C)	$\dot{N}$ $\frac{1}{\text{cm}^3\ \text{sec}}$
630	0·5
625	1·7
615	7·3
605	52

Further work is being conducted on various uranium alloys, and also attempts are being made to synthesize theoretical *TTT* curves from the measured G and calculated $\dot{N}$ values, and to compare them with experimental *TTT* curves.

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MINERALOGY

Direct Electron-optical Resolution of Anti-phase Domains in a Silicate

In this communication the direct electron-optical resolution of anti-phase domain patterns in a plagioclase feldspar is recorded. (The specimen, from Frederiksvärn, Norway, was kindly provided by Dr. P. H. Ribbe. A partial chemical analysis, made by J. H. Scoon, yielded $KAlSi_3O_8$, 3·4 per cent; $NaAlSi_3O_8$, 45·2 per cent; $CaAl_2Si_2O_8$, 51·8 per cent by weight.)

Relevant X-ray diffraction data for material of this composition have been presented by Gay[1] and indicate that, in addition to primary Bragg maxima due to a triclinic unit cell (appropriate to this member of the solid-solution series albite-anorthite), additional maxima occur in pairs symmetrically disposed about the additional reciprocal lattice points due to a doubled unit cell (anorthite-type cell[2]). An electron diffraction photograph illustrating the character of these paired maxima is presented as Fig. 1*a*, where a single pair of the maxima have been ringed and the primary Bragg maxima have also been indicated, *B*.

In its essentials this distribution of intensity in reciprocal space can be compared directly with that observed by Glossop and Pashley[3] in the examination of anti-phase

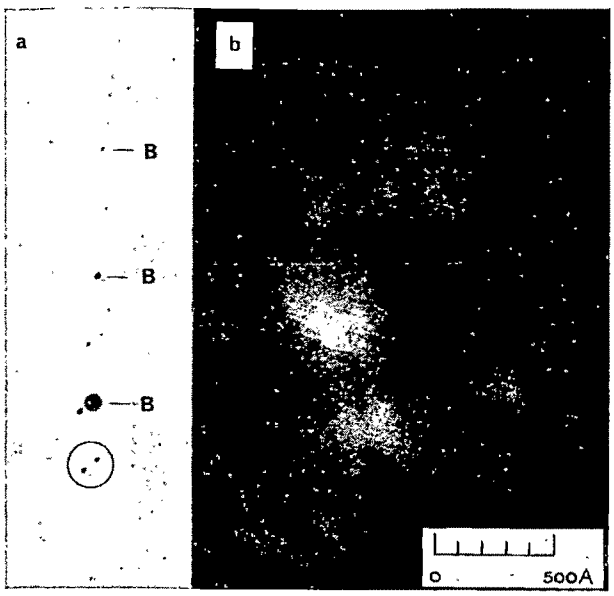


Fig. 1. *a*, Part of an electron diffraction photograph of the plagioclase studied. A pair of subsidiary intensity maxima have been ringed and the primary Bragg maxima have been indicated by letter *B*; *b*, part of the dark-field photograph obtained with a pair of diffraction maxima. The fringe pattern defines a distance of approximately 30 Å for the width of the individual domains

domains in an ordered copper–gold alloy; and implies, as suggested[4] in the case of nepheline where similar additional maxima are observed, that an antisymmetrical distribution in respect of a multiple cell is involved.

It has been possible in the feldspar, as in the alloys examined by Glossop and Pashley[3], to use the paired maxima for the direct electron optical resolution of the domain structure involved. This was done on finely crushed material mounted on a carbon film by selecting a single crystal orientated in such a way that a single pair of maxima, near the origin of the diffraction pattern, were in the reflecting position. The dark field image due to the pair of maxima was obtained by isolating them using a 25μ objective aperture but without introducing tilt in the direct beam. (The latter technique was used by Glossop and Pashley in order to obtain high resolution under dark field conditions.)

The anti-phase domains resolved during the present experiments were extremely regular and approximately 30 Å thick. The most striking feature of the anti-phase array (of which a small part is reproduced in Fig. 1*b*) was the absence of major dislocations in the pattern as studied in a semicircular area of 0·25μ radius.

The results of the present experiments were anticipated on the basis of a detailed thermodynamic analysis of anti-phase phenomena, as observed in several silicate systems, by one of us (J. D. C. McC.). This theoretical treatment, which will appear shortly, will deal with other aspects of the phenomena.

The present observations represent the first direct proof of the existence of anti-phase phenomena in crystalline solids other than the metal alloys. A model of similar character has recently been proposed for the plagioclase feldspars by Megaw[5].

The experiments described here were done on an *E.M.*6 which was kindly provided for the combined use of the Departments of Mineralogy and Geology at Cambridge by the Department of Scientific and Industrial Research.

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CHEMISTRY

Dielectric Loss and Hydration of Sodium Chabazite

WE have measured the apparent permittivity, ε', and loss, ε'', of a sodium chabazite both anhydrous and partially hydrated. Powdered Bay of Fundy chabazite containing Na^+ and Ca^{++} in the approximate equivalent ratio 1 : 4 was cation exchanged to give a 95 per cent exchanged sodium chabazite. The powder was sieved through a 200-mesh sieve and packed in a concentric cylindrical cell described elsewhere[1]. It was then outgassed under vacuum for two days at 400° C. From the unit cell dimensions and the chemical analysis, the density of the anhydrous zeolite was calculated as 1·659, while the packing fraction of the powder in the cell was 0·445.

Measurements were then made of the apparent values of ε', ε'' and tan δ at 115°, 102°, 85°, 60°, 30° and −196° C, over a range of frequencies using apparatus already described[1]. From the apparent values of ε' and ε'', 'real' values ε_0' and ε_0'' were estimated from Bottcher's[2] approximate formula. Typical values are summarized in Table 1 for several temperatures. Both permittivity and loss are high and frequency dependent, although the limitations of Bottcher's formula means that accuracy in the figures may be rather low.

Table 1. 'REAL' DIELECTRIC CONSTANT AND LOSS FOR MASSIVE ANHYDROUS SODIUM CHABAZITE

Temperature (° C)		Frequency (kc/sec)					
		4	6	10	20	30	40
115	ε_0'	—	19·6	18·4	16·8	16·0	15·4
	ε_0''	—	5·54	5·47	5·72	6·01	5·53
102	ε_0'	18·0	17·0	15·5	13·6	12·6	11·9
	ε_0''	4·92	5·14	5·40	4·00	3·21	2·71
85	ε_0'	15·3	14·0	12·3	10·0	9·3	—
	ε_0''	4·49	4·80	4·40	2·44	2·03	—

As Fig. 1 shows, a loss peak was observed in curves of tan δ_{app} versus log f (f = frequency of a.c. field), at 115°, 102° and 85° C. The maximum moved to lower frequencies as the temperature was reduced and lay outside the practicable range of frequencies at 60°, 30° and −196° C. Even at the lower temperatures the zeolite was lossy, except at −196° C for the highest frequencies. At these temperatures, curves of log ε''_{app} versus log f had slopes differing from the value −1 which would correspond to d.c. conduction losses, except for a limited frequency range at −196° C.

Measurements were next made on the same specimen of sodium chabazite which had been allowed to take up first 4 per cent of zeolitic water and finally 11 per cent of water. The crystals when saturated sorb about 21 per cent by weight of water. In each of the partially hydrated states the loss peaks observed at 115°, 102° and 85° in the anhydrous state had moved out of the practicable frequency range as shown by the curves of ε''_{app} versus log f in Figs. 2*a* and *b*. Thus there is an interaction between water and the cause of the loss peaks.

The value of the activation energy for the process responsible for the loss peaks in Fig. 1, calculated by means of the Arrhenius equation from the temperature dependence of the frequency of maximum loss, is 7·5 ± 1 kcal per Avogadro number of unit loss processes. It has been established that, in other anhydrous zeolites at temperatures as low as these, the main charge transfer phenomena are due to cation movement[3]. Thus, at first sight it seems reasonable to ascribe the above value of the energy of activation to cation jumps within the crystal framework. It has been found that the self-diffusion of sodium and other monovalent cations in hydrated chabazites requires activation energies in the range 7–8 kcal per Avogadro number of unit diffusion processes[4], a value very close to that now observed for the dielectric loss process in

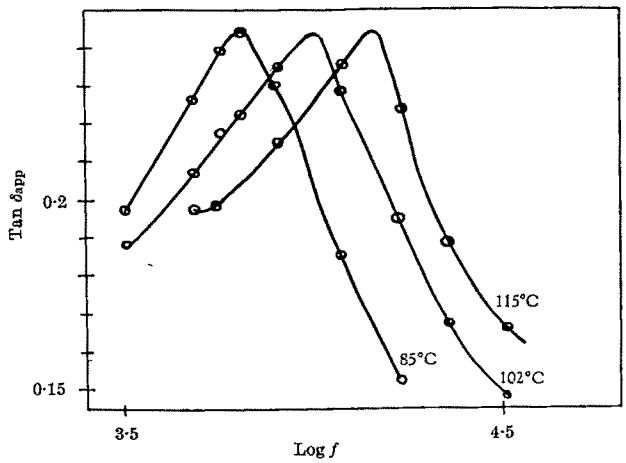


Fig. 1. Dielectric properties of anhydrous chabazite. tan δ_{app} versus log f

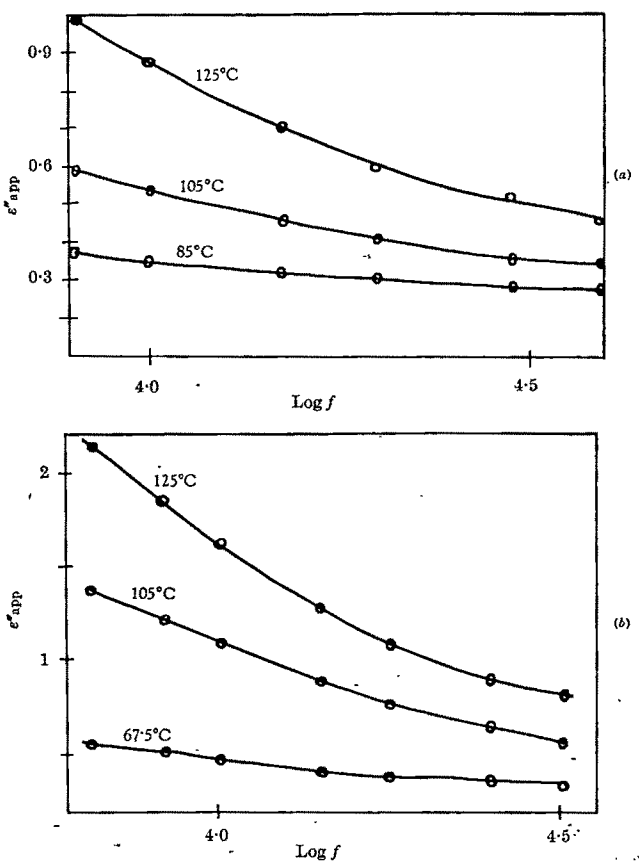


Fig. 2. Dielectric properties of partially hydrated chabazite. ε''_{app} versus log f. *a*, containing 4 per cent of zeolitic water; *b*, containing 11 per cent of zeolitic water

anhydrous sodium chabazite. On the other hand, for d.c. conductivity in anhydrous sodium-chabazite the larger energy of activation of 14–15 kcal was reported[5]. In another synthetic zeolite[3] it has also been found that the energy barriers for conduction differ considerably as between anhydrous and hydrated states, being larger in the anhydrous crystals. Thus, the loss peaks shown in Fig. 1 cannot with certainty be identified with intracrystalline d.c. cation jumps. Nevertheless, the interaction with water seems readily explained if the cause of the loss peaks is the sodium cations. It may, therefore, be that the loss peaks are due to sodium ion jumps in a partly hydrolysed surface skin on the sodium chabazite, or at dislocations or grain boundaries. It is interesting that

Ducros[6], in a hydrated calcium-rich chabazite containing a little sodium, found two kinds of loss peak associated with energy barriers of 16·1 and 8·0 kcal per Avogadro number of unit loss processes. The larger of these peaks was attributed to Ca^{++} and the smaller, possibly wrongly in view of ref. 4, was attributed to water. However, neither of the relaxation processes found by Ducros can be related to that which we have observed.

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Mass Spectrometric Investigation of Reactions of Electronically Excited Neutral Particles with Alkali Metal Atoms

Recent development of radiation chemistry, gaseous electronics, plasma physics, and reaction kinetics in flames requires full understanding of elementary reactions occurring in highly energized systems. A substantial class of these reactions represent those between excited neutral particles and atoms or molecules (collisions of the second kind). However, knowledge of these processes is so far very limited, even though their important role, for example, in ionization phenomena in gaseous mixtures (Penning effect) has been known for a long time[1]. The need for further investigation became particularly apparent when collisions of the second kind were found, in which unexpected ions like $NeAr^+$, ArN_2^+, ArI^+, CH_4Xe^+, $C_2H_2Hg^+$ or N_2Hg^+ are formed[2–6].

We examined the reactions occurring in mixtures of noble gases and nitrogen with alkali metal vapours. The reactions occurred in an ordinary ionization chamber of the Nier type mass spectrometer[7]. The alkali metal vapour was introduced into the reaction region from a small furnace attached from one side to the ionization chamber. During experiments, the temperature of the furnace was controlled to ensure a constant pressure of the alkali metal vapour in the ionization chamber of the order of 10^{-5}–10^{-4} mm mercury. The total pressure of gaseous mixture varied up to 2×10^{-3} mm mercury. No special provision for homogenizing the electron energy was made.

The type of reaction and the nature of reacting particles were identified from the dependence of product ion current on the pressure of gaseous reactants, from appearance potentials of the ions formed, and from the shape of the molecular ion efficiency curves. The results are summarized in Table 1.

Table 1. Molecular Ions formed in Mixtures of Alkali Metal Vapour with Noble Gases and Nitrogen

Mixture	Product ion	Appearance potential (V)	Mixture	Product ion	Appearance potential (V)
He + Li	$HeLi^+$	19·3 ± 0·5	Kr + K	KrK^+	9·9 ± 0·5
Ne + K	NeK^+	16·7 ± 0·5	Kr + Cs	$KrCs^+$	9·8 ± 0·5
Ar + Li	$ArLi^+$	?	Xe + Na	$XeNa^+$	8·6 ± 0·5
Ar + Na	$ArNa^+$	11·3 ± 0·5	Xe + K	XeK^+	8·6 ± 0·5
Ar + K	ArK^+	12·0 ± 0·5	N_2 + Na	N_2Na^+	7·1 ± 0·5
Ar + Cs	$ArCs^+$	11·5 ± 0·5	N_2 + K	N_2K^+	7·3 ± 0·5
Kr + Na	$KrNa^+$	10·1 ± 0·5	N_2 + Cs	N_2Cs^+	6·8 ± 0·5

All the reactions found are of the same type

$$X^* + Me \rightarrow XMe^+ + e \qquad (1)$$

where X^* denotes an electronically excited atom (molecule) and Me an alkali metal atom. These reactions presumably occur together with concurring reactions leading to the ionizations of the metal atoms:

$$X^* + Me \rightarrow Me^+ + X + e \qquad (2)$$

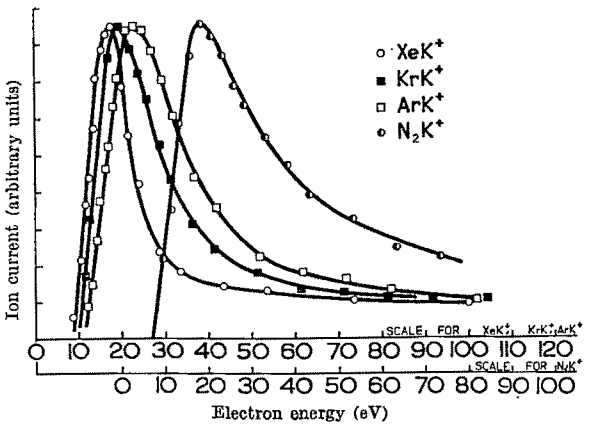


Fig. 1. Efficiency curves for ions XeK^+, KrK^+, ArK^+ and N_2K^+ (electron energy scale for N_2K^+ is shifted)

However, whereas the occurrence of reactions of type (2) can be *a priori* expected (ionization potential of Me is lower than the excitation energy of X), reactions (1) have not yet been reported. The formation of a molecular ion XMe^+ in reaction (1) is made possible by the fact that the electron released carries away the heat of reaction in the form of kinetic energy and stabilizes thus the molecular ion XMe^+.

In all mixtures with noble gases the appearance potentials of molecular ions XMe^+ correspond within experimental error to excitation potentials of the known metastable states of noble gas atoms (19·81 V for $He(2^3S)$, 16·61 V for $Ne(^3P_2)$, 11·54 V for $Ar(^3P_2)$, 9·91 V for $Kr(^3P_2)$ and 8·31 V for $Xe(^3P_2)$ (ref. 8). In mixtures with nitrogen the appearance potentials correspond to excitation potential of the metastable $A^3\Sigma_u^+$ state of N_2(7 V) (ref. 9). Reactions with molecules (atoms) in higher lying long-lived states cannot be excluded, but the measurement of appearance potentials of XMe^+ alone is not sufficient to decide the question.

The efficiency curves of XMe^+ ions exhibit in the case of reactions in noble gases a maximum at electron energies about 17–25 eV and decrease markedly with increasing energy. In the mixtures of alkali metals with nitrogen the maxima on efficiency curves are located at 18 eV (Fig. 1). The efficiency curves for various metals and the same gas coincide, within experimental error. The shape of the curves resembles that of the excitation functions of optically forbidden transitions. In our case, under the given pressure conditions, these curves reflect the abundance of excited metastable particles formed by electron impact both directly from the ground state via optically forbidden transitions and by cascade processes from higher lying excited states. The participation of meta-

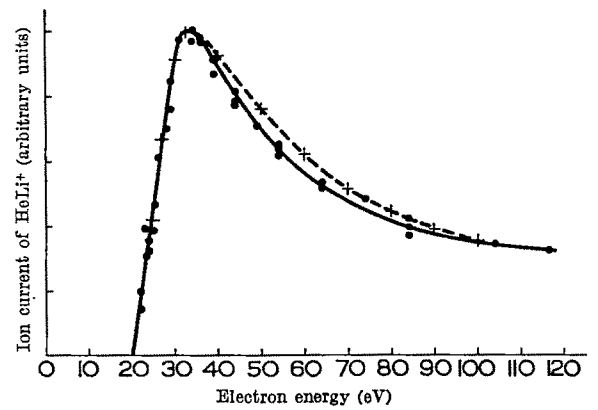


Fig. 2. Efficiency curve for $HeLi^+$ ion. Crosses and dashed line indicate the position of Dorrenstein's excitation curve for metastable helium atoms

stable particles in reactions (1) is supported also by the fact that the efficiency curve for $HeLi^+$ ion is practically identical with the electron excitation curve for metastable helium atoms which was obtained in a quite different way from secondary electron emission due to the arrival of separated metastable atoms at a metal surface[10] (Fig. 2).

The reaction cross-section may be at present only roughly estimated. Taking the mean path of the excited particle in the reaction zone $\approx 0{\cdot}25$ cm and the ratio of the corresponding excitation and ionization cross-sections (in the maximum of the excitation function) for argon $Q_e/Q_i \approx 0{\cdot}1$, the cross-sections for formation of $ArNa^+$, ArK^+ and $ArCs^+$ are $1{\cdot}5 \times 10^{-15}$ cm^2, 2×10^{-15} cm^2 and 5×10^{-15} cm^2, respectively. These results indicate that reactions (1) may effectively compete with ionization processes (2) for which a cross-section of the order of the gas kinetic cross-section may be expected.

It can be concluded that the formation of molecular ions in reactions with excited neutrals is a rather common process. Further investigation of these reactions promises to discover new ionic species, the knowledge of which may be of value in the aforementioned fields. Moreover, the formation of molecular ions may serve as a mean for the detection of excited molecular states. For example, if the molecular beam of metastable particles is used and the kinetic energy of the electron released in reaction (1) is measured, there may be obtained useful data on the energy levels of metastable biatomic and polyatomic molecules, data on the shape of excitation curves for intercombination transitions and an estimate of the bond strengths in new, unusual ions. Preliminary experiments in these directions have been commenced.

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Equilibrium Coverage of Copper by Chemisorbed Hydrogen

Several instances have been given in the literature[1-3] in which copper will catalyse the *para-ortho* hydrogen conversion. This process requires the chemisorption of hydrogen, regardless of the reaction mechanism which may be prevailing.

Recently[4] the chemisorption of hydrogen by copper has been examined calorimetrically and volumetrically and the equilibrium surface coverage was found to be at a value of $\theta = 0{\cdot}15$, where the heat of adsorption was determined as 10 kcal/mole.

Confirmation of these values has been obtained in the course of a study of the kinetics of the *para-ortho* hydrogen conversion on evaporated metal films. It has been shown previously[3] that an investigation of the pressure dependency of the *para-ortho* hydrogen conversion enables a determination of the heat of adsorption to be made. In this manner the heat of adsorption of hydrogen on copper at equilibrium coverage was found to be 8·0 kcal/mole.

The Stevenson equation[5] has been used[6] to calculate a value for the heat of adsorption of hydrogen on copper, giving a value at zero surface coverage of $\Delta H_{\theta=0} = 25{\cdot}6$ kcal/mole. An experimental value of 28·0 kcal/mole has been reported[6] for silica-supported copper. The dipole moment of the hydrogen–copper bond may be calculated from the electronegativities of the elements as 0·73 Debye units, using Sanderson's[7] value for the electronegativity of hydrogen and calculating that for copper[6] from the work function as given by Michaelson[8].

The fall in the heat of adsorption with increasing surface coverage is related to the change in work function by the following equation[9]:

$$\delta(\Delta H) = e\Delta V \qquad (1)$$

where e is the electronic charge and ΔV the change in work function. The change in work function may be related to the surface coverage in the following manner:

$$\Delta V = 4\pi\sigma\mu\theta \qquad (2)$$

where σ is the number of surface sites per cm^2 and μ the dipole moment of the bond.

In the present case values of ΔH at equilibrium coverage of 10 kcal/mole and 8 kcal/mole were available, and the change in work function was calculated from equation 1. This value was substituted in equation 2 to obtain a value for the equilibrium surface coverage, θ. A value of σ for the 111 plane of copper was used. These calculations give, respectively, values of $\theta = 0{\cdot}17$ and $\theta = 0{\cdot}15$, which may be compared with the previously reported value of $\theta = 0{\cdot}15$.

The calculations assume no change in the dipole moment of the copper–hydrogen bond with increasing surface coverage. This seems reasonable as the surface of copper is sparsely populated and has been shown to be correct for hydrogen chemisorbed on nickel[10].

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Behaviour of Polarons produced by the Action of Ionizing Radiation in Aqueous Media

It has been suggested[1,2] that the action of, for example, γ-rays on water leads primarily to the formation of radiation-produced electrons and the corresponding positive holes and that these electrons, and possibly also the holes, become self-trapped in the polar medium, water, leading to what should be regarded as 'polarons', in the sense of Landau, as discussed by Mott[3] and Pekar[4]. Afterwards, some definite experimental evidence was produced for the negative polaron[5,6]. This was based on its chemical reactions and reactivity, which in many cases (for example, with CO_2, leading to the formation of the CO_2^- radical ion[6]) were found to be different from hydrogen atoms which are known to be present in irradiated aqueous solutions under certain conditions. The chemical evidence concerning negative polarons has now been corroborated by the recent work using the methods of pulse radiolysis[7]. In pure water, the majority of the positive and negative polarons, $(H_2O)^+$ and $(H_2O)^-$, annihilate each other according to:

$$(H_2O)^+ + (H_2O)^- \rightarrow 2H_2O \qquad (1)$$

On the other hand, as suggested previously[1,2], the interaction of two polarons of the same species ('bipolarons') can lead to the 'molecular yield' processes. In the case of

the negative polarons, the formation of molecular hydrogen, according to:

$$(H_2O)^- + (H_2O)^- \rightarrow H_2 + 2OH^- \quad (2)$$

has now been confirmed by the recent pulse radiolysis work of Dorfman and Taub[8]. Formation of such bipolarons, even if rather short-lived, would be energetically favoured if the repulsion between the like charges is overcompensated by the increased polarization energy of the doubly charged species.

The theory of Pekar[4] allows to give an estimate of the region of optical absorption of the polaron or rather a relation between this and an effective mass of the electron (μ), according to the equation:

$$\hbar\omega_m \approx 0{\cdot}07\ \frac{\mu e^4 C^2}{\hbar^2} \quad (3)$$

where ω_m is the maximum (circular) frequency of absorption; $C = (\varepsilon_0^{-1} - \varepsilon_s^{-1})$, where $\varepsilon_0 = n^2$ is the optical dielectric constant and ε_s the static dielectric constant; $C \approx 0{\cdot}55$ for water at room temperature; the other symbols having their usual significance. In this theory the polar medium is regarded as a continuum and characterized by its optical and static dielectric constants; it is therefore applicable, in this approximation, not only to polar crystals but also to polar liquids.

Taking the observed maximum absorption of the negative polaron at $\lambda_{max} = 7000$ Å, one can obtain an estimate of the effective mass of the electron from equation (3) as $\mu \sim 3$, in units of the rest mass of the electron. A similar value can also be estimated from Feynman's theory[9].

The above value for the effective mass of the polaron leads to an estimate for its radius of the order of $r_p \sim 5$ Å, which means that the electron is not associated with a particular molecule but, corresponding to its de Broglie wave-length, is 'smeared out' over a number of water molecules (~ 10). These polarons could thus be written as $(H_2O)_n^+$ and $(H_2O)_n^-$; for the purpose of this and of our previous publications the subscript is omitted for simplicity.

It is clear from the above that it is more appropriate, therefore, to designate the self-trapped electron as a 'polaron' rather than as a 'hydrated electron', as the latter expression bears an obvious connotation to a hydrated ion, where the charge sits on a specific ion, with the water dipoles oriented around it.

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Separation of Gases by Flow under Pressure through Porous Membranes

The separation of gases by permeation through membranes has been reviewed by several authors[1–4]. Since the observed separations do not always follow Graham's law, the mechanism is explained on the basis of solubility, diffusion and re-evaporation in the case of flow through 'non-porous' plastic films, and sorbed-flow through porous media.

The concept of preferential sorption at the gas-solid interface together with the flow of the interfacial fluid through capillary pores of size equal to, or less than, a critical diameter characteristic of the system offers a new approach[5]. The experimental technique consists simply in letting the gas mixture flow under pressure through an appropriate porous medium; the gas leaving the porous medium is enriched in the preferentially sorbed constituent. For a given separation problem, the physicochemical nature of the film material and the number of pores of appropriate size on the area of the film at the interface constitute the governing criteria for the development of films with superior separation and flow characteristics.

There is sufficient evidence in the literature to show that the physicochemical nature of the film surface affects the degree of separation of different gases[6]. Oxygen permeates faster than nitrogen in rubber and plastic films[7–9], and the reverse is true of flow through porous glass[10]. Different samples of the same type of film obtained from different sheets varied widely in their relative permeability values[9]; this is understandable since preferential sorption is a surface property.

Flow through microporous media has been discussed by Rutz and Kammermeyer[11]. For the mechanism of separation proposed here, gas flow through the films can still be laminar, and its rate can be increased, without affecting the degree of separation, by increasing the number of pores of appropriate size on the film surface. The effects of the different operating variables on the separation and flow characteristics may be expected to be similar to those discussed earlier with respect to liquid-solid systems[5].

A few experiments with air have recently been carried out to test the foregoing approach to gas separation. Porous cellulose acetate membranes (supplied by Schleicher and Schuell Co.) were initially shrunk in hot water, then dried and used. The film was held on a porous stainless steel backing plate suitably mounted on to a pressure chamber connected to a compressed air cylinder. The other side of the porous plate was open to the atmosphere. The experiments were carried out at laboratory temperature under static conditions of compressed air on the film surface. The compositions of the feed and permeated air were analysed using the mass spectrometer. The permeated gas was collected over water and its rate determined. A set of typical results is given in Table 1.

Table 1

Film No.	Operating pressure psig.	Flow-rate of permeated gas (c.c./min)	Composition of permeated gas* (%)		
			N_2	O_2	A
1	1,000	1·0	76·7	22·4	0·9
2	250	0·19	74·2	24·7	1·1
2	500	0·42	72·0	26·8	1·2
3	1,000	0·15	73·4	25·6	1·0
3	1,500	0·22	73·7	25·3	1·0
4	1,000	(not determined)	71·0	27·2	1·8

Area of film surface: 9·0 cm². Feed air composition* (per cent): nitrogen, 78·1; oxygen, 21·0; argon, 0·9.
* The feed air contained about 0·06 per cent carbon dioxide. The permeated gas showed an enrichment in carbon dioxide also. Since the analysis for carbon dioxide is not considered accurate in this investigation, the gas compositions here are given on carbon dioxide-free basis.

The permeated air was found to be enriched in oxygen, argon and carbon dioxide. The degree of separation increased with decrease in average pore size. Increase of operating pressure increased the rate of production of enriched gas almost proportionately without affecting the degree of separation. Even though the experimental conditions used here are far from ideal for the given separation problem, yet the results obtained are significant both with respect to the degree of separation and flow rate; they illustrate the essential validity of the new approach.

The foregoing technique is obviously applicable for the separation of vapours also[12]; in this case, the relative ease of condensibility of the vapour components under the

experimental conditions becomes an important additional factor affecting their separation and flow characteristics.

Thus, based on the concept of preferential sorption and capillary flow through critical and sub-critical pores, it seems possible to develop a general process for the separation of substances in gaseous, vapour or liquid solutions. It is clear that properly oriented researches on the precise physicochemical criteria of preferential sorption and the development of process techniques for making suitable high flow porous films can contribute much to the emergence of a new and versatile field of separation technology.

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Calculation of Valence State Energies from Thermochemical Data

THE predictive power inherent in the concept of intrinsic bond energies has led to many attempts to calculate the energy of the hypothetical valence state of an atom in a molecule[1–7]. The intrinsic bond energy I can be defined[1] by the equation:

$$\Delta H_f = \Sigma V - \Sigma I \qquad (1)$$

where ΔH_f is the atomic heat of formation of a molecule, V is the energy of the valence state of an atom, and the summation is over all atoms and bonds in the molecule. The valence state of an atom in a molecule can be defined as the hypothetical state in which the free atom would find itself if the bond involved were broken without any change in the electronic configuration of the atom. Long has summarized the attempt to calculate the valence state energy of the tetrahedral carbon atom from thermochemical data[1]. He concluded that the probable limits for the energy of this state are approximately 60–70 kcal/mole. This value is quoted[6] as the only experimental value presently available for this quantity. (In several communications with people in this field it has been brought to our attention that this number is still widely quoted.)

One of the primary purposes of this communication is to point out that the limits for the valence state energy of the tetrahedral carbon atom given by Long were based on a value for the heat of atomization of graphite that is no longer accepted. Whereas Long used a value of 125 kcal/mole, it is now known that the heat of atomization of graphite is 171 kcal/mole[8–10]. Long himself stated that if the controversy over the value for the latter atomization energy were resolved in favour of the higher quantity, then his conclusions were invalid. As often happens in science these qualifications have been commonly ignored when the value of 60–70 kcal/mole is quoted for the tetrahedral valence state energy of the carbon atom. If Long's procedure is applied with atomic heats of formation based on presently accepted thermochemical data the lower limit becomes 6·8 and 0·8 kcal/mole instead of the previously reported 58·6 and 48·4 kcal/mole.

It is instructive to consider why Long's valence state energies become trivial when correct values for the thermochemical data are inserted in his procedure. He used equation 1 to set up simultaneous equations of the type:

$$\Delta H_{f,CH_4} = V - 4I$$
$$\Delta H_{f,CH_3} = V - 3I$$

based on the assumption that the valence state energies and intrinsic bond energies were essentially equivalent for CH_4 and CH_3. This is apparently unjustified. In point of fact the bond distances in CH_4 and CH_3 are 1·094 (ref. 8) and 1·066 Å (ref. 11), respectively, indicating that the C–H bond in CH_3 is stronger than in methane. This point is also verified by the respective overlap integrals, calculated from Mulliken's tables[12]. The overlap for $sp^2\,\sigma - 1s$ at 1·066 Å is greater than that for $sp^3\,\sigma - 1s$ at 1·094 Å; it is believed that CH_3 is planar with sp^2 hybridization of the carbon atom[13,14]. The stronger C–H bond in CH_3 compared to CH_4 requires consideration of appreciably larger intrinsic bond energies for the CH_3 molecule, compared to CH_4.

Successful application of equation (1) to the calculation of the various valence state energies of carbon from thermochemical data requires either prior knowledge of the intrinsic bond energies, or exact relationships between the intrinsic bond energies in a dissociation sequence such as CH_4, CH_3, CH_2 and CH. Such information is not available at present. Actually, the only system that might presently be solved satisfactorily by Long's procedure is that for BeH_2 and BeH. Since the ground-state of the beryllium atom has the $2s^2$ electronic configuration, the principle of minimum energy for molecules requires that the beryllium atom in both BeH_2 and BeH be essentially sp hybridized, assuming that these molecules are mostly covalent. For the latter reason the intrinsic bond energies in both of these hydrides should be essentially equivalent, and V identical for the sp beryllium atom in both molecules, since the valence state energy is based on isolated atoms. One can then solve the following equations to obtain V and I.

$$\Delta H_{f,BeH_2} = V - 2I$$
$$\Delta H_{f,BeH} = V - I$$

Unfortunately the atomic heat of formation for BeH_2 has not been experimentally determined. Using an empirically estimated value of $-153 \pm 3{\cdot}5$ kcal/mole (ref. 15) for this quantity with -53 ± 7 kcal/mole for BeH (refs. 8, 16) leads to $V = 47 \pm 17$ kcal/mole and $I = 100 \pm 10{\cdot}5$ kcal/mole. Better values for these energy quantities can be obtained only when better thermochemical data are available.

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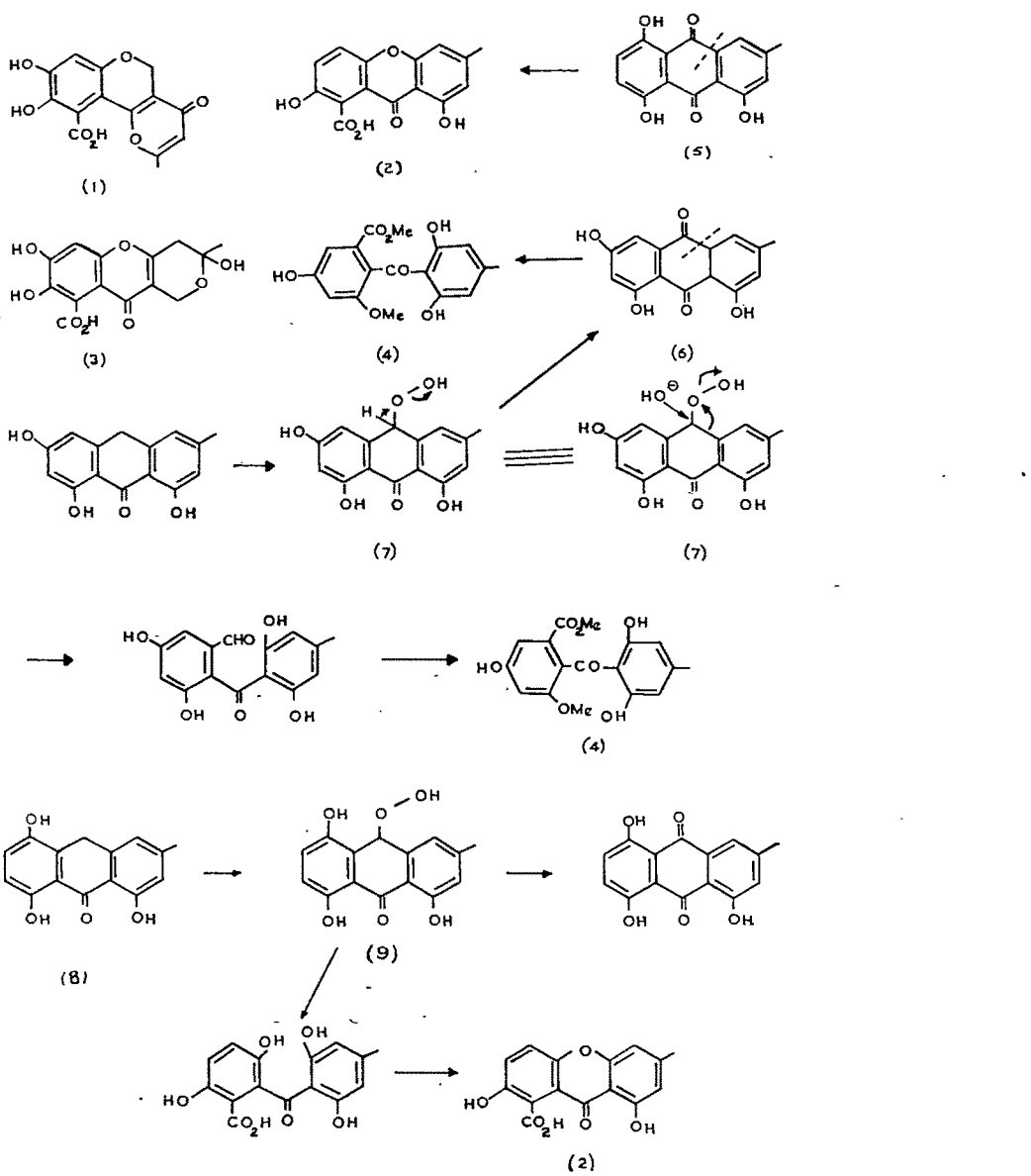
A Postulated Biosynthesis of Some 'Anomalous' Natural Phenolic Compounds

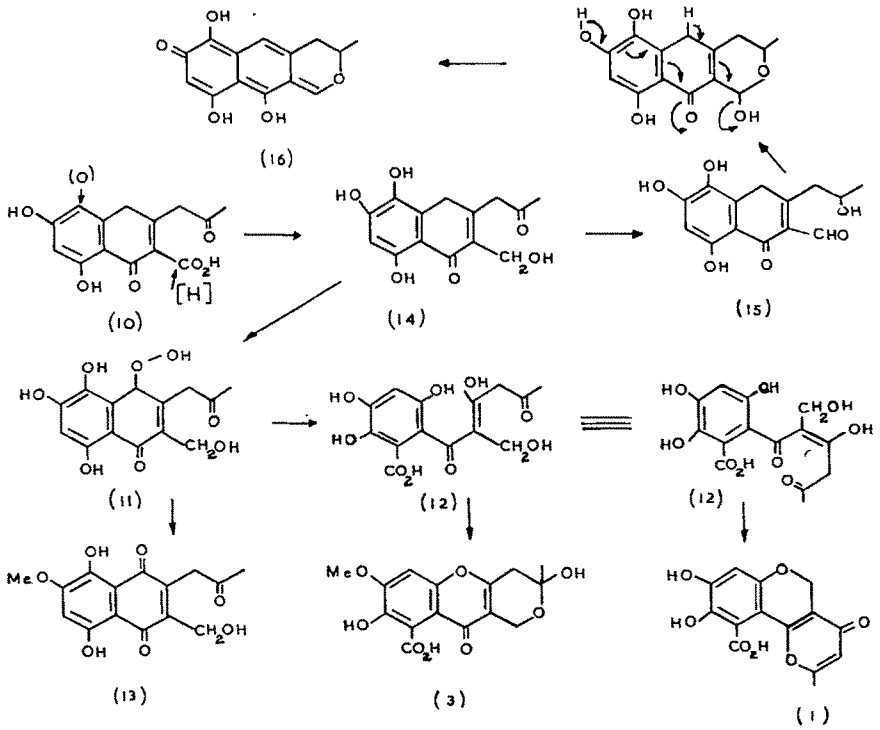
Considerable experimental evidence has been provided in recent years to substantiate Birch's biogenetic proposals[1] for the polyacetate derivation of a number of classes of naturally occurring phenolic compounds. However, certain phenolic substances are known which, although 'acetate-derived', cannot be formulated directly via a linear polyketide chain. Included in this 'anomalous' category are sulochrin (4), citromycetin (1), fulvic acid (3), and pinselic acid (2). To rationalize the structures of these compounds, Birch[1] and Thomas[2] have each postulated a branched polyketide precursor.

An alternative biogenetic pathway has been proposed[3] which involves an unelaborated 'oxidative cleavage' of well-known hydroxyanthraquinones. Thus, the structure of sulochrin and pinselic acid can be derived by application of this process to the anthraquinones emodin (6) and helminthosporin (5) respectively. These ideas are supported by the established co-occurrence of emodin 5-methyl ether and sulochrin[4]. It has further been suggested that citromycetin could be derived from oxyjavanicin (fusarubin) by a similar oxidative cleavage followed by cyclization[4,5].

It is proposed here that the oxidative cleavage reaction occurs at an earlier stage in the biosynthetic pathway by means of a rational mechanism based on plausible chemical analogies. Thus, in the case of sulochrin (4) the hypothesis envisages the initial formation of emodin anthrone by the usual cyclization of a linear polyketide chain, followed by oxygenation at the reactive meso-methylene position of the anthrone or anthranol to form the hydroperoxide (7). By the acceptable rearrangement mechanism shown, this hydroperoxide can be transformed to the sulochrin structure (for close chemical analogies see 6); the ready conversion of this intermediate to emodin is, of course, straightforward. In an exactly analogous manner pinselic acid (2) may be derived from helminthosporin anthrone (8) via fission of an intermediate hydroperoxide (9) followed by cyclization to produce the xanthone structure.

In the corresponding naphthalene series, cyclization of a linear polyketide chain in the usual manner would provide a naphthol tautomer (10), capable of transformation into the hydroperoxide (11). By the foregoing rearrangement this furnishes the intermediate (12), which possesses two





plausible modes of cyclization; the first leads to the structure established for fulvic acid (3) while the second produces citromycetin (1). Transformation of the hydroperoxide (11) without re-arrangement leads, of course, to oxyjavanicin (fusarubin) (13). It is also significant that the intermediate (14), without involving a change in oxidation state, can be transformed plausibly via (15) to the structure (16) already established for purpurogenone.

The ready oxidation of anthranols to hydroperoxyanthrones has indeed been demonstrated *in vitro* in these laboratories[7], and thus, practical precedent has been provided for the primary key-step in the above rationalizations. Further work is now in hand on these synthetic hydroperoxides to parallel the subsequent transformations.

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RADIATION CHEMISTRY

Ultrasonic Effect on Neutron-irradiated Inorganic Solid Compounds

Intense ultrasonic waves are known to cause chemical effects in liquids and solutions similar to those caused by ionizing radiation. It is also well known that neutron-irradiated solid compounds can be annealed by heating (thermal annealing) as well as by treatment with ionizing radiation (radiation annealing) and, in some cases, by ultra-violet light (photochemical annealing). Therefore, it was of interest to see if it were possible to anneal neutron-irradiated solid compounds under the influence of intense ultrasonic waves. As model substances for this purpose potassium sulphate and ammonium sulphate were chosen because their thermal and photochemical annealing behaviour had recently been examined[1–4].

Samples of solid potassium sulphate and ammonium sulphate, both reagent-grade, were irradiated for 3 and 4 weeks respectively in the thermal column of the *Bepo*-pile, Harwell, at a thermal flux of 10^{10} n.s.$^{-1}$ cm^{-2}. As an ultrasonic source a quartz-oscillator with a frequency of 800 kc/s and an output power of 2 W/cm^2 was used. The samples of the neutron-irradiated material (about 500 mg) were kept in thin nylon bags in a suspension of water-free carbon tetrachloride. The bags were closed so that no air bubbles were present and then placed in a beaker, having a polished bottom, containing carbon tetrachloride. For a good join of the beaker to the oscillator glycerine was used. The duration of the ultrasonic treatment was varied from a few minutes up to 50 h and the temperature of the samples kept between 18° and 20° C by a water-cooling system.

Because of the recoil energy of the ^{35}S atom after the nuclear reaction $^{34}S(n, \gamma)^{35}S$, a part of the ^{35}S is present in form of sulphite. The separation of sulphate and sulphite as well as the separation of ^{32}P and ^{33}P, which are also produced ($^{32}S(n, p)^{32}P$, $^{33}S(n, p)^{33}P$), was achieved by using suitable inactive carriers and hold back-carriers, as described earlier[1,2]. The activity of ^{35}S of the sulphate and the sulphite was measured by a Geiger–Müller counter with a very thin window. The standard deviation was less than 1 per cent.

It was found with the neutron-irradiated potassium sulphate that the retention (ratio between ^{35}S activity in the sulphate form and the total ^{35}S activity) at first rises rapidly with duration of ultrasonic treatment, reaching a plateau of between 8 and 12 h, and then becomes successively smaller. Some typical results are shown in Table 1.

From these experimental results it can be assumed that ultrasound causes two processes in the neutron-irradiated potassium sulphate, namely, 'ultrasonic annealing' and

Table 1. Retention (*R* per cent) of Neutron-irradiated Potassium Sulphate as a Function of Time of Ultrasonic Treatment

Duration of ultrasonic treatment (h)	Without	2	8	12	25
% *R*	79·0	83·5	89·5	90·3	82·7

'ultrasonic damage' ('phonolysis'). In other words: the introduction of vibratory motion at ultrasonic frequencies into the neutron-irradiated potassium sulphate leads to a significant annealing process simultaneously with a damage effect. Only a small part of the fragments formed due to the recoil after the (n, γ)-process, possibly that which has a smaller activation energy, seem to be annealed under the given circumstances. As soon as this process has reached its plateau, phonolysis becomes more and more visible. Both processes, ultrasonic annealing and phonolysis, are supposed to take place simultaneously and to compete with one another.

From the neutron-irradiated ammonium sulphate the bigger crystals (corn size 1–2·5 mm) have been separated and the smaller ones ground to a corn size of 10^{-2} to 10^{-1} mm. The ground crystals showed under the influence of intense ultrasonic waves a small rise of retention in comparison with the selected rough crystals. Both parts, fine ground and rough crystals, have been treated separately in small portions by ultrasound as described above.

Table 2 shows some average results.

Table 2. Dependence of Retention (*R* per cent) of Neutron-irradiated Ammonium Sulphate on Duration of Ultrasonic Treatment

Duration of ultrasonic treatment (h)	Without	2	10	20
Rough crystals (1–2·5 mm) *R* %	59·6	61·8	57·0	54·6
Ground crystals (10^{-2}–10^{-1} mm) *R* %	61·2	60·5	52·2	46·7

It has been found that the effect of ultrasound on the ground crystals was much higher than in the rough ones. Obviously the displacements caused by the grinding-process of the fine crystals possess high absorption properties for ultrasound, which leads to more prominent chemical effects.

It was established recently[4] that hydrazine has been formed in solid ammonium sulphate under the influence of neutron irradiation. This can be explained due to both nuclear recoil and the chemical effect of the γ-radiation in the pile. Therefore, the amount of hydrazine produced depends on the dose of neutrons as well as on the dose of γ-radiation. Hydrazine formation was also observed when neutron-irradiated ammonium sulphate was post-irradiated with ultra-violet light[4].

Apart from the above it was found that small amounts of hydrazine were also produced by ultrasonic treatment of inactive finely ground ammonium sulphate. By ultrasonic treatment of neutron-irradiated ammonium sulphate an additional amount of hydrazine is found. Its yield in this case is a function of the duration of the ultrasonic treatment. The curve for the rough crystals shows a plateau, while it passes through a maximum in the case of the finely ground crystals. For the explanation of this fact one can assume that as a result of the higher ultrasonic absorption in the fine crystals, which leads to an increased formation of hydrazine, an increase of its diffusion is also achieved. Consequently the hydrazine can react more efficiently with the recoil fragments of the sulphate and reduce them to sulphite, and on the other hand, because of the small size of the crystals, the hydrazine can also diffuse out more easily. It is also very probable that simultaneously with these processes the effect of ultrasonic damage appears as with potassium sulphate. The hydrazine in all samples was determined after the method of Watt and Chrisp[5]. Table 3 shows some experimental results.

Table 3. Formation of Hydrazine in Solid Ammonium Sulphate by Neutron Irradiation and Ultrasonic Treatment

Duration of ultrasonic treatment (h)	Without	10	20	50
μg N_2H_4/mg $(NH_4)_2SO_4$ in ground crystals without neutron irradiation	0·0	0·003	0·004	0·006
μg N_2H_4/mg $(NH_4)_2SO_4$ in ground crystals neutron irradiated	0·275	0·370	0·361	0·348
μg N_2H_4/mg $(NH_4)_2SO_4$ in rough crystals neutron irradiated	0·275	0·310	0·326	0·334

Finally, it can be stated that by ultrasonic treatment of neutron-irradiated solid compounds, of the same type as potassium sulphate, two processes are in competition, namely, ultrasonic annealing and ultrasonic damage. In the case of neutron-irradiated solid ammonium salts an additional reducing process occurs, caused by the hydrazine, which can be formed due to the γ-radiation and recoil effects in the pile as well as because of the ultrasonic influence.

I thank Prof. B. Karlik for her advice, Prof. E. Schmid for the loan of an ultrasonic apparatus and for helpful assistance by Dr. D. Oelschlägel in carrying out the ultrasonic treatment. I also acknowledge the financial support awarded by Bundeskanzleramt of Republic Austria, Sektion IV, Verstaatlichte Betriebe.

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Radiation Yield of Citraconic and Mesaconic Acids in Aqueous Solution

Experiments have been carried out in order to ascertain whether the double-bound radiolability of citraconic acid is different from that of its isomer mesaconic acid in aqueous solution. Mesaconic and citraconic acids were purchased from Fluka and were used without further purification. The water was prepared by distillation of de-ionized water from potassium permanganate. The solutions were irradiated with a cobalt-60 source at $(4{\cdot}20 \pm 0{\cdot}12) \times 10^{15}$ eV/(ml. × min). The source calibration was made with the Fricke dosimeter[1]. The determinations of mesaconic and citraconic acids were carried out spectrophotometrically at 220 mμ by a Beckman spectrophotometer model *DU*. When carboxylic group is conjugated with C=C, its absorption band in the ultra-violet regions is shifted to longer wave-lengths and the molecular extinction coefficient is increased at the same time by a factor of about 10^2 (ref. 2).

Therefore the decrease of optical densities of our irradiated solutions is due mainly to the opening of double bound. Indeed the solutions lose at the same time their strong absorption band and their ability to reduce potassium permanganate while their acidity is little reduced; for example, for a 5×10^{-2} M solution of irradiated citraconic acid the decrease of optical density was found to be 65·5 per cent and that of acidity was 2·7 per cent; for a 5×10^{-2} M solution of irradiated mesaconic acid the analogous data are 62·0 and 8·0 per cent.

Solutions of mesaconic and citraconic acids of the following molarities were irradiated in opened glass test

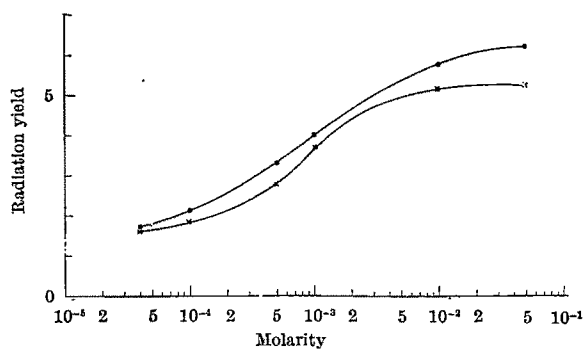


Fig. 1. ×, Citraconic acid; ●, mesaconic acid

tubes at room temperature: 4×10^{-5}; 10^{-4}; 5×10^{-4}; 10^{-3}; 10^{-2}; 5×10^{-2}. The G values (number of events for every 100 eV absorbed) were calculated at every concentration by least-squares method from the initial slope of the plot of destroyed molecules number against absorbed eV. The G values obtained are reported in Table 1 and plotted in Fig. 1.

Table 1

Initial molarity	G values Citraconic acid	Mesaconic acid
4×10^{-5}	1·56 ± 0·02	1·73 ± 0·06
10^{-4}	1·84 ± 0·02	2·13 ± 0·10
5×10^{-4}	2·76 ± 0·11	3·26 ± 0·12
10^{-3}	3·69 ± 0·09	3·99 ± 0·07
10^{-2}	5·15 ± 0·10	5·74 ± 0·04
5×10^{-2}	5·22 ± 0·06	6·19 ± 0·07

Table 2

Initial molarity	Maleic acid	Fumaric acid
$5{\cdot}00 \times 10^{-4}$	2·50 ± 0·02	2·54 ± 0·03
$5{\cdot}00 \times 10^{-4}$	3·64 ± 0·03	3·52 ± 0·02
$5{\cdot}00 \times 10^{-3}$	6·45 ± 0·08	6·01 ± 0·16
$1{\cdot}25 \times 10^{-2}$	8·87 ± 0·15	8·99 ± 0·05
$2{\cdot}5 \times 10^{-2}$	9·68 ± 0·14	10·75 ± 0·36

It can be seen that the radiation yields increase for both compounds with the concentrations of the solutions to attain almost a saturation value and that the radiation yields of the two isomers are different; particularly the radiation yields of *cis* form are lower than those of relative *trans* form. It is interesting to compare these results with those which we obtained[3] for the fumaric and maleic acids. They are summarized in Table 2. It can be observed that no difference exists between their G values in the range of experimental concentrations and that the substitution of a hydrogen atom of fumaric and maleic acids with a methyl group lowers the radiation yield of the double bound. This effect is more significant for the *cis* isomer. Therefore it seems that a steric factor plays a part in the double-bound stability of γ-ray-irradiated solutions of fumaric, maleic, citraconic and mesaconic acids.

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Light Emission during the Neutralization Reaction in Presence of a Chemiluminescent Radical Indicator

Recent publications by Stauff *et al.*[1-3] of the results of experiments in which light emission during neutralization of sodium hydroxide with sulphuric acid was observed have prompted us to submit herewith the results of somewhat similar experiments of our own which were conducted some time ago.

In the course of experiments designed to determine whether the emission observed by Audubert[4] during neutralization extended into the visible wave-length, it was discovered that greater than expected yields of light were obtained. Since addition of chemiluminescent substances increased the yield without altering the characteristics of the light pulses the light was attributed to contamination of the solutions used with minute amounts of such substances. So far as we were then aware this type of 'activation' of chemiluminescence had not previously received attention. Since chemiluminescence of the phthalazdiones is observed as a result of reactions involving free radicals[5-8] some experiments were performed to try to determine whether in this case the flash of light emitted was the result of radical formation initiating an oxidation

Table 1. Magnitude of Light Flashes following Sequential Injection of 0·5 ml. Portions of Various Solutions into 5 ml. of 0·2 M Sodium Hydroxide containing Initially 0·15 μmole of Luminol

Substance added	Quantity (μmoles)	Maximum intensity * (galv. deflexion) (amp × 10^9)
None ('steady state')	—	1·0
'Versene' †	6	1·9
'Versene' †	6	0·09
KCl	100	0·12
KCl	100	0·12
Demineralized water	—	0·09
HCl	100	> 300
CCl_3COOH	100	> 1,000
None ('steady state')	—	0·45
Luminol	0·6	1·8
NaOH	400	1·8
HCl	100	2,800
CCl_3COOH	100	2,800
None ('steady state')	—	1·0
KNO_3	24	1·0
HCl	100	0·06

* A reading of approximately 3×10^{-9} amp is given by a blue light source emitting 10^8 quanta per second.
† Added as the tetrasodium salt.

of the luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) used. The luminol was prepared by the method of Drew and Pearman[9], but commercially obtained material was also found to be effective.

Table 1 shows the effect of injection of 0·5 ml. portions of 0·2 M acid into 5 ml. of 0·2 M sodium hydroxide containing 0·15 μmole of luminol in front of a photomultiplier arrangement similar to that described by Cormier[10] and capable of easily measuring light emission from a source emitting about 10^7 quanta per second of blue light. Air was bubbled through the solutions throughout the experiments. Also shown is the effect of adding tetrasodium ethylenediamine tetraacetate, a substance the presence in solution of which improves the reproducibility of the results. Figs. 1 and 2 show characteristic recordings of results with and without the chelating agent. It may be noted from Table 1 that injections of potassium chloride or of demineralized water did not cause a light flash. The type of acid does not seem to be important as is shown by the response to trichloroacetic acid. Other acids tested which gave positive results were sulphuric, nitric, acetic, carbonic and succinic acid. The alkali may be replaced by sodium carbonate.

In order to test for radical involvement, additional luminol was injected (as shown in Table 1) until sufficient light intensity extending over a period of time was obtained. The addition of a small amount of potassium nitrite had no effect but the subsequent injection of acid brought the light emission to background at once instead of causing a flash. In the absence of more likely alternative explanations it may be assumed that production of NO during acid addition provided an effective radical trapping agent. Similarly, the addition of mercaptoethanol in quantities insufficient to remove the dissolved oxygen dimmed or abolished both the 'steady state'

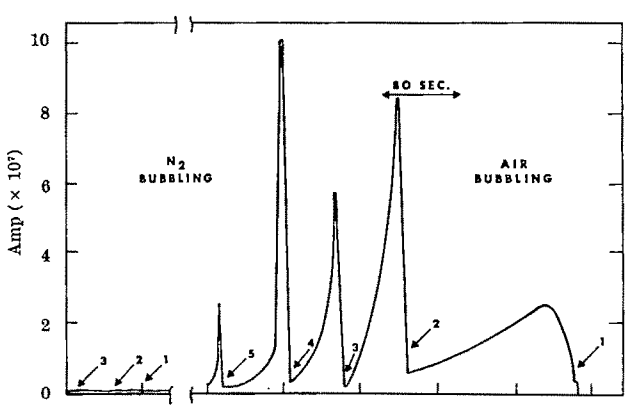


Fig. 1. Typical light pulses following injection of 0·5 ml. portions of 0·2 molar HCl into 5 ml. of 0·2 molar NaOH containing 0·15 μmole of luminol. The injections were made sequentially as indicated by the numbered arrows. Also shown is the effect of replacing air by nitrogen

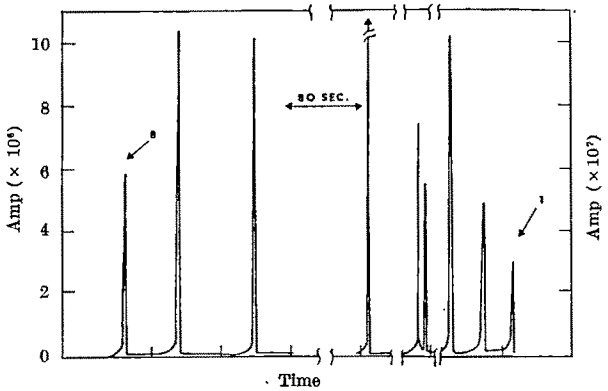


Fig. 2. Effect of versene on light pulses during neutralization in the presence of luminol. Conditions same as for Fig. 1 except that 6 μmoles of tetrasodium ethylenediamine tetraacetate were added. The fourth injection was divided and the full-scale deflexion is 3×10^{-6} amp

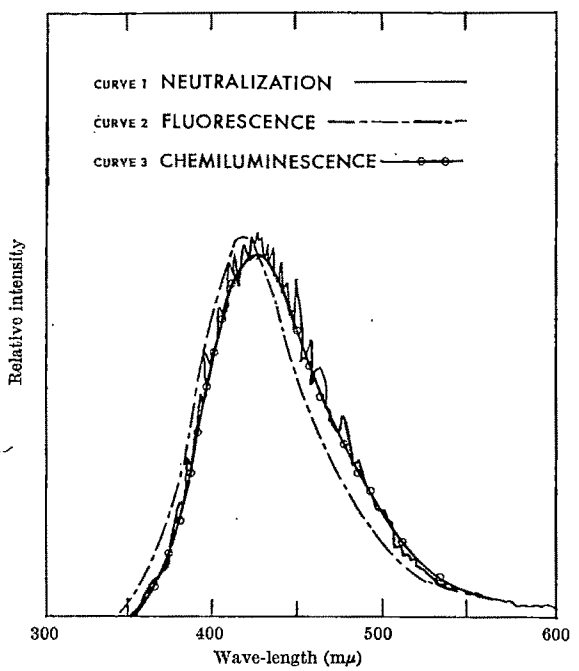


Fig. 3. Spectrum of light emitted during partial neutralization of 0·2 M NaOH with 0·2 M HCl in the presence of about 2×10^{-4} molar luminol compared with chemiluminescence spectrum and with fluorescence spectrum of luminol under similar *p*H conditions

emission and the neutralization flashes. Replacement of the air with nitrogen drastically reduced the intensity or abolished the flashes also (Fig. 1).

To show conclusively that the emission observed was that of the typical chemiluminescent oxidation of luminol an experiment was conducted in which 0·2 M hydrochloric acid was mixed with 0·2 M sodium hydroxide 3×10^{-4} M in luminol in a continuous-flow apparatus in such a manner that there always remained a slight excess of alkali. The mixing tube was placed in front of the slit of a monochromator (Aminco-Keirs spectrophosphorimeter) and the spectrum recorded during the mixing. The results of this experiment are shown in Fig. 3 (curve 1), together with the emission spectrum of the fluorescence of the sodium hydroxide solution of luminol excited by light of wavelength 350 mμ from the same tube placed in the same position (curve 2). The chemiluminescence emission spectrum was also taken by adding an excess of $K_2S_2O_8$ as a source of 'radicals' to the same tube. This is shown in curve 3. The results leave no doubt that the neutralization emission is due to chemiluminescence and not to fluorescence of luminol excited by some other means.

These results appear to suggest that hydroxyl radicals are present in not insignificant amounts during the formation of water in the neutralization reaction. The only alternative explanation that appears to us to have any probability is that the acid may change the state of some very effective catalyst present as a trace impurity. Such a catalyst might be expected to increase light emission in the presence of small amounts of peroxide. In this connexion freshly prepared luminol solutions were equally as effective as those which had stood for some days.

Whatever explanation is ultimately found to be the correct one, it is clear that neutralization may have unexpected effects on organic solutes. Even though quantitatively the effect may appear small when compared with the total chemical change, alterations in materials of high molecular weight might be profound. Should this phenomenon prove to be general, its importance in biological material may be highly significant, especially with respect to spontaneous mutation and to senescence.

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BIOPHYSICS

Adsorption Chromatography of Direct Azo Dyes

Direct azo dyes contain in most cases a number of coloured by-products which may adversely influence the shade of the dye, its stability in light and distort its biological action. In view of the importance of direct azo dyes in industry as well as in biology and medicine a number of authors[1–10] worked on the problem of their purification. So far it has not been possible to separate these dyes completely (strong adsorption of dyes on cellulose in paper chromatography or electrophoresis, losses during crystallization, etc.). This communication describes a simple method of separation which excludes use of cellulose. Tests with agar electrophoresis, though there was some movement of dyes, did not lead to satisfactory separation. Therefore, I carried out adsorption chromatography on aluminium oxide. This method, though it has been frequently used for the separation of other dyes[11–15], has not been used in practice for direct azo dyes.

For preparation and analytical purposes the chromatography was carried out on columns 200 × 40 mm; for analytical purposes only, columns 150 × 10 mm were also used. In most cases a commercial aluminium oxide was used for chromatography (LACHEMA, Č.S.S.R.) which was not processed in any special way. On the column (200 × 40 mm) 0·01–1·00 g of dye was applied, dissolved in about 50 ml. 96–80 per cent ethanol, the concentration being dependent on the type of dye and on alkalinity of aluminium oxide. For elution the same solvent was used. When any fraction was eluted too slowly or not at all, I increased the proportion of water in the solvent up to 40

per cent. The fractions were collected by means of an automatic fraction collector and their absorption spectra were assessed. Thus we obtained from commercial congo red or congo red synthesized by myself two dyes, the first having an absorption maximum at 485 mμ, and a second one (real congo red) with a maximum at 498 mμ. The course of the absorption spectra of individual fractions is shown in Fig. 1. In a similar way from trypan blue (synthesized from commercial preparations of *o*-tolidine and 1 - amino - 8 - hydroxynaphthalene - 3,6 - disulphonic acid) three fractions were obtained: the first one with an absorption maximum (λ_{max}) of 560 mμ, a second with λ_{max} 610 mμ, and a third weak, and not closely defined fraction. Direct blue 2*B* (*C.I.* 406) was also separated into three fractions: one with λ_{max} 555 mμ, a second one with λ_{max} 590 mμ, and a third one with λ_{max} 600 mμ. From direct green *B* (*C.I.* 593) five dyes were isolated with the following absorption maxima: (1) 388 mμ; (2) 376 mμ, 644 mμ; (3) 384 mμ, 534 mμ; (4) 374 mμ, 640 mμ; (5) 382 mμ, 556 mμ.

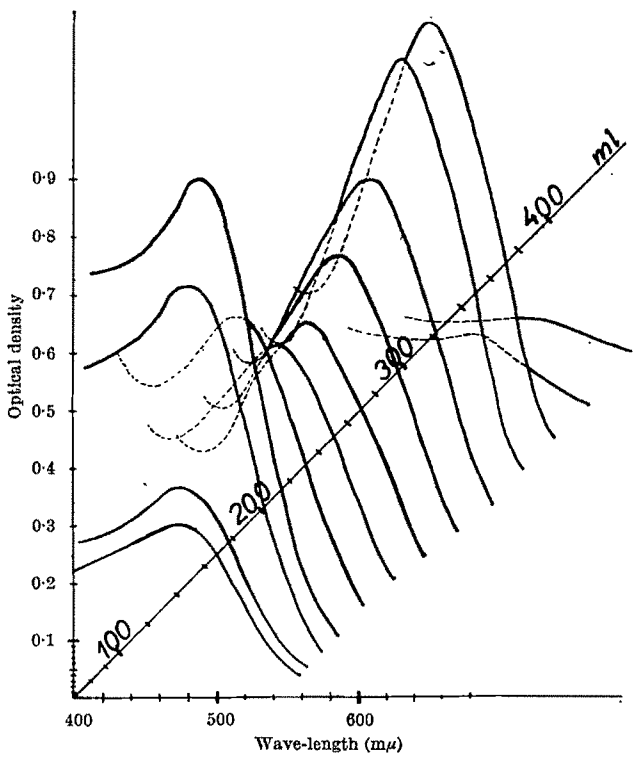


Fig. 1. Dependence of optical density on wave-length for fractions obtained from commercial congo red

I also used chromatography on thin layers of aluminium oxide with ethanol–water as a solvent, the ratio depending on the type of aluminium oxide and the dye. The dye was applied in the form of a 0·5 per cent solution in 50 per cent ethanol. By this method I was able to separate into their individual components congo red, trypan blue, direct blue 2*B*, direct green *B*, benzopurpurin, and direct red *F* (*C.I.* 419). Better separation of dyes was obtained as compared with paper chromatography (Whatman No. 1; 80 per cent ethanol or ethanol/ammonia/water (10 : 5 : 85) as a solvent).

As the commercial preparations of aluminium oxide are more or less alkaline and the degree of separation of dyes depends on the alkalinity of the aluminium oxide used, it is important to test the most suitable ratio of ethanol and water for every new type of aluminium oxide. This procedure is not required using neutral aluminium oxide prepared in a standard fashion. Moreover, ethanol with a higher water content may be used for elution, in which the dye is more easily soluble.

The importance of the purity of direct dyes in medicine and biology has often been demonstrated. The toxicity of non-purified preparation of congo red is much higher (LD_{50} 102 mg/kg) than in a preparation purified by crystallization (LD_{50} 320 mg/kg)[10]. In the case of Evans blue and trypan blue, which are used for estimation of the blood volume, other dyes present seemed to distort the results or give results which are not reproducible. A similar situation may develop when congo red is used in the diagnosis of amyloidosis. The importance of the purity of trypan blue was confirmed also by Beck, Spencer and Baxter[5]. The method of adsorption chromatography appears to be suitable for obtaining gram amounts of pure direct dyes as well as for assessing the content of coloured admixtures in commercial preparations of these dyes.

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BIOCHEMISTRY

Effect of Phenolase on Anthocyanins

Several anthocyanin-decolorizing systems have been reported as present in leaves and fruits[1–4]. Although oxidizing enzymes are apparently involved, none has been isolated from these systems. In this communication the effect of a mushroom phenolase ('Tyrosinase', General Biochemicals, Inc.) on purified tart cherry anthocyanins is described. In view of the wide distribution and low specificity of phenolases, the testing of an enzyme not originating in the same tissue with the possible substrate seems justified, at least as a first step in the search for the enzyme responsible for the *in situ* reaction. Furthermore, phenolase activity has been detected in the cherries used here[5].

The anthocyanins were extracted from red, tart cherries (*Prunus cerasus*, var. Montmorency) with boiling ethanol, sorbed on 'Dowex 50W-*X*8' resin, eluted with methanol 0·3 N in hydrochloric acid, concentrated *in vacuo* and streaked on sheets of 3*MM* Whatman paper. After irrigating the paper ascendingly for 15–20 min with 1 M acetic acid solution, the two cherry anthocyanins appeared as well-separated zones. The pigment of each zone was eluted separately with methanol containing a trace of hydrochloric acid and the eluates were again concentrated *in vacuo* and further purified by zone electrophoresis on cellulose powder as described previously[6].

Before the enzymatic reaction was initiated, 3·0 ml. of a mixture of equal volumes of 0·1 M citric and 0·1 M phosphoric acids were mixed with 1·0 ml. of anthocyanin solution, 1·0 ml. of a catechol solution varying in concentration from 0 to 20 mM, varying volumes of 5 N sodium hydroxide solution, depending on the desired *p*H in the region 2·5–7·5, and water to make up a total volume of 6·8 ml. From this mixture, 0·5 ml. was transferred to a test tube containing 2·5 ml. of 1 M citrate buffer *p*H 2·0 and 0·1 ml. of 0·5 M potassium cyanide; another 0·5

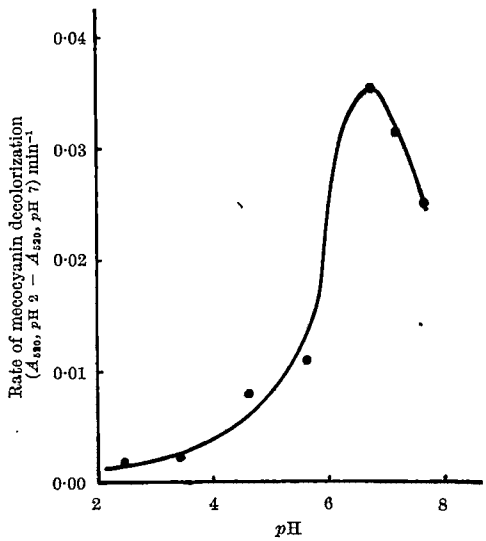


Fig. 1. Effect of *p*H on the mecocyanin decolorization by phenolase. 3·4 mg enzyme/100 ml. reaction mixture, 2·8 × 10^{-5} M catechol; 25° C

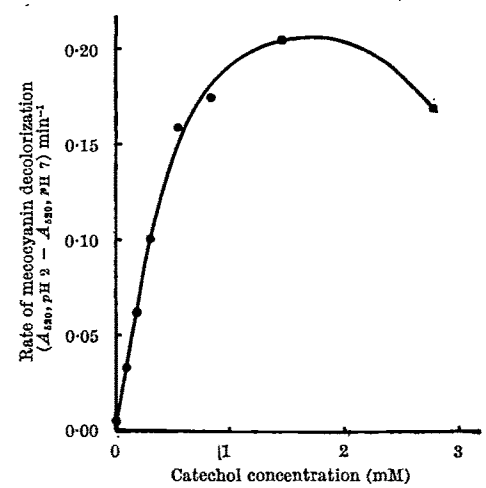


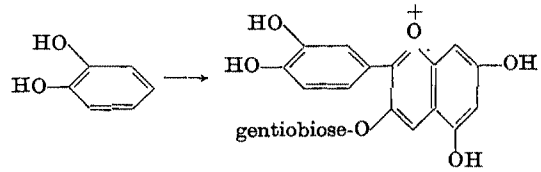
Fig. 2. Effect of catechol concentration on the mecocyanin decolorization by phenolase. 3·4 mg enzyme/100 ml. reaction mixture; *p*H 6·5; 25° C

ml. of the mixture was transferred to a second tube containing 2·5 ml. of 1 M citrate buffer *p*H 7·0 and 0·1 ml. of 0·5 M potassium cyanide. These tubes served to obtain a measure of the zero time anthocyanin concentration.

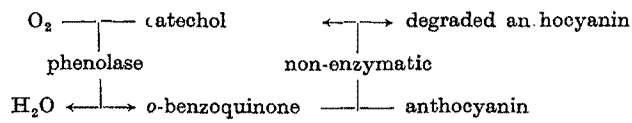
To the remaining 5·8 ml. of anthocyanin–buffer–catechol mixture, 0·2 ml. of enzyme solution was added and at intervals of time as short as 30 sec two 0·5 ml. aliquots of reaction mixture were transferred to citrate–cyanide solutions similar to those used for the zero time anthocyanin determination. The reaction mixture was kept under vigorous magnetic stirring to ensure air incorporation. Fifteen min after each transfer the absorbancy of the tube contents was measured in a Beckman *DU* spectrophotometer at 520 mμ for the mecocyanin (cyanidin-3-gentiobioside) and 515 mμ for the antirrhinin (cyanidin-3-rhamnoglucoside) of the cherries. The difference in absorbancy at *p*H 2·0 and 7·0 has been found a better measure of anthocyanin concentration than the absorbancy at one *p*H, because of the interference of the concurrent browning[7]. The reaction rates are given here as decrease in this differential absorbancy per min and are based on the first few min during which the reaction was of zero-order.

The effect of *p*H on the degradation of mecocyanin by phenolase is illustrated in Fig. 1. A *p*H optimum of about 6·5 is apparent.

The effect of catechol concentration on the reaction rate is shown in Fig. 2. It is obvious that in the absence of catechol the reaction is very slow, although mecocyanin can be considered a benzopyrylium derivative of catechol.



In the presence of catechol the rate of mecocyanin decolorization increases rapidly with catechol concentration. Excessive catechol, however, inhibits the reaction. The probable mechanism of the phenolase–catechol–anthocyanin reaction is as follows:



An attempt will be made to support this scheme by using free *o*-benzoquinone.

The effect of temperature was also examined using 3·4 mg of enzyme per 100 ml. reaction mixture, 2·8 × 10^{-5} M catechol, and *p*H 6·5. The reaction rate increased as the temperature was raised from 5° to 50° C; at higher temperature it rapidly declined.

When the amount of enzyme was varied at *p*H 6·5, 2·8 × 10^{-5} M catechol and 25° C, the typical linear rise of reaction rate with enzyme concentration was observed. Above 3 mg of enzyme per 100 ml. reaction mixture the rate levelled off.

The results obtained with antirrhinin were very similar to those of mecocyanin.

It is concluded that anthocyanins, of the cyanidin types at least, are poor phenolase substrates; but they are readily decolorized by this enzyme in the prosence of a better substrate, such as catechol.

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Effect of Puromycin on Cholinesterase Activity of Embryonic Chick Intestine in Organ Culture

The acetylcholinesterase (AChE) activity of a number of chick embryo tissues cultured *in vitro* can be influenced by the presence of acetylcholine (ACh) or other substrates in the nutrient media[1–4]. For example, in the presence of 0·02 M ACh, the AChE activity of primary explants of chick embryo lung increased 3–7-fold over a period of four days[3]. It would seem that a case of enzyme induction had been demonstrated. However, no direct evidence has been provided which would support this hypothesis. This report presents evidence that certain changes in AChE and cholinesterase (ChE) activity of chick embryo intestine maintained *in vitro* are due to changes in protein synthesis, a finding that is compatible

with the hypothesis that the synthesis of AChE can be induced.

When fragments of chick embryo small intestine were maintained in organ culture for 48 h using Waymouth's synthetic medium, *MB* 752/1, the AChE activity of the tissue medium usually caused no further increase. However, when medium No. 199 was used instead of Waymouth's medium, no 'spontaneous' increase in enzyme activity was observed, and addition of ACh to the 199 caused an increase in AChE activity of the cultured intestine. These observations with 199 are similar to those published previously[1–3]. Although the exact reasons for the differences in results with the two culture media are unknown, Waymouth's medium is superior to 199 in the maintenance of the tissue. Also, the choline concentration in Waymouth's medium is 250 mg/l. compared with 0·5 mg/l. in mixture 199. This may be important since choline is a precursor in the biosynthesis of ACh. The 'spontaneous' increase in enzyme activity occurring when using Waymouth's medium is much like that recently reported for cultured chick skeletal muscle by Goodwin and Sizer[4]. One question raised by all these studies is whether the increase in enzyme activity is due to increased synthesis of enzyme protein. This problem was approached by studying the effects of puromycin, a known inhibitor of protein synthesis[6,7].

Fragments of small intestine from 16-day-old chick embryo were maintained in organ culture for 48 h, using Waymouth's defined medium containing puromycin. The fragments were then homogenized and the colorimetric method of Ellman *et al.*[8] was used to determine enzyme activity. Both acetylthiocholine and butyryl thiocholine were used as substrates, and enzyme activity was based upon both wet weight and protein content. The results are shown in Table 1.

Table 1. CHOLINESTERASE ACTIVITY OF EMBRYONIC CHICK INTESTINE

	μM/g tissue/min	% of control	μM/g protein/min	% of control
Acetylthiocholine hydrolysis				
Control (not cultured)	2·06*	100	33·7	100
Cultured 48 h	3·56	173	57·6	171
Cultured 48 h + 5×10^{-5} M puromycin	0·42	21	10·4	31
Cultured 48 h + 5×10^{-6} M puromycin	1·74	84	31·4	93
Butyrylthiocholine hydrolysis				
Control (not cultured)	0·93	100	15·2	100
Cultured 48 h	1·13	121	18·0	118
Cultured 48 h + 5×10^{-5} M puromycin	0·01	1	0·1	0·07
Cultured 48 h + 5×10^{-6} M puromycin	1·15	123	22·1	146

* Each number is the average of three independent determinations.

Addition of 5×10^{-5} M puromycin not only blocked the 'spontaneous' increase of AChE activity (reflected by acetylthiocholine hydrolysis) but decreased it to less than control levels. ChE activity (butyrylthiocholine hydrolysis) was virtually abolished by this concentration of puromycin. At 5×10^{-6} M, puromycin blocked the increase in acetylthiocholine hydrolysis without decreasing butyrylthiocholine hydrolysis. These effects of puromycin were not due to enzyme inhibition since addition of the same concentrations of puromycin to the enzyme assay system had no effect on the hydrolysis of either acetylthiocholine or butyrylthiocholine. 5×10^{-5} M puromycin caused a 30 per cent decrease in the average protein content of the cultured tissue. This decrease was statistically significant ($P < 0{\cdot}01$). The small decrease in protein content (16 per cent) caused by 5×10^{-6} M puromycin was not statistically significant.

The mechanism of the 'spontaneous' increase, or of the ACh induced increase in AChE activity of these embryonic tissues is not known. The results with puromycin indicate that, at least for the 'spontaneous' increase, protein synthesis is involved and that the increased activity probably reflects an increase in synthesis of enzyme protein. Further studies are required to clarify the biochemical mechanisms, and to determine the physiological significance of these observations since both ACh and AChE are involved in cholinergic synaptic transmission, and histochemical localization of the AChE in embryonic chick intestine revealed that this enzyme was almost exclusively located in the Meissner's and Auerbach's plexi of the intestine[5].

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Possible Turnover of DNA with an Increased 6-Methylaminopurine Content

THE 6-methylaminopurine content of the DNA of a thymine-requiring mutant of *E. coli* strain 15, normally 2·1 per cent of the adenine, increases when 5-aminouracil is added to a logarithmically growing culture[1]. We have previously reported that the 6-methylaminopurine content of the DNA of a culture of a similar strain (strain *I*) continues to increase after net DNA synthesis ceases[2]. We have also shown that this increase could not be attributed to additions to the ends of DNA molecules since 6-methylaminopurine was not localized at these ends[2].

The increase in the 6-methylaminopurine content of DNA in the absence of net DNA synthesis can be explained by any of the following three proposals.

(1) The adenine in polymeric DNA is methylated[2–5].

(2) DNA molecules (possibly only one strand of the two) are degraded and replaced by new ones having an increased 6-methylaminopurine content (DNA-turnover). Although DNA-turnover in bacteria is not an established phenomenon, it has been indicated in the case of another abnormal DNA, that is, DNA containing 5-bromouracil[6]. In addition, incorporation of thymidine-2-^{14}C into DNA in the absence of net DNA synthesis has been reported in bacteria treated with alkylating agents[7]. It should be pointed out that DNA breakdown and resynthesis could also be effected by cell turnover, that is, the breakdown of old cells and the concomitant formation of new cells. However, normal cells of *E. coli* do not lyse easily in 24 h (refs. 6 and 8). Cell turnover, therefore, is an unlikely explanation unless the condition of the cells with respect to lysis in the presence of 5-aminouracil is very different from normal; this cannot be rigorously excluded since such cells are abnormal in shape[2].

(3) There may be net synthesis of DNA involving a small, undetectable fraction of DNA with a high 6-methylaminopurine content. The existence of small fractions of DNA of unusual base composition (but not involving 6-methylaminopurine) has been reported[9].

It is to be noted that these three proposals are not mutually exclusive. For example, the proposals (2) and (3) do not preclude the possibility that methylation of polymeric DNA is the mechanism by which 6-methylaminopurine appears in DNA.

The experiments reported here concern the incorporation of thymine-2-^{14}C into DNA during the period when

net DNA synthesis has ceased but the 6-methylaminopurine content increases. Under our conditions, the final 6-methylaminopurine content was 6 moles per 100 moles of adenine. The bacterial strain and the methods used have been discussed in a previous publication[2]. A small amount of radioactive thymine (14·8 μc./μmole was added to cultures containing 5-aminouracil and to control cultures without 5-aminouracil, after cessation of net DNA synthesis. (This represents the beginning of the stationary phase (culture about 17 h old).) The incubation was continued until the termination of the increase in the 6-methylaminopurine content of DNA (2 h)[2]. During the incubation with radioactive thymine the DNA content of the cells (determined by a micro-modification of the diphenylamine test[10]) remained constant. (μg of DNA per ml. of culture: with 5-aminouracil 10·0 ± 0·6; without 5-aminouracil 11·3 ± 1·0; the error is expressed as the standard deviation among samples removed from the medium at different time intervals.)

Table 1. Specific Activity of Thymine in DNA of Thymine-requiring Strain *I* of *E. coli*

(Exposed to thymine-2-^{14}C for 2 h after cessation of net DNA synthesis)

Experiment No.	Specific activity of thymine in DNA: Culture with 5-aminouracil (c.p.m./μmole) × 10^{-4}	Control* (c.p.m./μmole × 10^{-4})
1	9·5 ± 0·9	3·8 ± 0·3
2	5·5 ± 0·4	2·5 ± 0·2
3	5·6 ± 0·4	4·0 ± 0·2
Average	6·9	3·4

* One half of the culture, without 5-aminouracil, served as the control. The error is expressed as the standard deviation of four thymine samples isolated and counted independently.

The specific activity of thymine in DNA is presented in Table 1. Although the thymine in DNA of the control is radioactive, the amount of thymine actually incorporated (about 6–10 per cent of total thymine calculated using specific activity of thymine in the medium as 100 per cent) during the period under investigation is low enough to be attributed to a small amount of cell division and/or DNA synthesis undetected by our methods. However, the specific activity of thymine in the DNA with an increased 6-methylaminopurine content (culture grown in the presence of 5-aminouracil) is consistently higher than the control (on the average, twice that of the control).

These results do not allow one to rule out completely any of the three proposals already presented here. However, the proposal concerning the synthesis of a small fraction of DNA with a very unusual base composition appears unlikely; if the incorporation of thymine were due to net synthesis of DNA, the increase in DNA, as indicated by the specific activity of DNA thymine, should have been detectable. The results given here indicate a possibility of DNA turnover; thus, experiments designed to demonstrate methylation of polymeric DNA in this system must be interpreted with caution.

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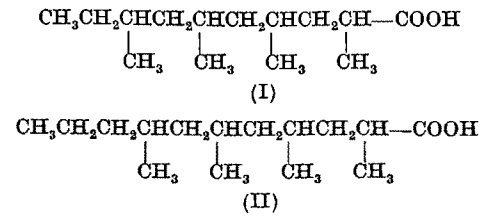
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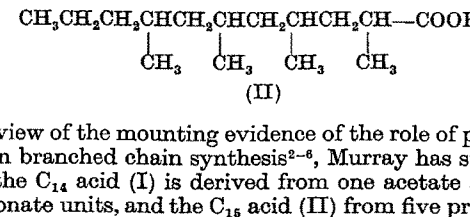
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Incorporation of Propionic Acid into a Branched-chain Fatty Acid of the Preen Gland of the Goose

Murray[1] has recently isolated the two main acids of the secretion of the preen gland of the goose and has shown that they are 2,4,6,8-tetramethyldecanoic (I) and 2,4,6,8-tetramethylundecanoic acids (II).



(I)



(II)

In view of the mounting evidence of the role of propionic acid in branched chain synthesis[2–6], Murray has suggested that the C_{14} acid (I) is derived from one acetate and four propionate units, and the C_{15} acid (II) from five propionate units. The present investigation was undertaken to test this hypothesis by tracer technique.

Recently, Lennarz *et al.*[7] have demonstrated that the synthesis of tuberculostearic acid (10-methylstearic acid) occurs via addition of a 'C_1 unit' from methionine on to oleic acid. Hence, parallel experiments with methionine-$^{14}CH_3$ were conducted here for the purpose of comparison.

Fifty μc. each of sodium propionate-3-^{14}C (0·025 m/moles) and L-methionine-$^{14}CH_3$ (0·010 m/moles) (purchased from the New England Nuclear Corp., Boston, Mass.) dissolved in 0·5 ml. of physiological saline were injected directly into the preen gland of each of two adult geese. Five h later the fowls were killed and their preen glands dissected out entirely. The glands were washed with water, cut open and the waxy contents removed by gentle scraping with a spatula.

Fatty acids (average yield = 820 mg) were isolated according to methods previously used in the examination of mycobacterial lipids[8]. The fatty acids obtained from the methionine-$^{14}CH_3$ experiment were found to contain no detectable radioactivity, whereas the propionate-3-^{14}C fatty acids showed incorporation of tracer to the extent of 19 c.p.m./mg (^{14}C-activity determined in a Geiger–Müller counter operating at 6 per cent efficiency) (Table 1).

Table 1. Radioactivity of Total Fatty Acids of Preen Gland after Administration of Radioactive Precursors

	Activity (c.p.m.)	Background (c.p.m.)	Activity—background (c.p.m.)	Weight fatty acids (mg)	C.p.m./mg fatty acids
Methionine-$^{14}CH_3$ fatty acids	38	37	1	31·1	~0
Propionate-3-^{14}C fatty acids	779	37	742	37·7	~19

The propionate-3-^{14}C fatty acids were esterified with diazomethane and chromatographed on a 30 per cent 'Apiezon *L*'/'Chromosorb *P*' column, using an F and M vapour phase chromatograph. The fraction corresponding chromatographically to the methyl ester of 2,4,6,8-tetramethyldecanoic acid (I) (The identity and the structure of I were verified by mass spectrographic analysis by Dr. W. Vetter. Acid II was present only in small quantity. Meanwhile, Prof. E. Stenhagen (Göteborg) has informed us that he too has confirmed the structure of I proposed by Murray using mass spectrometry), as described by Murray[1], was collected and found to be radioactive to the extent of 22 c.p.m./mg. Kuhn–Roth degradation[9] of this compound resulted in 24 per cent recovery of the original radioactivity in the formed acetic acid. (The low yield obtained is due to the loss of acetic acid during the course of the degradation. One millimole of carrier acetate was added at the termination of the reaction.) The acetic acid was purified[10], counted on a proportional gas phase counter and further degraded by the Schmidt

method[11] to determine activity of each carbon atom. The degradation data, showing the exclusive presence of radioactivity in the methyl group (Table 2), provide further evidence that propionic acid is indeed incorporated into 2,4,6,8-tetramethyldecanoic acid (I) in the predicted manner.

Table 2. SCHMIDT DEGRADATION OF ACETIC ACID FORMED FROM KUHN–ROTH DEGRADATION OF 2,4,6,8-TETRAMETHYLDECANOIC ACID OBTAINED FROM PROPIONATE-3-^{14}C EXPERIMENT

Activity of CH_3COOH (c.p.m./mM*)	Activity of CH_3-group (c.p.m./mM*)	Activity of —COOH group (c.p.m./mM*)
1,756	1,725 (99%)	22 (1%)

* ^{14}C activity determined in a proportional gas phase counter operating at 90% efficiency.

The incorporation of propionic acid into branched-chain aliphatic compounds has already been proved for bacterial products: erythromycin[2,3], methymycin[4], mycocerosic acid[5,6] and phthiocerol[6]. In the animal kingdom, Saz and Weil[12] have shown that in the *Ascaris* 2-methyl-butyric acid is synthesized *in vivo* from one molecule of acetic and one molecule of propionic acid and that 2-methyl-valeric acid is formed in a similar way from two molecules of propionic acid.

This work seems to prove that propionic acid can also be used by vertebrates to produce branched-chain compounds.

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An Inverse Relationship of the Effects of Oxygen and Iron on the Production of Fluorescin and Cytochrome *c* by *Pseudomonas fluorescens*

IT has been suggested by Burton *et al.* that fluorescin, a yellow-green fluorescent bacterial pigment, is related to respiratory enzymes. Observations from this laboratory on the control of cytochrome c production in *Pseudomonas fluorescens*[2–4] are in general accord with Burton's hypothesis, and suggest that fluorescin is an alternative product of porphyrin metabolism.

Burton *et al.*[1] and Totter and Mosley[5] demonstrated that production of fluorescin by *Ps. aeruginosa* is inversely related to the amount of iron present in the media. Using *Ps. fluorescens*, Koepsall[6] reported that medium obtained from cultures grown without iron "contains sufficient pigment of a fluorescent nature to give a marked greenish yellow colour, while the pigment is almost absent in fermentations containing iron".

In the experiments illustrated in Fig. 1, *Ps. fluorescens* grown at a high oxygen tension in the absence of iron produced large amounts of fluorescin while less than 0·1 per cent of the protein in the cells was accountable as cytochrome *c*. Conversely, when the organism was grown at a low oxygen tension in the presence of iron, 3·5 per cent of the soluble proteins of the cell consisted of cytochrome *c* while scarcely any fluorescin was produced. These results, in agreement with Burton, suggest that in the absence of iron and at high oxygen tensions *Ps. fluorescens* cannot make cytochrome *c*; the accumulated metabolic precursors of the porphyrin, therefore, might be metabolized to yield fluorescin.

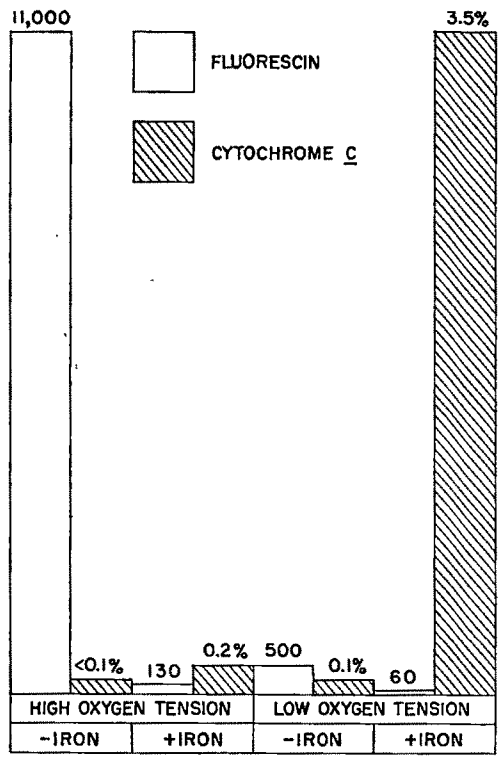


Fig. 1. A comparison of the effects of oxygen and iron on the production of fluorescin and cytochrome *c* by *Pseudomonas fluorescens*. *Pseudomonas fluorescens* was grown at conditions of high oxygen tensions (shaking) and low oxygen tensions (non-shaken) in media with or without iron prepared as previously described[3]. A unit of fluorescin is described as the reading of 1 ml. of the clear media as measured by the Coleman fluorimeter equipped with *B*–1 and *PC*–1 filters. The cytochrome *c* is expressed as the percentage of the soluble protein of the bacterial extract[2]

If Burton's postulate is true, then it might seem likely that the 'respiratory precursor' of fluorescin is also fluorescent. Indications to this effect are shown by examining washed cells of *Ps. fluorescens* previously grown at a low oxygen tension in the presence of iron (10^{-5} M or greater ferric chloride). Suspensions and aqueous extracts of these cells fluoresced red. The extract fluoresced red, however, only when the cytochrome *c* was in the reduced state. If the cytochrome *c* were oxidized, the extract fluoresced yellow. Extracts of cells grown so that no observable cytochrome *c* was present did not fluoresce either in the presence or absence of oxidizing or reducing agents. Furthermore, in contrast to the red fluorescing cultures, similar cells grown at low oxygen tensions containing less than 8×10^{-6} M ferric chloride fluoresced blue, while a trace of fluorescin was excreted into the medium.

Fluorescin has been considered to be a flavinic pigment[7,8]. However, my observations (see also refs. 4, 9) and consideration of its empirical formula as determined by Turfitt[10], $C_4H_9O_2N$, rather suggest that fluorescin might be a derivative of either pyrolle or pyrollidine.

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Purine Catabolism, a Source of Endogenously Formed Urea in Homogenates of Amphibian Kidney

CLASSICAL thinking holds that the bulk of urea formed in the body is derived from reactions of the Krebs–Henseleit cycle[1] occurring in the liver of mammals and of amphibia[2]. Although the cycle is incomplete in mammalian kidney due to a relative lack of ornithine transcarbamylase and of carbamyl phosphate synthetase[3], the enzymatically catalysed pathway, citrulline + aspartate ⇌ arginine ⇌ urea + ornithine provides a potential source of urea in kidney cells.

Recent results have suggested the existence of alternative chemical pathways for urea production in mammalian liver and in amphibian kidney.

Cedrangolo *et al.*[4] injected rats with α-D-L-methyl aspartate (α-MA), a potent inhibitor of arginino-succinate synthetase in liver homogenates[5]. Even though the Krebs–Henseleit cycle was completely blocked in liver slices of rats treated with α-MA, the daily excretion of urea in treated was the same as that in untreated rats. We[6] have demonstrated that urea production of bull-frog kidney was not inhibited by lysine, a well-known inhibitor of arginase activity.

This communication, an investigation of the source of urea produced by homogenates of amphibian kidney, involved testing for the activity of three possible reaction paths—that from citrulline to arginine to urea; that from protein to arginine to urea; and that from uric acid to urea.

Kidneys of 10–20 frogs (*Rana catesbiana*) were homogenized together with 3–10 volumes of diluent solution, 60 mM sodium chloride plus 60 mM potassium chloride. Aliquots of diluted homogenate were boiled for determination of pre-incubation levels of urea. Other aliquots of the same homogenate were incubated in a Dubnoff shaker at 23° C. for varying times after which they were boiled for determination of post-incubation levels of urea. Tissues, incubated with or without exogenously added substrates, were buffered to a *p*H of 7·3. Analyses, performed routinely on supernatant fluids of boiled homogenates before and after incubation, were those of urea and ammonia[7]. In some instances, arginine was measured enzymatically. No free ammonia was detected in more than 30 pools of frog kidney homogenate under present conditions. All exogenous materials added to homogenates were tested for their effects on urease and arginase activity in synthetic systems.

Table 1 presents results on concentration of exogenously added substances and on urea production by homogenates of bull-frog kidneys. No extra urea production was induced by addition of citrulline or of arginino-succinate, suggesting that neither arginino-succinate synthetase nor arginino-succinase was active in homogenates. Extra urea production was induced by indicating the presence of some a frog kidney.

Table 1. EFFECT OF EXOGENOUSLY ADDED OF THE ORNITHINE CYCLE ON PRODUCTION GENATES OF BULL-FROG

Homog. pool (No.)*	Added substance	Conc. added substance: Amount per ml. ambient (μM/ml.)	Amount per g homog. (μM/g)	
1	Citrulline	6	60	
	None	0	0	
2	Arginino-succin.	0·5	5·0	
	None	0	0	
3	Arginine	1·25	12·5	
	None	0	0	
4	Lysine	20	200	
	None	0	0	
5	Leucine	5	50	
	None	0	0	
6	Ornithine	20	200	
	None	0	0	

* Data from each pool of kidney homogen first column) were from paired aliquots of homogenate was prepared from kidneys of 8–
† $\Delta U_e/\Delta U_c$ = amount of urea appearing in homogenate with exogenously added substanc in the same time in other aliquots of the s genously added substance (control aliquots). urea; subscripts, *e* and *c*, denote experime given homogenate.

Table 2. EFFECT OF EXOGENOUSLY ADDED REACTION SERIES ON PRODUCTION OF UREA OF BULL-FROG KI

Homog. pool (No.)*	Added substance	Conc. added substance: Amount per ml. ambient (μM/ml.)	Amount per g homog. (μM/g)	
1	Xanthine	1·5	15	
	None	0	0	
2	Hypoxan-thine	1·5	15	
	None	0	0	
3	Uric acid	1·5	15	
	None	0	0	
4	Allantoin	1	10	
	None	0	0	
5	Glyoxylic	3	30	
	None	0	0	
6	Glyoxylic	33	330	
	None	0	0	

* Format of experiments from paired aliqu same as that in Table 1.
† $\Delta U_e/\Delta U_c$ is defined in same manner as i

Having verified that lysine and ase in synthetic systems buffered t in the renal homogenates, we ad frog kidney homogenate. Neith reduced urea production of frog l urea formed in the homogenate orgi reactions other than those termi arginine. This was supported by Table 1. Addition of ornithine, hydrolysis, had no effect on end urea. The concentration of ornitl to reduce the velocity of arginine h in a control synthetic system. A have found that ornithine, citru cinate depress arginase activity buffered at *p*H 7·2–7·4.

If endogenously formed urea in r not derived from arginine hydro inate two well-known metabolic citrulline to arginine to urea, an peptide to arginine to urea. O include those of purine or of py The purine pathway, a well-kno primitive vertebrates, was tested urea production in the frog kidney

Table 2 shows that neither xant induced any change in the 2-h homogenates of bull-frog kidney addition of uric acid and allantoin and 160 per cent, respectively, of urea.

orming urea in unfortified
oicase-catalysed breakdown
plus glyoxylic acid, then
nates with buffered glyoxy-
genous urea production by
rmed, as shown in Table 2,
of 75 and 56 per cent in the
te loaded homogenates. In
n glyoxylate addition, not
mogenates was decreased by

homogenates of frog kidney
substances by degradation of
ie reaction chain, uric acid ⇌
rea + glyoxylic acid, provides
duced in renal homogenates
Hydrolysis of arginine, activ-
ginine loading, is a potential
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kidney under present con-

by grant *A*–461, U.S. Public
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gh sucrose density gradients
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n examined individually. Such a
ning and lacking in sensitivity and
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natically obtaining a continuous
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ie stable and adaptable optics, and
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se the Cary model 14.
n in the *SW* 39 rotor of the Spinco
e ultracentrifuge, the 'Lusteroid'
sucrose gradient is placed in the
'Perspex' block (Fig. 1), from which the upper screw has been removed. This screw carries a rubber washer and a fine stainless steel tube through which the gradient will be extruded. Underneath the 'Lusteroid' tube is a No. 18 gauge hypodermic needle, sheathed by a rubber bung and connected by a fine polythene lead to a syringe which can be driven by a synchronous motor. The needle and syringe are filled with a sucrose solution of a density greater than the maximum used in preparing the gradient. When the upper screw is replaced the washer seals the top of the centrifuge tube and the needle penetrates the bottom, the compression of the rubber bung effectively preventing any leakage from the point of entry. The syringe motor is then started and the contents of the tube are steadily floated upwards on the denser sucrose, through a short polythene lead, and into a specially designed micro flow-cell (Fig. 2). The cell is held in a detachable mounting that is fixed to the edge of the platform in the sample compartment of the spectrophotometer. Four screws in the mounting permit accurate adjustment of the position of the flow-cell so that it contains the slit image (Fig. 3). A polythene lead is taken from the top of the flow-cell out through a special lid to the cell compart-

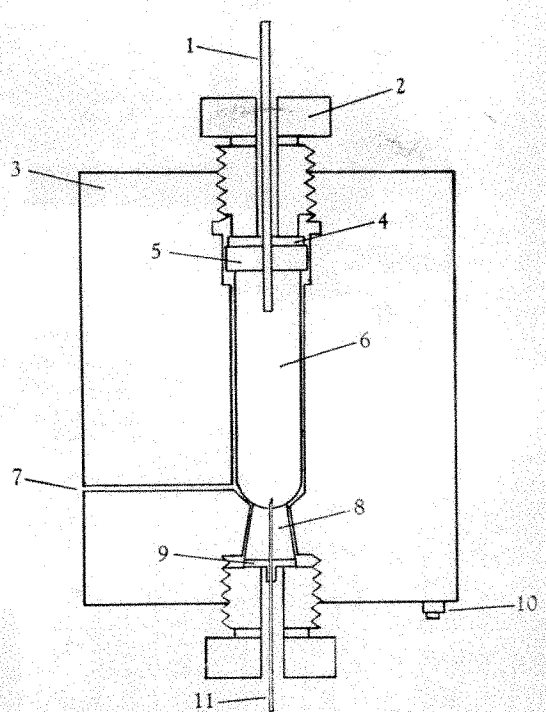


Fig. 1. 'Perspex' holder for lusteroid centrifuge tube. 1, Stainless steel tube; 2, upper screw; 3, 'Perspex' block; 4, brass plate; 5, rubber washer; 6, centrifuge tube; 7, drainage channel; 8, rubber bung; 9, lower screw; 10, clip for polythene lead; 11, hypodermic needle

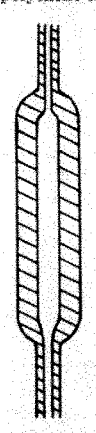


Fig. 2. Micro-flow cell. Path-length is 0·2 cm and the height is 2 cm

ment. The density gradient itself serves to prevent mixing in the leads and flow-cell. A few seconds before the liquid column reaches the empty cell, the chart motor of the spectrophotometer is started. The passage of the meniscus through the cell chart. The distance sedim ponent may be calculated f syringe and chart motor spe concentration are made fro been heated to 100° for a f fully to equilibrate with air ture (usually about 3°) to av of small air bubbles in the flo warms. The contents of the emerging from the spectro drops formed at the end of t photo-electric drop counter. has to be made when correlat record to allow for the dea cell and the tip where drops a

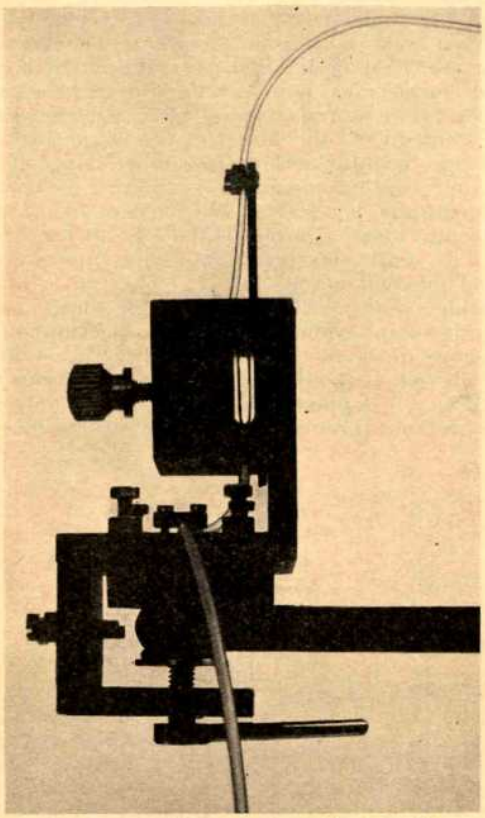


Fig. 3. Detachable mounting for the flow cell

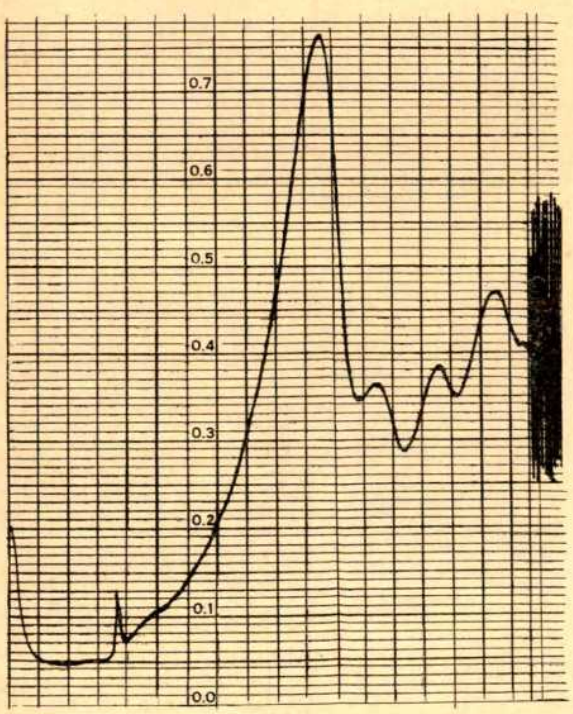


Fig. 4. Sedimentation analysis of ribosomes prepared from *Pseudomonas aeruginosa*. Centrifugation: 1 h at 37,000 r.p.m. in the *SW* 39 rotor of the Spinco model using a gradient of 5–2 per cent sucrose in 0·01 M *tris*/hydrochloric acid *p*H 7·2, 0·01 M Mg, 0·05 M KCl. 1 horizontal chart division represents 0·21 cm depth in the centrifuge tube. The passage of the meniscus through the flow cell is indicated by the start of a steady trace on the right-hand side of the chart

The equipment has already the particle size distribution from rabbit reticulocytes, be and when used in conjunctio slide-wire of the spectropho dissociation of proteins into record is shown in Fig. 4.

We thank Mr. J. H. Trend their assistance, and also th purchase of a recording Medical Research Council (J. G. E.).

Department of Bio University Coll

Biological A Pigment pro Ae

It has previou pigments could when a strain presence of vita pigment (pigm shown to be de vitamin B_{12} in phoretic and ch to air and light an converted to aquocoba cyanide.

The biological activity examined. The pigment u fermentation medium with previously[1]. It was purified b N acetic acid containing 0·01 pe followed by paper chromatograph with water/acetic acid/potassium with 10 mg potassium cyanide electrophoresis a second time i system.

The amount of pigment I refe was calculated from its light absor and its molecular extinction coeffic as an approximation its molecula that of vitamin B_{12} (ref. 1).

Table 1. MICROBIOLOGICAL ASS

Micro-organism	Activ of th
Ochromonas malhamensis *	
Lactobacillus leichmannii †	
Euglena gracilis ‡	

* Carried out by Dr. H. R. V. Arnstein, Research, Mill Hill, using the growth test des
† Carried out by Mr. R. Brækkan, Vitamin using strain A.T.C.C. 4797.
‡ Carried out by Dr. A. Schrumpf, St. J Norway, using the method of Killander[3].
Solutions of pigment I were sterilized by fil

It is seen from Table 1 that pigment I has virtually no activity for *Ochromonas malhamensis* and *Lactobacillus leichmannii*. For *Euglena gracilis* a small activity is found. Bioautography with the *E. coli* mutant (*M* 200) of a chromatogram of 25 μg of pigment I run in the solvent system described showed no growth-promoting properties. The chromatogram indicated traces of vitamin B_{12} and Factor *B*, both of which were present in quantities less than 0·01 per cent of pigment I. (This experiment was kindly carried out by Dr. E. Lester Smith, Glaxo Laboratories, Greenford, England.)

Pigment I has been given intramuscularly as a single dose with no apparent side-effects to 9 patients with megaloblastic marrow and histamine fast achlorhydria typical for pernicious anæmia. The reticulocyte responses are shown in Table 2. The administration of 30–50 μg of pigment I gave some response. Subsequent administration of vitamin B_{12} or liver extract gave an increased response in all but one case (patient 4). When 100 μg of pigment I was injected, subsequent injection of 1 mg and 5 mg of vitamin B_{12} in patients 5 and 6 respectively did not produce additional reticulocyte responses. The time required for maximum reticulocyte response following injection of pigment I is longer (6–9 days) than is usual with vitamin B_{12}. The increase in red blood cells over a period of 15 days following the injection of 100 μg of pigment I is seen in Table 3. Using the formula of Ungley[4], who found that the increase in red blood cells was linear to the logarithm of the dose of vitamin B_{12}, it can be calculated that the activity of pigment I in man corresponds to 8 per cent (by weight) of that of vitamin B_{12}.

Table 2. Reticulocyte Response of Patients receiving Pigment I

Patient No.	Reticulocytes before treatment per cent	Dose (μg)	Maximum of reticulocyte response Per cent	Day
1	0·8	30	4·0	7*
2	0·5	50	2·0	4†
3	1·0	50	4·2	9‡
4	1·0	50	11·7	6§
5	0·1	100	5·8	8¶
6	0·8	100	13·1	7‖
7	0·8	100	15·5	6
8	2·0	100	15·8	9
9	1·5	100	10·8	8

Reticulocytes were counted using a wet method with nile blue.

* 1 mg of vitamin B_{12} on the 14th day gave a second reticulocyte response of 8 per cent on the 20th day.
† 5 ml. of Pernami, Nyegaard and Co. *A/S*, on the 11th day gave a second reticulocyte response of 7 per cent on the 15th day.
‡ 10 ml. of Pernami on the 12th day gave a second reticulocyte response of 8·3 per cent on the 21st day.
§ 10 ml. of Pernami on the 12th day gave a second reticulocyte response of 5 per cent on the 18th day.
¶ 1 mg vitamin B_{12} on the 18th day gave no additional reticulocyte response.
‖ 5 mg vitamin B_{12} on the 10th day gave no additional reticulocyte response.

Table 3. Number of Red Blood Cells after Administration of Pigment I

	Days after injection			
	0	5	10	15
Patients No.*	No. of red cells (millions)			
7	1·6	1·9	2·3	2·8
8	1·8	2·1	2·4	2·8
9	1·9	2·0	2·4	3·0

* See Table 2.

It appears from these results that pigment I produces in patients with pernicious anæmia a hæmatological response typical for vitamin B_{12}. Maintenance therapy has not been attempted.

A number of B_{12}-analogues differing in the nucleotide portion of the molecule have in the past been examined for biological activity in micro-organisms and in man[5]. Certain of these analogues exhibit activity in man. The results so far indicate that all the substances which are active in pernicious anæmia patients also exhibit microbiological activity. Pigment I, being active in man, shows a different pattern as no activity was found with *E. coli*, *L. leichmannii*, *O. malhamensis* and only slight activity with *E. gracilis*.

The activity of pigment I in man is most likely explained by assuming that part of the pigment injected is converted to a biologically active substance. Its negligible microbiological activity makes it unlikely that the unchanged molecule should have any effect.

The structure of pigment I is not known. It has recently been suggested by Hill, Pratt and Williams[6] that pigment I may have an additional group on one of the methene bridges. Whether man is capable of converting such a structure to a biologically active substance is not known.

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Aromatic Acids of Fermented Cacao

A resinous material with a 'balsamic' or 'honey-like' aroma can be recovered from chloroform-extracts of fermented cacao; it has been regarded as an important component of chocolate flavour[1]. Paper- and gas-liquid chromatography have now revealed in this material several aromatic acids, some of which have been identified.

Fermented and dried cacao beans (*CPA* brand) were homogenized and defatted with petroleum solvent (b.p. 80°–100°). The fat-free residue was acidified with N sulphuric acid (20 ml. per 100 g) and extracted with chloroform in a Soxhlet apparatus. Acids were extracted from the chloroform solution with 5 per cent sodium bicarbonate solution and taken into chloroform again after acidification of the bicarbonate solution. The chloroform solution was reduced to small volume and used for chromatography.

On paper chromatograms five main spots are revealed with diazotized *p*-nitroaniline after pre-treatment with ammonia[2]. A sixth spot is detected by its fluorescence in ultra-violet light. The R_F, colour reactions and tentative identifications are given in Table 1.

Table 1

Spot No.	Solv. *A*	Solv. *B*	Solv. *C*	Colour	Identification
1	0·51	0·71	0·78	mauve	*o*-hydroxyphenyl-acetic acid
2	0·43	0·89	0·77	red	phenylacetic acid
3	0·20	0·60	0·79	violet	*p*-hydroxyphenyl-acetic acid
4	0·12	0·61	0·60	brownish-red	*p*-hydroxybenzoic acid
5	0·09	0·82	0·57	violet	vanillic acid

Solvent *A*, *sec* butanol–conc. ammonia–water (8 : 1 : 1).
Solvent *B*, chloroform–acetic acid–water (34 : 15 : 1).
Solvent *C*, 2 per cent aqueous acetic acid.

Only short runs (5 in.) were routinely used and there was some variation in R_F, but the identifications were checked by eluting the individual components of the extract from large one-dimensional chromatograms of rows of spots and co-chromatographing the isolated component with the pure compound it was thought to be. Further corroboration of the identifications was obtained from gas-liquid chromatography.

A portion of the chloroform solution was evaporated. The residue was treated with diazomethane in ether and reaction allowed to continue until methylation was

complete ($K_3Fe(CN)_6/FeCl_3$ reaction for phenolic groups negative). Evaporation of the ether left a brown oily residue which was analysed by gas chromatography on polyethylene glycol adipate at 150° C. Methyl phenylacetate was readily detected as a major constituent, and methyl veratrate was also present. The methyl esters of *p*-methoxyphenylacetic acid and *o*-methoxyphenylacetic acid and/or *p*-methoxybenzoic acid were present in small amount. The principal constituents of the mixture appeared to be methyl esters of the fatty acids C_{14} upwards. The relevant known compounds were prepared by treatment of the acids in the table with diazomethane.

Phenylacetic and vanillic acids are both strongly odorous but are not alone responsible for the honey-like aroma. Carbonyl compounds also present probably contribute to this.

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PHYSIOLOGY

Aortic Wall as a 'Two-phase' Material

Comparison of the orientation of the collagen and elastin components of the rabbit aorta at various distending pressures indicates that the media functions as a 'two-phase' material. At physiological pressures, collagen fibres of high tensile strength and relatively high modulus of elasticity bear most of the stressing force; an intricate network of elastin lamellæ and fibrils of relatively low modulus of elasticity distributes stressing forces uniformly.

The microscopic arrangement and interrelation of collagen and elastin fibres was examined in 40 rabbit aortæ each of which was fixed while distended by a constant intra-luminal pressure. In each instance a 5-cm segment of abdominal aorta was cannulated at both ends and restored to *in vivo* length on an adjustable frame. During 2–4 h of fixation in 10 per cent formalin or 2 per cent osmic acid intra-luminal pressures ranging from 0 to 200 mm mercury were maintained by a constant pressure perfusion apparatus. For light microscopy sections of formalin-fixed material embedded in paraffin were stained with Weigert and Van Gieson stains for elastin and collagen. For electron microscopy, osmium-fixed tissues were embedded in 'Epon' or 'Araldite'; phosphotungstic acid or uranyl acetate stains were used. Microscopic measurements included vessel diameter and wall thickness, thickness of elastin lamellæ and distances between elastin lamellæ.

The relation of vessel radius, vessel wall thickness and total tangential tension to distending pressure is shown in Fig. 1. As intra-luminal pressure increased, the radius of the aorta increased and thickness of the wall decreased. The differences between radii and wall thicknesses were large below physiological pressures. However, differences between radii and wall thicknesses were small at physiological distending pressures between 80 and 120 mm mercury and remained small at higher pressures up to 200 mm mercury. The wall tension is related to both the intra-luminal pressure and the radius (according to

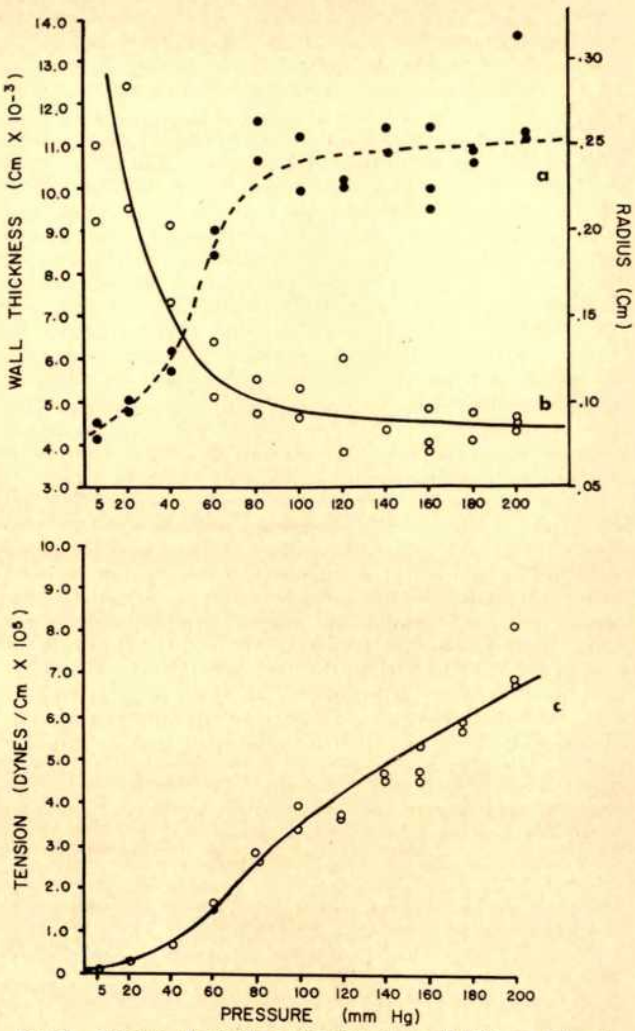


Fig. 1. Relation of rabbit aortic radius (*a*), wall thickness (*b*) and wall tension (*c*) to distending pressure

Laplace's law, as applied to blood vessels, $T = Pr$, where T is the total tangential tension (dynes/cm), P is the intra-luminal pressure (dynes/cm²), and r is the radius (cm) of the vessel). Since there is relatively little change in radius at and above physiological pressures, the tension in this range was almost entirely a function of pressure. These findings, based on measurements of many fixed aortæ, are in essential agreement with those of investi-

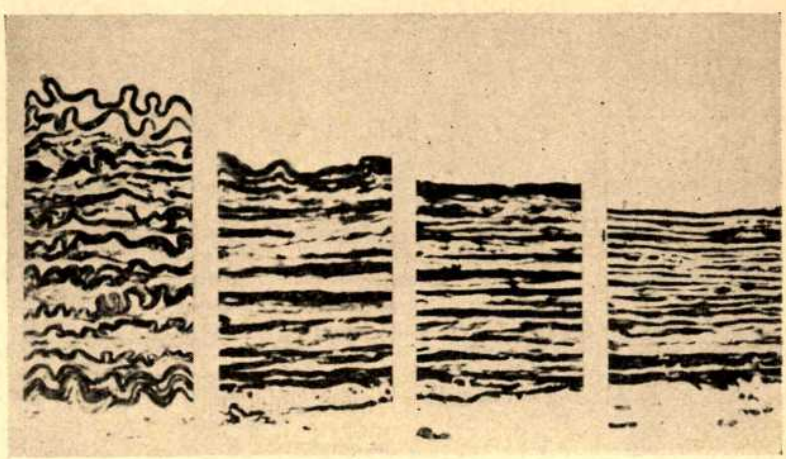


Fig. 2. Transverse sections of rabbit aortæ at distending pressures of 40, 60, 80 and 100 mm mercury, left to right. Weigert–Van Gieson stain (× *c.* 470)

gators who have measured the relation of volume or diameter to intra-luminal pressure or wall tension in individual fresh specimens[1,2]. The modulus of elasticity of the aortic wall is relatively high at and above physiological pressures.

The appearance of the concentric elastin lamellæ of the rabbit aortic wall at distending pressures of 40, 60, 80 and 100 mm mercury is shown in Fig. 2. At pressures below 80 mm mercury cross-sections of the lamellæ were wavy and crumpled; and above physiological pressures the lamellæ were straight. The media appeared to function as a whole, for the orientation of each of the lamellæ throughout the thickness of the wall changed with each increase in distending pressure. With increasing pressures below physiological values straightening of the lamellæ was uniform throughout the wall; interlamellar distances decreased uniformly and markedly; lamellar thicknesses decreased uniformly but very much less than interlamellar distances. Tangential and oblique sections revealed an abundant network of fine elastin fibrils which connected the thicker tubular elastin lamellæ (Fig. 3). At and above pressures of 60 mm mercury, the fine elastin fibrils between adjacent thick lamellæ were nearly parallel and arranged obliquely with respect to both the circumferential and longitudinal directions of the vessel. Thus, at physiological pressures lamellæ were straight, interlamellar distances were uniform and the extensive fine elastin net connecting lamellæ had a distinct orientation.

Electron photomicrographs revealed the orientation of the abundant collagen fibres within the media of the distended aorta. Below physiological pressures with elastin lamellæ and fibrils wavy and crumpled, collagen fibres showed no consistent arrangement. At and above physiological pressures collagen fibres appeared to be dispersed uniformly and arranged very nearly circumferentially. In transverse sections through the vessel wall most of the collagen was seen as long fibres. In longitudinal sections through the wall the collagen was seen as round or oval cross-sections of the fibres (Fig. 4).

Materials consisting of 2 components of different tensile strength and elastic modulus have been called 'two-phase' materials. Some of the advantageous properties of these systems have been described[3,4]. In a 'two phase' material

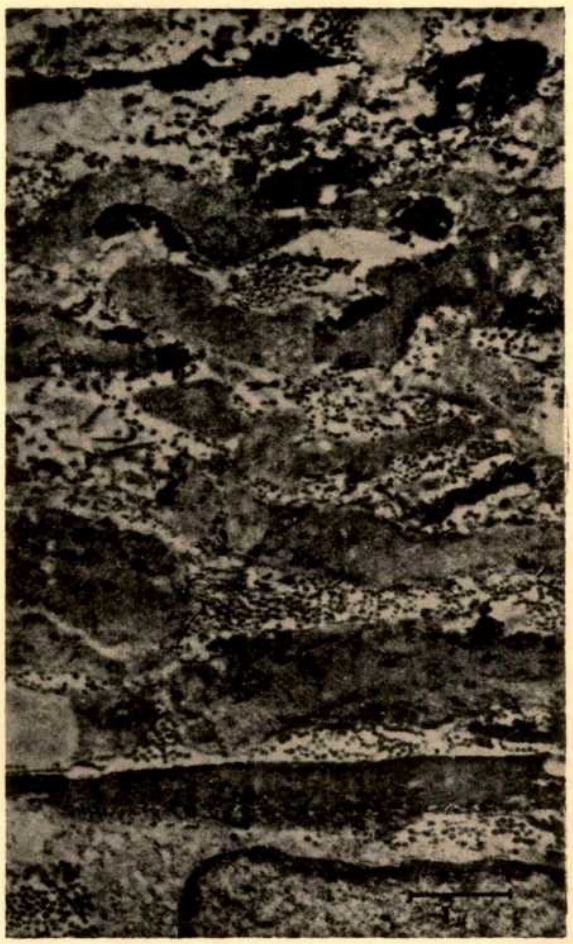


Fig. 4. Electron photomicrograph of a longitudinal section of a portion of the media of a rabbit aorta fixed while the vessel was distended by an intra-luminal pressure of 150 mm mercury (× *c.* 13,500). Most of the collagen fibres are seen in cross-section as small round and oval osmophilic spots



Fig. 3. Oblique section of a rabbit aorta fixed while the vessel was distended by an intra-luminal pressure of 60 mm mercury. Weigert–Van Gieson stain (× *c.* 620). The fine elastin fibrils are almost parallel and arranged obliquely between adjacent thick lamellæ

the elastic modulus is less than that of the highly elastic component but greater than that of the less elastic components; the absolute value of the modulus is related to the cross-sectional area occupied by each component. Substances of high tensile strength may yield at much less than intermolecular binding forces because of structural defects; a 'two-phase' material may have an actual tensile strength greater than that of the stronger component alone. This occurs because the more extensible (less elastic) component distributes the stressing forces uniformly; stresses about flaws are transferred to other parts of the stronger component, preventing the extension of structural defects and permitting the tensile strength to approach the theoretical value. Glass fibres embedded in plastic and laminated metals are examples of industrial 'two-phase' materials. The usefulness of bamboo and other woods with special properties is attributable to the presence of oriented cellulose fibres dispersed in lignin[3]. The strength of felted fur results from the intimate admixture of fibres of different composition[5]. Currey has recently discussed bone as a 'two-phase' system of collagen and apatite crystals[6].

Collagen and elastin are suitable components for a 'two-phase' material. Collagen has a high tensile strength and a modulus of elasticity of about 1×10^9 dynes/cm^2; elastin has a relatively low tensile strength and a modulus of elasticity of about 3×10^6 dynes/cm^2 (ref. 2). Though the relative quantities of elastin and collagen may vary somewhat in different segments of an aorta[7], the aortic wall has an elastic modulus approximating that of elastin at.

low intraluminal pressures and increasing markedly at and above physiological pressures[1,2,3]. Although distensibility is comparatively low at and above physiological pressures, the wall rarely breaks despite rapid fluctuations in distending pressure and numerous irregularities of composition and structure which increase in size and number with age. Thus, under usual conditions of distention at physiological pressures, the elastic modulus is relatively high as the oriented collagen fibres bear most of the stressing force; elastin fibrils serve to distribute the stressing forces uniformly. The mechanical properties of the wall at low, non-physiological pressures of 0 to 80 mm mercury, associated with the straightening of the wavy elastin lamellæ and the aligning of the disarrayed collagen fibres, may be only indirectly related to normal vessel function.

Attempts to assess the role of pressure and tension gradients in the aortic wall in disease processes must take into account the special mechanical features of this 'two-phase' material. The role of local defects and zonal strain may be relatively insignificant.

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'Rebound Effect' of Ovulation-inhibiting Steroid in Rats

While interpreting the results of studies in women and the dual role of 'Norethynodrel', Rock describes it as 'rebound effect' possibly on the pituitary gland[1]. It has been shown that syntheses of gonadotrophins are affected by administration of 'Norethynodrel'[2,3]. Our recent experiments on this rebound effect phenomenon and the results obtained have brought forth some new thoughts on the role of anterior pituitary gland in gonadotrophic hormone elaboration.

In the test animals (adult rats, Holtzman strain) which received 'Norethynodrel' over a period of 100 days, a marked decrease in ovarian weight was observed; but no other major differences were from our control animal experiments (Table 1). Structural changes in the anterior pituitary of test animals were not significant, and the variations were negligible. Somewhat similar observation has been recently reported[4]. In a second group of experimental animals the drug was administered for 100 days and the animals killed 50 days later. Salient differences in the number of corpora lutea and eggs (recovered from tubes at autopsy) were noticed in the test groups. Average number of corpora lutea were 6·4 per ovary in controls and 9·2 per ovary in test animals; the number of eggs 4·4 per tube in controls and 7·2 per tube in test animals, indicating a definite increase in the number of eggs in 'Norethynodrel' treated (1·0 mg/day)–recovered individuals. No other highly significant differences were noticed in the organs of test groups from controls, except slight variation in the weight of kidneys (Table 2). Microscopically the uterine cells which line the lumen in the treated group appeared more columnar than in the controls. This is possibly due to direct effect of steroid on the uterus, and this observation, to a certain extent, lends support to Rock's interpretation of expansion of womb in his post-medication subjects with progestins. We were a little surprised to see microscopically no outstanding differences in the pituitary glands of treated, treated–recovered and control groups.

Ever since the pioneering work of Bayliss and Starling[5], it is believed that a hormonal homeostatic mechanism exists in a normal individual depending on age and sex. They suggested that secretions of the pituitary gland are regulated by the needs of the body. Zuckerman gives a good review of earlier workers regarding this aspect[6]. It has already been reported that imbalance of ratio of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) results in anovulation[7,8]; the former seems to be more potential than the latter, since it is believed that twice the dose of suppressants is required for FSH inhibition than for LH in the pituitary gland (W. O. Nelson in discussion in *Control of Ovulation*, Pergamon, 1961, 243). As such the exact mode of action of pituitary gonadotrophin suppressants is not completely understood. However, it is assumed that exogenous steroids affect the pituitary gland in the synthesis and release of gonadotrophins[9]. Also the steroids can act as augmenting agents along with pituitary hormones in frog ovulation[10]. There is a common and perhaps important resemblance between endocrine glands and the brain. Both essentially control the vital processes occurring in the body. Whether the hormones affect changes by acting directly on the enzyme systems, or by modifying the permeability of cells and thus making particular substrate available, or some other method, remains to be explored.

The pituitary gonadotrophic hormone possibly unites with some receptor of the cells of the ovary, resulting directly or indirectly in the formation of a new biochemical entity which becomes potent to trigger some metabolic reaction to result in liberation of the egg. Hence, it seems that reproductive function is not merely a hormone to hormone relationship but involves an intricate system of glandular production, neural stimulation and vascular transportation. In the investigation recorded here it is noticed, in spite of no structural variations in the pituitary glands of treated animals, the ovaries were very small and did not show any grown follicles, indicating cessation of ovulation. Therefore, it may not be incorrect to assume that 'Norethynodrel' exerts its primary effect on the neurohumoral system which in turn affects the adenohypophyseal secretions.

There are many unanswered questions concerning hormonal activity and there are many aspects of total reproductive function as affected by hormones that are not completely understood. The role of the hypothalamus in reproduction presents a relatively new field of study, promising to answer many questions when its role is better understood. A series of experiments regarding the effect of 'Norethynodrel' in hypophysectomized and gonadectomized rats and changes in the hypothalamic nuclei, in correlation with normal intact ones, are in progress.

In the investigation recorded here it is noticed that the action of 'Norethynodrel' is not directly on the pituitary gland so far as basophil changes go. However, the depletion of gonadotrophins is not ruled out as it is known that synthesis is affected for administration of 'Norethynodrel'[2,3]. In the treated animals it is clearly seen that ovaries are highly reduced in size and weight, and with underdeveloped follicles. 'Norethynodrel' seems to exert its primary effect on the neurohumoral system which in turn affects the hypothalamo-hypophyseal complex to decelerate the secretion of gonadotrophins. This observation could lend support to Harris's interpretation of pituitary control by neural mechanisms[11] through a neurohumoral system. The pituitary gland plays a secondary part in the elaboration of trophic hormones but certainly not a dispensable one.

The 'rebound effect' seems to result in increase of number of eggs in rats; although at this juncture we are

Table 1

Group	Body-weight Initial	Body-weight Final	Ovaries	F. tubes	Uterine horns	Adrenals	Pituitary	Thymus	Heart	Kidneys	Thyroid	Dose and duration	C.L./ ovary	Eggs/ tube
1	195	200	12·0	10·2	249·5	28·4	5·9	116·9	404·3	894·9	8·3	1·0 mg/day—for 100 days	—	—
2	196	244	23·7	9·9	220·9	23·5	4·3	74·1	344·3	832·8	7·9	0·25 mg/day—for 100 days	—	—
3	198	272	31·1	8·2	188·8	22·7	4·7	101·2	334·8	777·2	8·4	Control group	6·5	5·1

Table 2

4	194	248	31·7	10·5	228·4	26·9	4·8	86·5	346·6	802·6	7·2	1·0 mg/day for 100 days and autopsied 50 days later	9·2	7·2
5	191	254	28·8	10·5	204·9	26·7	5·6	111·2	334·3	763·8	8·7	0·25 mg/day for 100 days and autopsied 50 days later	8·7	7·6
6	193	282	27·2	8·7	179·7	23·1	5·4	81·4	334·6	720·2	7·5	Control group	6·4	4·4

Organ weights are calculated in mg/100 g body-weight. Six animals in each group, except group 5 with four. Groups 3, 4, 5, and 6 killed 12 h after initiation of œstrus.

unable to say whether this increase in number of eggs in the recovered animals is transitory or a permanent one. The structure of anterior lobe of pituitary gland in treated, recovered and control groups did not show any significant microscopic differences, indicating that 'Norethynodrel', in the doses used in this experiment, does not significantly alter the morphology of the pituitary gland. Further experiments to elucidate the pathways are continuing and others planned for the future.

We thank G. D. Searle and Co. for the supply of 'Norethynodrel'.

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Biochemical and Physiological Function of Thiamine in Nervous Tissue

A considerable amount of evidence has been accumulated, primarily as a result of studies by von Muralt *et al.*[1], to suggest that thiamine may play a vital part in peripheral neurophysiology that is independent of its classical role as a coenzyme. Experiments in our laboratory suggest that thiamine may function as a neurohumour in the central nervous system, and, in addition, may be involved with sodium transport.

The spinal cord of a large pithed frog (*Rana catesbiana*) was stimulated via the sciatic nerve at a frequency of 1 pulse/sec; the amplitude of each stimulus was 6–12 V and its duration was 1 msec. At the conclusion of the stimulation period (15–60 min), the frog and a control (unstimulated) frog were decapitated and the spinal cords dissected out and extracted with either acid-alcohol (50 per cent ethanol; 0·1 N hydrochloric acid) or 5 per cent trichloroacetic acid. Using the procedure of Burch[2], the thiamine-content of an aliquot of the deproteinized extract was determined; the remainder of the extract was dried, taken up in a small volume of 50 per cent alcohol and chromatographed on either Whatman No. 3 *MM* or No. 4 paper using a solvent system composed of propanol : ammonium formate solution (2 M; *p*H 5) : water (65 : 15 : 25). Reference standards were thiamine, thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), α-hydroxyethylthiamine (HET), and α-hydroxyethylthiamine pyrophosphate (HETPP). After chromatography, either the spots were visualized by spraying with alkaline ferricyanide or the paper was arbitrarily sectioned into 0·5-in. strips which were eluted and the eluates assayed by the procedure of Burch.

Table 1. Release of Thiamine from Spinal Cord Thiamine μg/g Spinal Cord

Exp.	Control	Stimulated	Increase (%)
1	3·33	4·25	27·6
2	7·30	9·85	34·9
3	4·56	6·35	39·1
4	2·09	3·81	82·3
5	3·07	8·02	160·0

Values are expressed in terms of thiamine. Details on experimental procedures are given in the text. Exps. 1–3 are *in vivo*, experiments 4 and 5 are *in vitro*.

As shown in Table 1, stimulation of the spinal cord caused the appearance of an increased amount of thiamine compounds in extracts of the tissue, as compared with those of unstimulated control frogs. Exps. 1, 2 and 3 were performed as already indicated; Exps. 4 and 5 were done on isolated spinal cords (*in vitro*); some cords were kept as controls, while the others were stimulated as described above. Using the perfusion method of Angelucci[3], similar results were obtained with perfusates of the spinal cord of the frog. These results, which suggest that thiamine or its metabolites are released from a bound form on stimulation of the cord, are in agreement with those obtained by Minz[4] and von Muralt[5] with isolated peripheral nerves of the ox, frog, and rat. Since the thiamine assay of Burch does not distinguish between free thiamine and its various naturally occurring forms, paper chromatography of the spinal cord extracts was used for identifying the thiamine compound which is released on excitation of the tissue. The qualitative results obtained so far have indicated that both free thiamine and TMP appear to be responsible for the increase; in one experiment a trace of HETPP appeared to be present in the extract of the stimulated cord, but not in that of the control animal. Reliable and reproducible quantifications of the thiamine metabolites are difficult to obtain with this procedure because of the lability of the coenzyme forms of the vitamin.

For this reason these techniques do not permit a conclusion concerning the form of the vitamin that is released during the initial response to electrical stimulation. Although the neurophysiological activity of thiamine and its antimetabolites has been well documented by Kunz[6], von Muralt[1] and Petropulos[7], no direct evidence is available to separate this role from the metabolic role of TPP in carbohydrate metabolism. Enough indirect evidence has accumulated[8–10], nevertheless, to suggest that thiamine may have dual functions in the body.

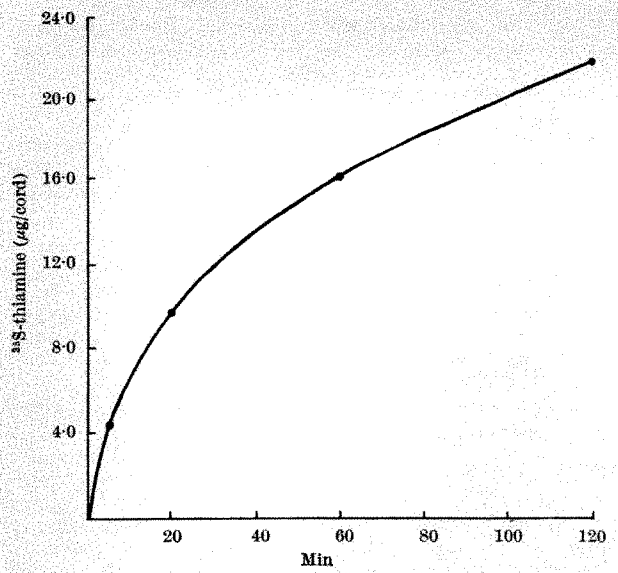


Fig. 1. Intact spinal cords of frogs were individually pre-incubated in 1 ml. of frog Ringer solution for 10 min at room temperature with oxygen as the gas phase. After the pre-incubation period, 300 μg of ^{35}S-labelled thiamine was added to each beaker and the incubation continued. At various times, the spinal cords were removed, rinsed quickly in cold Ringer's solution, blotted, and homogenized with 2 ml. of 5 per cent trichloroacetic acid. After centrifugation an aliquot of the supernatant solution was plated and the radioactivity determined in a gas-flow counter. In Fig. 1, the radioactivity measurement has been converted to μg of thiamine

That the neurophysiological activity is not mediated by thiamine *per se* is suggested by observations showing that large amounts of the vitamin are required to elicit a neurophysiological response. Although thiamine is a cationic molecule which contains a quaternary nitrogen atom, and it might be assumed that it does not readily penetrate lipoidal membranes, this explanation is untenable in the light of our findings; thus, when thiamine labelled with sulphur-35 was incubated with intact spinal cords, a rapid uptake of the vitamin occurred (Fig. 1). The radioactivity in the spinal cord was shown by paper chromatography to be unchanged thiamine; it is probable, however, that the neurophysiologically active form of the vitamin is some derivative of thiamine. Both pyrithiamine and oxythiamine produce signs of thiamine deficiency, but only pyrithiamine causes polyneuritis[10]. Since pyrithiamine, again in contradistinction to oxythiamine, inhibits the phosphorylation of thiamine, it might be inferred that the aforementioned active form of the vitamin is a phosphorylated derivative.

Present investigations in this laboratory[11] on the possible cation requirements of pyruvic oxidase in heart and brain have shown that in both tissues this enzyme complex is specifically activated by sodium ions (Table 2). In some preliminary experiments, a marked accumulation of HETPP (the primary intermediate in the oxidation of pyruvate by this enzyme system)[12,13] occurred when sodium ions were included in the incubation mixture.

It is tempting to speculate that the following sequence of reactions may occur during excitation of nervous tissue. The influx of sodium ions into the cell activates pyruvic oxidase, which then causes the rapid formation of HETPP The HETPP may itself initiate a response which is terminated by the hydrolysis of HETPP to another form of the vitamin, or it may, under appropriate conditions, lead to the rapid synthesis of acetylcholine, which then initiates the response. Although the foregoing hypothesis is highly speculative, experiments by von Muralt[5] and by Kunz[6] with the thiamine-binding extract of ferns, as well as with pyrithiamine, have indicated a relationship between the sodium transport system and thiamine.

A critical evaluation of this hypothesis is at present under investigation in this laboratory.

We thank Dr. H. Holtzer for a sample of HETPP, Dr. J. M. Sprague for the HET and Hoffmann-LaRoche for ^{35}S-labelled thiamine. We also thank Dr. L. O. Krampitz for details concerning the preparation of HETPP.

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Table 2. Sodium Ion Activation of Pyruvic Oxidase

Cation in medium	Citrate formed (μmoles) Beef heart	Pig brain
K^+	1·89	1·35
Na^+	2·75	2·57
Increase in activity with Na^+	45·5%	90·5%

The incubation medium contained, in a volume of 2 ml., the following: phosphate buffer, *p*H 7·4, 100 μmoles; pyruvate, 20 μmoles; oxaloacetate, 10 μmoles; $MnCl_2$, 2·5 μmoles; cysteine HCl, 10 μmoles; coenzyme A, 100 μg; NMN, 2·1 μmoles; thiamine pyrophosphate, 1 μmole; lactic dehydrogenase (Worthington), 40 μg; condensing enzyme (ref. 14), 50 μg; pyruvic oxidase preparation from beef heart, 3·8 μg, or from pig brain, 3·9 μg. Incubation was carried out at 30° in a nitrogen atmosphere for 1 h and the mixture was deproteinized with trichloroacetic acid. Citrate was determined by the method of Stern (ref. 15).

HÆMATOLOGY

Role of Platelets in Dissolution of Blood Clots

Venous blood (1 vol.) diluted with 0·05 M phosphate buffer, *p*H 7·4 (9 vol.), and clotted with bovine thrombin (Parke, Davis and Co.; 1 unit per ml.) will afterwards dissolve in 2–8 h at 37° C if the diluted blood is handled in siliconized glassware and kept at 0° C for 30 min after the addition of thrombin[1]. The time required for the clot to dissolve is 12–24 h when the diluted blood is centrifuged at 1,800*g* for 10 min (4° C) and the supernatant alone is clotted. If the supernatant and infranatant are mixed and then clotted with thrombin, the clot will again dissolve in 2–8 h. Centrifugation at 1,800*g* for 10 min would be expected to remove only the cellular components from the supernatant.

Red blood cell and platelet counts on the supernatant (Table 1) show that the red blood cell count approaches zero before the dissolving time of the clotted supernatant increases. Only as the blood platelet count drops to $1{\cdot}0 \times 10^3$ per ml. does the dissolving time of the clot increase to 12–24 h. It therefore appears that blood

Table 1. Relationship of Red Blood Cell and Platelet Counts to Clot Dissolving Time*

Centrifugation of diluted blood for 10 min at 4° C (*g*)	Clot dissolving time		Supernatant cell counts	
	Supernatant alone (h)	Remixed samples (h)	Platelet count/mm³ (× 10³)†	Red blood cells/mm³ (× 10⁶)
0	4·3	4·3	25·0	513·3
200	4·1	4·3	18·2	0·26
1,200	4·6	4·3	8·1	0·29
1,800	> 12 < 24	4·3	1·1	0·05

* Average values from studies on five healthy adults at 9 a.m.
† Platelet counts carried out with a phase microscope (ref. 3).

platelets are required for short dissolving times of clots in the assay system used in this work. In investigations of blood from a patient with leukæmia and thrombocytopænia, Van Creveld and Mochtar[2] found that the dissolving times of clots were related to the level of circulating platelets. Apparently the dissolving time of clots will be prolonged if the platelet concentration is lowered by *in vitro* centrifugation or *in vivo*.

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PHARMACOLOGY

Pharmacological Characteristics of Neurohypophysial Hormones in Lungfish and Amphibians

THE morphological similarity between the neurohypophysis of lungfishes and amphibians[1] suggested a comparison of the neurohypophysial hormones of these groups of vertebrates. When extracts of the neural lobe of *Protopterus aethiopicus* Owen were chromatographed and pharmacologically characterized, we[2] found that they contained one active principle (8-arginine oxytocin, vasotocin) common to both teleosts and amphibians. The other oxytocic peptide found differed pharmacologically from the second hormone in teleost glands, that is, from ichthyotocin[3], which has now been identified as 4-serine 8-isoleucine oxytocin[4,5].

Further investigations of the unidentified oxytocic principle obtained from the African lungfish (*Protopterus*) and the Australian lungfish (*Neoceratodus* sp.) showed that it behaved in many ways like oxytocin: its R_F in the solvent system *n*-butanol/acetic acid/water (4 : 1 : 5) could not be distinguished from that of oxytocin. When assayed against oxytocin on the isolated rat uterus with or without magnesium in the suspending solution, the mean potency ratio (R_m = with Mg^{++}/no Mg^{++}) was 1·00 ± 0·08 for *Protopterus* and 1·19 ± 0·12 for *Neoceratodus*. Similar ratios (0·97 ± 0·16 for *Protopterus*, 1·06 ± 0·05 for *Neoceratodus*) were obtained by comparing milk ejection activity in the guinea pig with oxytocic action on the rat uterus (no Mg^{++}).

A difference from oxytocin was observed when the lungfish extracts were assayed on the blood pressure of the chicken. By definition the ratio of avian vasodepressor potency to rat uterus (no Mg^{++}) activity ($=R_a$) should have been unity had the eluates contained oxytocin alone. However, significantly higher values were consistently obtained (Table 1).

The active principles in amphibian neurohypophyses were investigated in the same manner. Extracts of frog (*Rana esculenta*) and toad (*Xenopus laevis*) glands were used. The characteristics of one of the hormones found were again compatible with the presence of vasotocin. The pharmacological properties of eluates from the other active region of the chromatograms resembled those of the unidentified oxytocic peptide in lungfish glands: the potency ratios in oxytocic assays with and without magnesium and in galactobolic assays were again near unity. The results of avian vasodepressor assays differed somewhat in *Rana* and *Xenopus*. The values of R_a in thirteen assays from three batches of *Xenopus* neurohypophyses varied from 1·64 to 2·30; assays on eluates from frog pituitaries (nine assays, four batches of glands) gave lower figures, varying from 1·18 to 1·58.

In order to obtain a measure of the precision of R_a, control experiments with mammalian oxytocin were performed. Oxytocin was prepared from pig neural lobes by the same method as the eluates from amphibian or lungfish glands. It was then assayed against synthetic oxytocin on the isolated rat uterus (no Mg^{++}) and by the avian depressor method.

Table 1 shows the mean values for R_a obtained in the species investigated. It also gives values for mammalian oxytocin and a synthetic analogue of oxytocin the pharmacological characteristics of which are similar to those of the eluates under discussion.

The high values for the ratio R_a obtained in lungfish and amphibians pose the following questions:

(1) Since R_a for vasotocin is much greater than unity (3·1 ± 0·14), could the high values found be due to contamination of the 'oxytocic' region of the chromatograms with vasotocin? In 26 out of 32 assays on lungfish and amphibian eluates R_a was greater than 1·4. To yield such a ratio, 20 per cent of the rat uterus activity would have to be due to vasotocin. A contamination of this magnitude is unlikely since it would have become apparent in rat pressor assays. Moreover the potency ratio R_m (rat uterus with Mg^{++}/rat uterus without Mg^{++}) would also have been raised whereas in all experiments it was not significantly different from unity. The fact that the African lungfish extracts contained little vasotocin should be added.

Table 1. MEAN POTENCY RATIOS AVIAN DEPRESSOR ACTIVITY TO RAT UTERUS ACTIVITY WITHOUT MAGNESIUM (R_a)

Material	No. of assays*		R_a ± S.E.†	P for difference from oxytocin
	No Mg	Chick B.P.		
Oxytocin	7	9	0·98 ± 0·07	—
8-Ileu oxytocin	6	6	1·56 ± 0·08	< 0·001
Protopterus	9	8	1·48 ± 0·13	< 0·01
Neoceratodus	3	2	1·81 ± 0·06	< 0·001
R. esculenta	13	9	1·40 ± 0·10	< 0·005
Xenopus	16	13	1·95 ± 0·13	< 0·001

* Each assay consisted of four (2 + 2) blocks.
† The potency ratios were calculated according to Sawyer, Munsick and van Dyke (ref. 6).

(2) Are the high values for R_a due to the presence of an as yet unidentified neurohypophysial hormone or to a mixture of several active peptides, including perhaps oxytocin? The activities present in the eluates could indeed be due to a single hormone, for a synthetic analogue of oxytocin, namely, 8-isoleucine oxytocin, is known the pharmacological spectrum of which resembles closely those of the eluates under discussion. Thus in rat uterus assays with and without magnesium and in milk ejection assays the potency ratios for 8-ileu-oxytocin are not significantly different from unity[7,8]. However, R_a for 8-ileu-oxytocin is considerably higher than 1·0; it is 1·67 according to Berde and Konzett[7] and was 1·56 ± 0·08 according to our own findings. 8-isoleucine oxytocin is intermediate in structure between the 'oxytocic' hormone of bony fishes (4-serine 8-isoleucine oxytocin) and oxytocin, the hormone of higher vertebrates. Its occurrence would therefore be in keeping with the intermediate phyletic position of dipnoans and amphibians. However, the possibility that the eluates contained more than one active principle cannot be excluded. The results of Maetz, Morel, Acher, Chauvet and Lenci[9] with neurohypophysial extracts of *Rana esculenta* are compatible with the presence of two active peptides in addition to vasotocin. A high natriferic activity was associated with one of these peptides whereas in our experiments the natriferic activity of eluates from *Protopterus*, *Rana esculenta* and *Xenopus* was low, and not different from that of oxytocin.

These results confirm the findings of Follett and Heller[2] that, besides vasotocin, lungfish glands contain an oxytocic principle different from that of bony fishes and similar to that of amphibians. They show, however, that the pharmacological spectrum of this oxytocic hormone (or mixture of oxytocic hormones) in the Amphibia and Dipnoi investigated differs from that of mammalian oxytocin. The occurrence of yet another neurohypophysial hormone, in addition to the five already identified, may thus have to be envisaged.

We thank Profs. A. H. Ennor and F. W. Landgrebe for supplying us with *Neoceratodus* and *Xenopus*.

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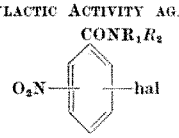
Anti-coccidial Activity of Halogenonitrobenzamides

In the course of a search for new anti-coccidial compounds we have prepared and examined the prophylactic activity of a number of halogenonitrobenzamides against *Eimeria tenella*.

The compounds, mixed in the food at 0·01 or 0·02 per cent w/w, were fed to young chicks for 8 days, starting one day before inoculation with sporulated oocysts. The criteria of activity were comparisons of deaths, blood in the droppings, and cæcal lesions.

Several compounds showed good prophylactic activity in these preliminary tests (Table 1), but after more detailed examination (Table 2) one of these, 2-chloro-4-nitrobenzamide[1] ('M and B 5921'), was selected for further investigation both alone and in association with sulphaquinoxaline. In particular, a mixture composed of 3 parts 'M and B 5921' and 2 parts sulphaquinoxaline showed good activity against experimental *E. tenella*, *E. necatrix* and

Table 1. Prophylactic Activity against *Eimeria tenella*



Halogen and position	Position of NO_2	R_1	R_2	Per cent in food	Relative activity
2–Cl ('M and B 5921')	4	H	H	0·01	+ + +
2–Cl	4	CH_3	H	0·02	+
2–Cl	4	C_2H_5	H	0·02	+ + +
				0·01	+ +
2–Cl	4	nC_3H_7	H	0·02	+
2–Cl	4	$isoC_3H_7$	H	0·02	+ +
2–Cl	4	nC_4H_9	H	0·02	0
2–Cl	4	CH_2	CH_3	0·02	+
2–Cl	4	C_2H_5	C_2H_5	0·02	+ + +
				0·01	+ +
2–Br	4	H	H	0·01	+ +
2–Br	4	CH_3	H	0·01	+ +
2–Br	4	C_2H_5	H	0·02	+ + +
				0·01	+ +
2–Br	4	CH_3	CH_3	0·02	+
3–Cl ('M and B 5888')	5	H	H	0·01	+ + +
3–Br	5	H	H	0·01	+ +
3–Br	5	CH_3	H	0·02	+ + +
				0·01	+ +
3–I ('M and B 5889')	5	H	H	0·01	+ + +
Reference compound—nitrofurazone				0·01	+ + +

Table 2. Comparative Prophylactic Activity against *E. tenella* in Chicks

Expt. No.	'M and B' No. or name	Per cent in food	Survivors in treated groups: Proportion*	Corrected (per cent)†	$E.D._{50}$ ‡ as per cent drug	Survivors in untreated controls
1	5888 §	0·025	5/5	100	0·007	5/15
		0·0125	9/10	85		
		0·006	4/9	17		
		0·003	1/5	20		
	5889 ¶	0·025	15/15	100	0·01	
		0·0125	14/15	90		
		0·006	5/15	0		
	5921	0·0125	15/15	100	0·005	
		0·006	11/14	68		
		0·003	4/10	10		
	Nitrofurazone	0·02	10/10	100	0·007	
		0·01	14/15	90		
		0·005	4/10	10		
2 and 3 ‖	5888	0·025	20/20	100	0·01	9/20
		0·0125	17/20	73		
		0·006	11/20	18		
	5921	0·0125	20/20	100	0·005	
		0·006	18/20	82		
		0·003	5/20	0		
	Nitrofurazone	0·01	20/20	100	0·004	
		0·005	17/20	73		
		0·0025	11/20	18		

Medication from 1 day before until 7 days after infection when results were determined
* Numerator = No. of survivors; denominator = No. of chicks infected.
† By Abbott's formula (ref. 5).
‡ Dose which saved 50 per cent of chicks obtained graphically using de Beer's method[6].
§ 'M and B 5888' = 3-chloro-5-nitrobenzamide.
¶ 'M and B 5889' = 3-iodo-5-nitrobenzamide.
‖ Results of two comparative tests added together.

E. acervulina infections when fed at 0·02 per cent w/w. The nitrobenzamide appeared to be less effective than sulphaquinoxaline against *E. acervulina*, but more effective against the other two species.

The mixture affected the development of the second generation schizonts of *E. tenella* and inhibited the early developmental stages of *E. acervulina*.

Detailed comparisons of activity against different laboratory strains of *E. tenella* have shown interesting variations.

The halogenonitrobenzamides are analogous to the active dinitrobenzamides[2,3] in which one nitro-group has been replaced by halogen. Hepworth[4] similarly found that replacement of one of the nitro-groups in 4,4′-dinitrocarbanilide by halogen gave a compound which retained anti-coccidial activity.

A full account of the biological investigations will be submitted for publication in due course.

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PATHOLOGY

Calcification in the Intervertebral Disk

The human intervertebral disk is a structure in which degenerative changes are very common, often appearing as early as the third decade. Consequently it is not surprising that foci of calcification are frequently found in the intervertebral disks of apparently normal subjects. However, little information is available on the exact nature of the mineral phases and the possible significance that these may bear in relation to the underlying changes in the matrix. At present X-ray diffraction is the most sensitive method for identification of the various calcium compounds which can occur in the body.

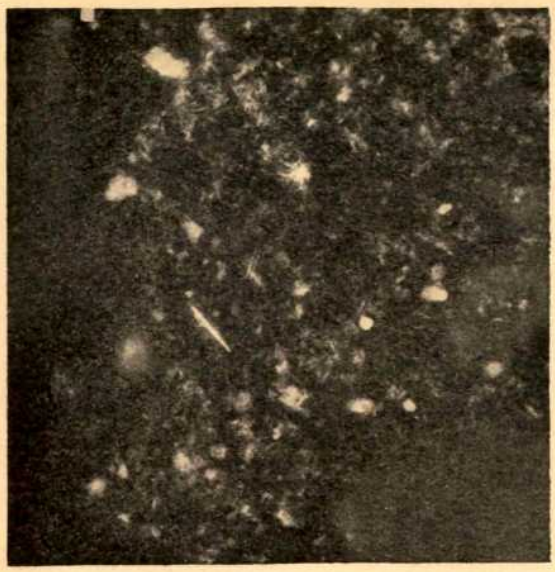


Fig. 1. Electron microscope photograph of prolapsed calcified thoracic intervertebral disk tissue showing crystallites of variable size on a very degraded matrix (× 9,000)

In an X-ray diffraction examination of intervertebral disk tissue which has been carried out by one of us (T. K. F. T.) hydroxyapatite diffraction patterns, similar to those obtained from bones, have been recorded from small calcified deposits at all spinal levels. However, when larger deposits have been examined, the crystallite size, as judged from the degree of broadening of the major reflexions, was larger than that which is usually recorded from pathological calcifications. When prolapse of a thoracic intervertebral disk occurs the extruded tissue is usually highly calcified and the dominant inorganic phase is hydroxyapatite. X-ray diffraction and electron microscope studies of such tissue have shown that although there is a variation in the crystallite size the majority of the crystallites are very large, approaching the size of those present in normal dental enamel. In the electron microscope the large crystallites have been observed in those areas devoid of living cells and fibrillar elements in the matrix (Fig. 1). This suggests that cell activity, in some as yet unexplained way, may be associated with the factors which regulate the size of the hydroxyapatite crystallites in a calcifying focus.

Hydroxyapatite is usually the dominant mineral constituent of pathological calcifications in the human body, but X-ray diffraction patterns of calcite, as a separate phase, have frequently been obtained from post-mortem and prolapsed disk tissue examined by us. It thus appears that calcium carbonate is a common inorganic component of the intervertebral disk. On twelve occasions (eleven of them when examining prolapsed tissue) the diffraction pattern shown in Fig. 2 has been recorded. The spacings do not correspond to those characterizing any compound yet described; the two strongest reflexions are 3·88 Å and 2·62 Å ± 0·05 Å. However, as water was eliminated (a matter of hours) the diffraction pattern changed to that of calcite. It is tentatively suggested that in this tissue the calcium carbonate, or a proportion of it, exists in a metastable form. On numerous occasions when calcium carbonate had not been found in disk tissue dried at room temperature the inorganic residue contained calcium oxide after heating to 950° C for 1 h. It is well known that mineralized tissues in the body contain 5–10 per cent carbonate, and this lack of separation as an independent phase is the normal finding[1].

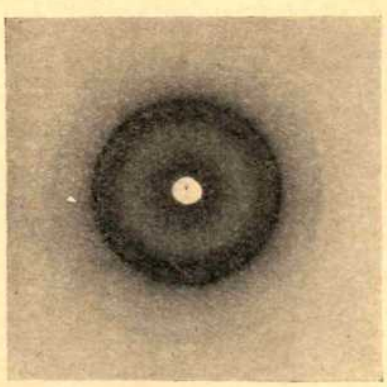


Fig. 2. Fibre X-ray diffraction pattern of prolapsed intervertebral disk tissue: Copper $K\alpha$ radiation of wave-length 1·54 Å, specimen to film distance ≈ 3 cm. The collagen diagram shows orientation with arching of the 2·86 Å reflexion. Two further rings are present, one at 3·88 Å (strong) and the other at 2·62 Å

X-ray diffraction patterns of whitlockite have been recorded from a substantial proportion of samples of air-dried disk tissue from subjects of more than sixty years of age. Preliminary measurements have indicated that the alterations in the lattice constants of this phase are compatible with a high atomic percentage of magnesium. In those subjects whose intervertebral disks contained whitlockite, it has been found that the cartilaginous tissues of the nasal septum, trachea, epiglottis and the wall of the abdominal aorta occasionally gave similar whitlockite diffraction patterns. The fact that whitlockite was not found in an extensive survey of the mineral phases in the bones of elderly people[1] suggests that the changes in the above tissues favouring the pathological deposition of whitlockite are due, at least in part, to local factors. Whitlockite diffraction patterns have not been recorded from a large number of specimens of prolapsed lumbar disk tissue dried at room temperature, and in this respect, as well as in some of the appearances of cells and matrix[2], prolapsed lumbar disk tissue differs from other disk tissue in the older age groups. A fuller account of this work and of other inorganic constituents of the intervertebral disk will be presented at a later date.

This work was carried out while one of us (T. K. F. T.) has been in receipt of a Nuffield Dominions Clinical Assistantship in Orthopædic Surgery at the University of Oxford. We thank Dr. J. Thewlis for his advice on the interpretation of some of the X-ray diffraction patterns.

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Carcinogenic Action of *N*-hydroxy-4-acetylaminostilbene

Recent investigations[1,2] have indicated that 2-acetylaminofluorene and 4-acetylaminobiphenyl are metabolized to *N*-hydroxy derivatives in the rat and these metabolites are more carcinogenic than the parent amides. Thus, *N*-hydroxy-4-acetylaminobiphenyl was as active as the parent amide as a mammary carcinogen and in addition induced a higher incidence of ear-duct carcinomata and forestomach papillomata[2].

The urinary excretion of *N*-hydroxy-4-acetylaminostilbene following oral administration of 4-dimethylaminostilbene or 4-acetylaminostilbene to rats has been demonstrated[3]. Furthermore, *N*-hydroxylation of 4-acetylaminostilbene has been observed in an *in vitro* system with rat liver homogenate or the mitochondrial supernatant fraction in the presence of oxygen and $NADPH_2$ (ref. 3). The carcinogenicity of *N*-hydroxy-4-acetylaminostilbene has

been examined, therefore, to assess further the importance of *N*-hydroxylation in amine carcinogenesis.

N-hydroxy-4-acetylaminostilbene was prepared by aluminium amalgam reduction of an ethereal solution of 4-nitrostilbene followed by direct acetylation of the reaction mixture with acetic anhydride. The *N*-hydroxy derivative was then extracted into alkali, re-extracted into ether/ethanol/acetone (3 : 1 : 1) from neutral solution and crystallized several times from benzene (m.p. 200° C).

The carcinogenic activity of this compound as well as that of the parent amide and 4-dimethylaminostilbene was assessed following oral administration to rats in a low protein diet[4]. The concentration of each compound in this diet was 40 mg/kg so that the daily intake of carcinogen per rat was approximately 0·5 mg. The *N*-hydroxy derivative proved to be highly toxic for female rats at this dose-level, as evidenced by marked weight losses after prolonged feeding. This toxicity was also manifested by a high mortality and only 10 rats (62 per cent) survived the period of treatment (22 weeks; total dose, 75 mg/rat). Toxic effects were less pronounced in male rats and all but two survived feeding with the *N*-hydroxy compound for 32 weeks (total dose, 112 mg/rat). Survival in rats fed either 4-acetylaminostilbene or 4-dimethylaminostilbene was good and these compounds were fed for 22 weeks or 32 weeks in female and male rats, respectively.

Following completion of carcinogen treatment, rats were maintained on the basal low protein diet until the experiments were terminated (50–60 weeks).

Ear duct tumours first became detectable after 20 weeks of feeding these compounds and, as shown in Table 1, the final tumour incidence in rats receiving *N*-hydroxy-4-acetylaminostilbene was almost identical to that obtained with the parent amide. In contrast, the incidence of ear-duct tumours in rats fed 4-dimethylaminostilbene was somewhat lower, while with this compound female rats were almost twice as susceptible as male rats.

Table 1. Carcinogenicity of Aminostilbenes following Oral Administration to Rats

Compound	Initial No. of rats	Sex	No. at risk*	Incidence of ear-duct carcinomata percentage
4-Dimethylaminostilbene	16	M	16	55
	16	F	11	55
4-Acetylaminostilbene	16	M	16	88
	16	F	13	85
N-Hydroxy-4-acetylaminostilbene	16	M	14	86
	16	F	6	83

* Tumour incidences calculated from number of rats alive when tumours were first observed.

Histologically the ear-duct tumours induced with the aminostilbene compounds were all squamous carcinomata. They were well differentiated with plentiful cell nest formation and often showed keratin-filled cysts. Polymorph infiltration going on to abscess formation was a frequent finding, and in the larger tumours central necrotic areas were present.

No other tumours were observed in rats fed either 4-acetylaminostilbene or 4-dimethylaminostilbene. In rats fed the *N*-hydroxy derivative, one had an abdominal fibrosarcoma and another had an intestinal adenocarcinoma.

The carcinogenicity of *N*-hydroxy-4-acetylaminostilbene has been observed also by Andersen *et al.*[5]. In confirmation of the present findings, they found that the *N*-hydroxy derivative was as active as the parent amide in inducing ear-duct carcinomata in the rat. In addition, however, they also found that the *N*-hydroxy derivative, but not the parent amide, induced tumours at other sites, particularly in the forestomach.

In considering possible mechanisms of the carcinogenic action of *N*-hydroxy-4-acetylaminostilbene, the high reactivity generally observed with arylhydroxylamines[6] may be relevant, since these compounds will be capable of undergoing a variety of interactions *in vivo* with intracellular components such as protein and nucleic acid. Thus, for example, hydroxylamine itself is a mutagen in *T4* phage[7] with a high degree of specificity of action and the genetic damage is produced, it is thought, through direct action on cytosine in DNA. Furthermore, hydroxylamine has been shown to induce chromosomal damage in mammalian cells grown *in vitro*[8].

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Differential Behaviour of Transplanted Mouse Lymphoma Lines in Genetically Compatible Homozygous and F_1 Hybrid Mice

Tumours induced in F_1 hybrids between two isogenic resistant mouse strains, differing at the histocompatibility-2 locus, occasionally give variant lines, compatible with one or the other of the parental strains, entering the F_1 cross[1,2]. Such variants have irreversibly lost the histocompatibility-2 antigens, determined by one of the parents. The mechanism of variant formation has been tentatively attributed to somatic crossing-over, even if deletion of a terminal chromosome segment cannot be excluded[1–4].

During further analysis of variant formation, variant sub-lines, compatible with one parental strain, were compared with the unselected F_1 tumour line, from which they have been derived, with regard to their ability to grow in mice of the original F_1 genotype. The results obtained were rather unexpected: the variant lines grew less well in the F_1 hybrids than the unselected lines.

A lymphoma designated *LNSF* was used. It originated in an *A/Sn* × *A.SW* F_1 hybrid and had been carried by serial subcutaneous transplantation in adult *A* × *A.SW* F_1 hybrids during 23 passages prior to the experiments recorded here. Four variant sub-lines were isolated, compatible with the *A.SW* strain, and two sub-lines, compatible with *A/Sn*. They were true variants, according to serological and transplantation experiments, performed as previously described[2], as judged by the loss of the histocompatibility-2 antigenic complex specific for *A* and *A.SW*, respectively.

Two *A.SW* variants were used for the tests recorded here immediately after one passage in the *A.SW* mouse where they were selected, while the other variants were carried for 1–3 transfers in the selective parental strain, followed by 1–2 passages in *A* × *A.SW* F_1 hybrids. Mechanically prepared cell suspensions of the variants or the F_1 line, *LNSF*, containing 60–80 per cent eosin unstained cells[5], were inoculated subcutaneously in a volume of 0·1 ml. containing 10^3 living cells to mice weighing 16–18 g. Approximately the same proportion of males and females was used in the majority of experiments. The mice were inspected every third day after inoculation, and the time required for the appearance of a tumour was recorded.

As exemplified in Fig. 1*A*, the variants grew less well in *A* × *A.SW* F_1 hybrids than the original unselected line, as judged by a more prolonged latency period and a slightly lower total frequency of takes. This was true for all variants tested, and particularly striking for the two *A*-variants. No differences were found between the sub-line tested directly after one passage in the selective

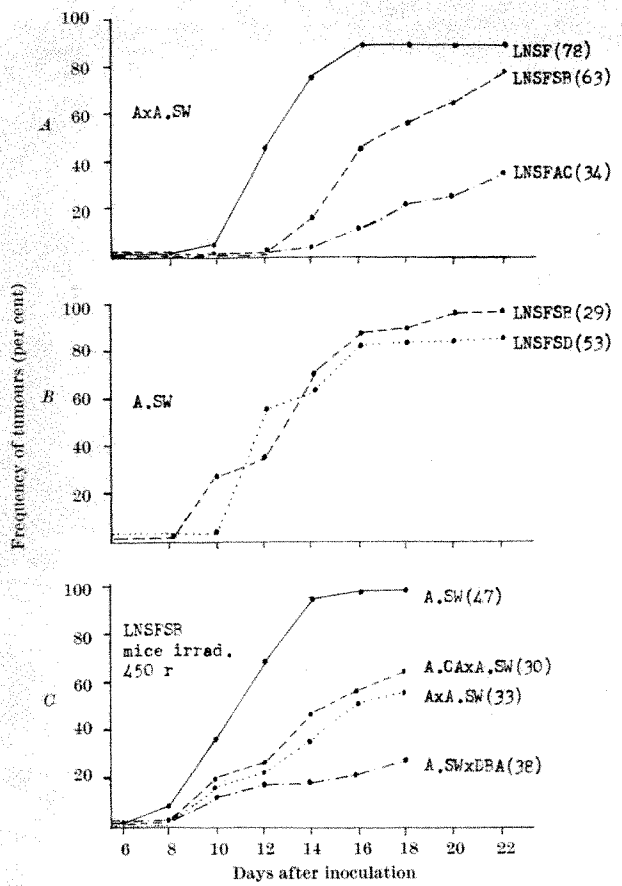


Fig. 1. *A*, Inoculation to *A/Sn* × *A.SW* F_1 hybrids of *LNSF* (unselected line in *A* × *A.SW*), *LNSFSB* (selected during two transfers in *A.SW*, followed by two transfers in *A* × *A.SW* prior to the current test) and *LNSFAC* (selected during two transfers in *A*, followed by one transfer in *A* × *A.SW* prior to the test). The numbers of mice inoculated in each group is shown within parentheses. *B*, Inoculation to *A.SW* of *LNSFSB* and of *LNSFSD* (tested directly after selection in *A.SW*). *C*, Inoculation of *LNSFSB* to *A.SW*, *A* × *A.SW*, *A.CA* × *A.SW* and *A.SW* × *DBA* F_1 hybrids. The mice had been pre-irradiated with 450 r. the day before inoculation

A.SW host or after subsequent serial passage in *A* × *A.SW* F_1. The difference between variants and the F_1 line disappeared when 10^6 cells were inoculated.

In order to test whether the differences between selected variant sub-lines and unselected F_1 hybrid lines with regard to their growth in the common F_1 hybrid recipient are related to the antigenic differential between the F_1 recipient and the variant cell, that is, the lack of certain histocompatibility-2 antigens in the latter, the variant cells were tested in an analogous way in the parental strain, where they had been selected and where no antigenic differential was known to exist. When 10^3 variant cells were tested in this way, they grew better than in F_1 hybrids and as well as the unselected lines grew in *A* × *A.SW* (Fig. 1*A* and *B*).

When 10^3 variant cells, compatible with *A.SW*, were tested in different F_1 hybrids other than the type of origin but containing *A.SW* as one contributor to the cross, it was found that they grew poorly in *A.SW* × *DBA*, slightly better in *A.SW* × *C3H* and still better in *A.SW* × *A.CA*, which was behaving similarly to *A* × *A.SW* F_1. The differences between the growth of *A.SW* variants in *A.SW* and in F_1 hybrids was not abrogated by irradiation of the hosts with 450 r. one day before grafting, in experiments with three variants and a minimum of 10 mice of each genotype tested for each variant. Analogous results were obtained with two *A*-variants, compared in untreated and irradiated *A* × *A.SW* and *A* × *A.CA* F_1. Fig. 1*C* exemplifies the growth of an *A.SW* variant in pre-irradiated mice of various genotypes. It may be of interest that pre-irradiated *A.SW* × *DBA* F_1 hybrids, while still showing relatively poor growth of 10^3 variant cells, nevertheless accepted an inoculum of 10^6 cells from a genetically incompatible (*A/Sn*) tumour, which did not grow in unirradiated hosts of this type, indicating that irradiation was efficient in abolishing the host versus graft reaction.

The mechanism of the phenomenon remains to be clarified. Two possible explanations may be considered:

A host-versus-graft reaction directed against antigens present in the variants and their parental strain hosts but absent from the F_1 hybrids could be postulated by assuming the existence of recessive antigens, determined by genes on the ninth chromosome and coming into expression in the variants as a result of somatic crossing-over (or, possibly, deletion of a chromosome segment). The fact that recessiveness to transplantation antigens has not, so far, been conclusively demonstrated by a variety of methods used and the finding that pre-irradiation does not abrogate the relative resistance of the F_1 hybrid hosts weakens this possibility. This latter finding also weakens the possibility that the results are due to antigenic differences between *A.SW* and *A* other than histocompatibility-2 or to the slight heterozygosity of the *A.SW* strain, detectable by skin transplantation techniques[6]. Furthermore, it would be difficult to explain on the basis of such a hypothesis that the unselected *LNSF* tumour grew as well in *A* × *A.SW* F_1 mice as the variants in the strain where they have been selected and both grew better than the variants in the F_1 hybrids.

These results may be more akin to a graft-versus-host reaction than a host-versus-graft response. The variant cells may recognize the host cells as antigenically different, and an intercellular reaction based on such recognition would be in line with the results obtained, although direct experimental confirmation is lacking. Alternatively, the mere exposition of the variant cells to the foreign antigens of the host may inhibit their growth. Both phenomena might act as homeostatic mechanisms, counteracting the appearance of antigenically deficient cells.

The results described appear to be similar to findings mentioned by Snell, obtained with homozygous lymphomas[7,8], which have been fully confirmed in (unpublished) experiments with two lymphomas of *A/Sn* origin, one lymphoma of *C57 B1* origin and one lymphoma of *A.SW* origin: they all grew better in the strain of origin than in various F_1 hybrids. The possible influence of recessive antigens or, for example, endocrine or metabolic differences may be more difficult to exclude in such situations, however, than when comparing tumour lines of a common origin, as presently described. It needs to be worked out whether similar findings can be made even with carcinomas and sarcomas.

The findings may suggest possibilities for the selection of cells having certain antigens from a large population of cells lacking them. Model experiments of that type are in progress. If successful, they might be of value for genetic investigations, trying to concentrate rare variants, resulting from, for example, fusion[9].

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RADIOBIOLOGY

Effects of X-rays on Electrical Activity in the Eye of the Cockroach *Blaberus giganteus*

Significant changes in electroretinograms (ERG) and in spontaneous electrical activity recorded from the compound eyes of cockroaches have been found here during investigations of the effects of radiation on insects. These electrical discharges, although similar to the activity recorded from the optic nerve of *Limulus*[1], do not exhibit as marked a change in frequency with light stimulation. In this communication, we present the results of preliminary studies of the effects of X-rays on both the ERG and the spontaneous electrical discharges in the eye of the cockroach *B. giganteus*.

Whole adult insects, wrapped in polythene, were fastened to a wax platform. Fine platinum wire electrodes were inserted into the eye and into the head capsule by means of a semi-micromanipulator. Most consistent results were obtained when the reference electrode was located immediately posterior to the margin of the eye. Electrical activity, displayed on a dual-beam 'Tecktronix' oscilloscope, was recorded with a 'Brush' pen recorder. The insects were irradiated with a 150 kV X-ray machine (inherent filtration 3 mm beryllium; target-specimen distance 20 cm) at a dose rate of 2,000 r./min. The source of light was fluorescent room-lights giving an illumination of approximately 4 ft.-candles. All experimental animals were light-adapted.

Fig. 1, tracing *A* shows the typical spontaneous electrical activity prior to irradiation. With irradiation, this activity gradually decreased and, at a total dose of approximately 120 kr, all activity had virtually disappeared (Fig. 1, *B*). Partial recovery from this effect occurred during a one-hour rest period without X-rays (Fig. 1, *C*). Subsequent irradiation-and-rest cycles showed that suppression and recovery would occur several times, although less radiation and shorter rest periods were required for succeeding cycles of irradiation and rest. The amplitude and frequency of the electrical activity never returned to the pre-irradiation values.

B. giganteus reacted to changes in light intensity with the typical 'on-off' response (Fig. 2, *A*) of some insects as described by Roeder[2]. Exposure to X-rays decreased the amplitude of the 'on' response to light, but no change in amplitude was observed in the 'off' response. The reduction in the 'on' response was small, reaching a maximum of 15 per cent only after an exposure of 60 kr. Recovery from this effect was rapid, occurring in approximately 30 sec after the cessation of exposure to X-rays. After short rest periods of about 5 min following irradiation, this decrease in amplitude could be produced with very small doses (about 300 r.). No attempt was made to measure latent periods in these preliminary tests. It was interesting that an 'on-off' response was also found with X-rays (Fig. 2, *B*).

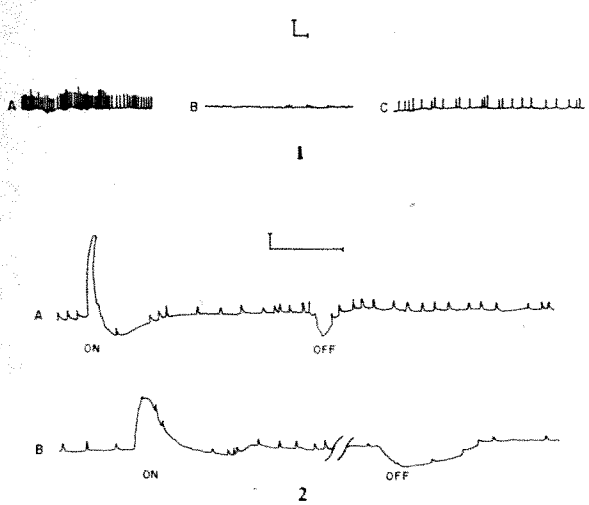


Fig. 1. Spontaneous electrical activity in the eye of *B. giganteus*. (*A*) Normal activity prior to X-rays. (*B*) after 120 kr; (*C*) recovery after a rest period of 45 min. Calibration values 275 μV and 1 sec

Fig. 2. 'On-off' response to (*A*) light and (*B*) X-rays. (Note that in tracing *B* a time lapse of 15 min occurred between the 'on' and 'off' responses.) Calibration values 200 μV and 1 sec

This work has demonstrated a marked effect of large doses of radiation in reducing the amplitude and frequency of the electrical activity recorded from the eye of *B. giganteus*. Also, an effect of X-rays was found in the 'on' response to light. Recovery from the effect occurred in both responses, but at very different rates. Further work will involve the study of recovery from radiation damage in these two systems.

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BIOLOGY

Species Identification of Animal Cell Strains by Immunofluorescence

Animal cells cultivated *in vitro* can be readily identified with respect to their species of origin by several immunologic procedures, including: hæmagglutination[1], and mixed cell agglutination[2], both involving detection of species antigens common to erythrocytes and cultured cells, and an immunofluorescence method[3], which provides direct visualization of species-specific antigen–antibody reactions on cell surfaces. By means of these procedures, a number of established cell lines have been shown to be of a species other than that originally claimed[4,5], a finding obviously due to heterologous cell contamination and overgrowth or other laboratory errors.

This communication describes the use of the immunofluorescence procedure to detect individual cells of different species within mixed cell populations (as occurs in contamination, especially in early phases), as well as its use in species identification of a number of established cell lines and sub-lines from various laboratories.

Antisera against verified prototype strains (human: Detroit-6, HeLa; monkey: *LLC-MK*$_2$; mouse: *L-M*, *S*-180; Chinese hamster) and/or primary kidney cultures of various animal species (human, monkey, mouse, rat, rabbit) were prepared by immunizing guinea pigs or rabbits with washed cells scraped from monolayers cultured in Eagle's medium supplemented with 5 or 10 per cent human or calf serum as previously described[3]. Antisera were heated at 56° C for 30 min and the globulins precipitated in 18 per cent sodium sulphate (1 vol. serum/2 vol. 27 per cent sodium sulphate) at 37° C for 16 h. Following dialysis, the resulting globulin solutions, adjusted to *p*H 9, were conjugated with fluorescein isothiocyanate (FITC) by adding them directly to a dry mixture of 1 part FITC (0·025 mg. FITC/mg protein) and 9 parts cellulose powder ('Celite') and shaken for 5 min[6]. The 'Celite' was removed by centrifugation and the unbound dye by 'Sephadex' gel filtration.

Cells to be tested were obtained by trypsinizing monolayer cultures to yield $3–4 \times 10^6$ cells per ml. After 3 washes in buffered saline (*p*H 7·5), 0·1 ml. of cell suspension was mixed with 0·1 ml. labelled antibody, agitated for 30 min, again washed 3 times, and a drop of the final sediment sealed under a coverslip and observed by fluorescence microscopy[3]. Specific staining reactions

head and the region containing the graft, were fixed in sublimated Bouin–Hollande[8]. The thyroid was studied in 5μ sections stained with a modified Masson technique[9], with periodic acid–Schiff (PAS), Cleveland–Wolfe (trichrome) and hæmalum-eosin. The pituitary grafts were recovered in 4μ or 5μ serial sections of the tail region and stained with Cleveland–Wolfe and various histochemical procedures[8]. The heads of the hypophysectomized fish were sectioned at 10μ to verify the absence of pituitary fragments.

As in most teleosts, the thyroid gland in *M. formosa* is not encapsulated, but consists of follicles scattered in the gular region associated with the inferior jugular veins and the ventral aorta, and contained in a mass of loose connective tissue into which the gland expands when strongly stimulated. In some fish, both intact and hypophysectomized, a few thyroid follicles have been found also in the region between the heart and the œsophagus. This region in grafted-hypophysectomized fish has not been examined.

Histological features of the thyroid in the three groups of fish are summarized in Table 1, and typical glands are illustrated in Fig. 1. It is clear that the transplanted pituitary maintained the thyroid in a more active state, by histological criteria, than the gland in the hypophysectomized controls. Furthermore, in six of the grafted-hypophysectomized fish, the thyroid was more strongly stimulated than in any of the intact controls, with marked hyperplasia in which epithelial cells and follicles filled nearly all the thyroid area and invaded the bases of the gill-arches.

Table 1. Summary of Histological Characteristics of the Thyroid Glands. Figures show the Number of Fish in which the Listed Characters were Predominant

	Intact	Hypophysectomized	Grafted-hypophysectomized
No. of fish	14	17	13
Thyroid epithelial cells			
Squamous	1	15	2
Cuboidal	5	2	6
Columnar	8	0	5
Nuclei rounded	12	0	9
Nucleoli prominent	10	0	9
Mean lowest cell height	2·7 ± 0·3μ	1·6 ± 0·2μ	2·9 ± 0·7μ
Mean greatest cell height	6·3 ± 0·6μ	2·8 ± 0·2μ	7·3 ± 1·0μ
No. of fish having cells 7μ or more high	2	0	6
Colloid			
Strongly PAS+	14	17	2
Granulated	10	0	9
Resorption vacuoles			
Many	9	0	8
Few	5	12	4
None	0	5	1
Hyperplasia of thyroid	4	0	8

In work now in progress we have identified the pituitary thyrotrophs in *Mollienesia* as PAS+ and AF+ cells in the proximal pars distalis (meso-adenohypophysis). In the eight grafts we have so far examined, there were large numbers of these cells, displaying signs of great secretory activity. All the grafts were healthy and well-vascularized, and besides the thyrotrophs contained many acidophils of the rostral pars distalis (pro-adenohypophysis), corticotrophs, inactive gonadotrophs, very few putative somatotrophs, and cells of the pars intermedia (meta-adenohypophysis). In two grafted-hypophysectomized fish which were kept for seven months, the grafts contained many active thyrotrophs and the thyroid glands were markedly stimulated and hyperplastic with extensive invasions of the gill-arches: evidence that function can continue in the transplanted pituitary for a considerable time.

These results indicate that TSH can be secreted at a greater rate by the transplanted pituitary than by the gland in its normal situation. The simplest explanation of this finding would be that the hypothalamus normally inhibits TSH-secretion, as suggested by other workers to account for enhanced TSH-output by heterotopic pituitary transplants in *Ambystoma*[4]. We have made no direct measurements of thyroid hormone (TH)-levels in the blood of these fish, but two lines of evidence suggest that the histological hyperactivity in the thyroid of grafted-hypophysectomized fish was accompanied by physiological activity and was not a false appearance analogous to the state of the thyroid after anti-thyroid drugs. First, the slight growth displayed by the grafted-hypophysectomized fish[11] suggests the presence of TH as well as small amounts of growth-hormone. Secondly, the state of the skin of these fish indicated at least normal blood TH-levels. In the hypophysectomized group, the skin was dull and poorly reflecting, due presumably to loss of guanine, but the grafted-hypophysectomized fish retained the silvery skin of the intact controls. In *M. latipinna* the dull skin of hypophysectomized fish is restored to the normal silvery state by treatment with thyroxine[10]. These observations indicate that the blood-levels of TH in the grafted hypophysectomized fish must have been at least as high as in the intact controls.

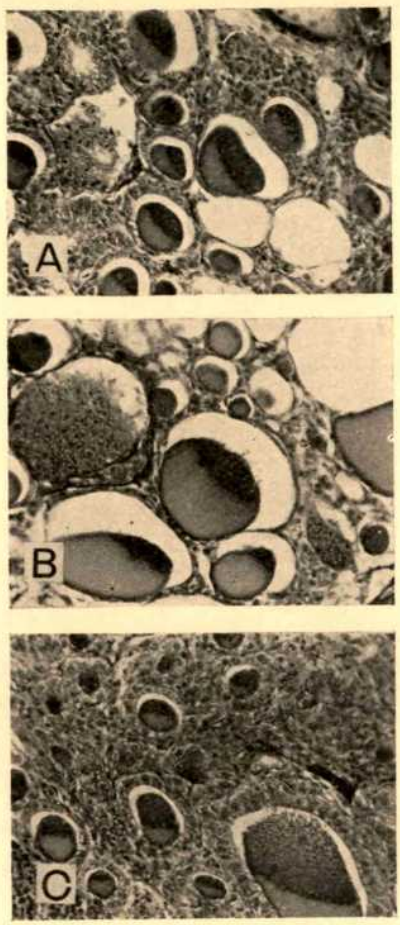


Fig. 1. Thyroid gland of *M. formosa* (× 230). *A*, Intact control; *B*, hypophysectomized control; *C*, hypophysectomized fish bearing pituitary transplant in caudal musculature

We have evidence, from the thyroidal response of intact *M. latipinna* to thiourea treatment[10], that negative feed-back operates between TH and TSH in these fish as in mammals. Thus we have to account for continuously elevated rates of TSH-production by the transplanted pituitary in the presence of normal (or even supra-normal) TH-levels in the blood. It may be that TH in the intact animal suppresses TSH-production by acting on the hypothalamus and reinforcing the hypothalamic inhibitory influence on TSH-secretion. Alternatively, TH may directly inhibit the activity of the thyrotrophs, the hypothalamus normally modifying this control by lowering the threshold for suppression of TSH-output by TH.

In conclusion, our results show decisively that the ability to secrete considerable amounts of TSH can be

retained by the teleost pituitary gland when removed from its normal connexion with the hypothalamus, and suggest that the teleost hypothalamo-hypophysial relationship with respect to TSH may differ considerably from that in mammals.

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Phototaxis and Green Rods in Urodeles

Many Amphibia are strongly phototactic, and it is generally found that this behaviour is more readily released by blue light than by light of any other colour[1]. It is reasonable to suppose that the green rod underlies such phototaxis, since this receptor has been shown to be strongly sensitive to blue light[2]. Although all anurans are said to have green rods, not all urodeles possess them, so that if this hypothesis is correct we should expect those urodeles which lack green rods also to fail to show any marked sensitivity to blue light when their phototactic behaviour is tested. In experiments recorded here two urodeles were tested, one of which has green rods (*Triturus cristatus*), and one of which lacks them (*Salamandra salamandra*)[3].

The experimental arrangement for testing phototaxis has previously been described in detail[4]. Animals were tested in a dark room in a *Y*-maze of black 'Perspex', in which they chose between two 4 in. × 4 in. translucent windows illuminated by means of Wratten filters and 500 W projectors. The average half band-width of the filters was 30 mμ, and the energy reaching the windows was approximately 23 μW/cm². Six filters were used, giving seven possible stimuli, for the windows could be illuminated by six different colours, or remain dark. These seven stimuli were presented on the windows in all 21 possible pairs, and the stimulus chosen by the animal noted in each case. In this way spectral response curves were obtained, which could be tested for statistical significance by means of Kendall's coefficient of agreement[5].

Twelve *Salamandra* and 14 *Triturus* were used. Eight of the *Salamandra* received two trials on each of the 21 possible pairs of stimuli; the other four only received one trial on each pair. All 14 *Triturus* received one trial each pair. Each individual received all its trials on one day,

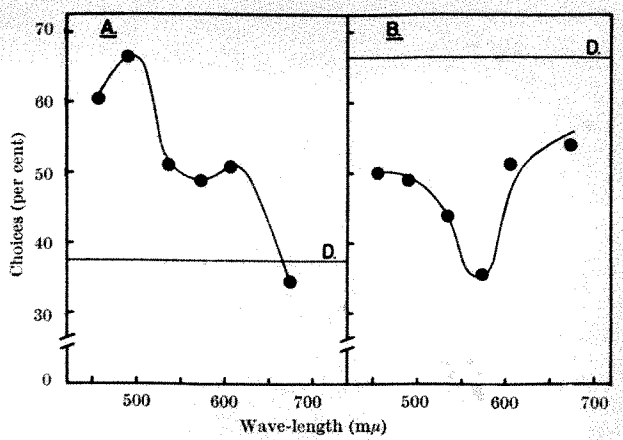


Fig. 1. *A*, Spectral response curve for *Triturus*; *B*, spectral response curve for *Salamandra*. The line labelled *D* shows the percentage of occasions on which darkness was chosen in each case

except for those animals which saw each pair of stimuli twice: these received one trial on all possible pairs on one day, and a second trial on all pairs a day later.

The spectral response curves thus obtained are shown in Fig. 1. The coefficient of agreement was in each case significant at considerably better than the 0·1 per cent level of confidence. It can be seen that the behaviour of the two species differed markedly. *Triturus* is positively phototactic, and responds to blue more often than to any other colour. The responsiveness to blue is less marked than it is in the frog[4], and the response curve also differs in having the maximum in the blue and the minimum in the green both shifted somewhat towards the longer wavelengths. The latter is presumably a consequence of the newt having visual pigments based on vitamin A_2 as opposed to vitamin A_1 like the frog. *Salamandra*, on the other hand, is negatively phototactic, choosing darkness more often than any other stimulus. The animal is maximally sensitive at about 570 mμ, and the spectral sensitivity curve is in line with the photopic sensitivity that would be expected for this animal[6].

The results therefore support the view that the green rod is involved in amphibian phototaxis. Previous results, however, show that other receptors must be involved as well[4].

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Movements and Co-ordination of the Ciliary Comb Plates of the Ctenophores *Beroe* and *Pleurobrachia*

Investigations by Afzelius[1,2] on the comb plates of *Mnemiopsis* showed that these plates are compound structures built up from several hundred thousand cilia of somewhat modified structure, the shafts of which are apparently cemented together in long rows. The comb plates of the smaller ctenophores used in this work contain fewer cilia, but even here many thousands of cilia must be incorporated in each plate.

Figures for parameters of ciliary beating published previously[3] refer to measurements made on a detached

Table 1. Some Parameters of Ciliary Activity in Ctenophores (Temperature, 19°–20° C)

	Pleurobrachia	*Beroe*
Length of comb plate cilia	1·1 mm	0·8 mm
Angular velocity of beat in effective stroke	6,100°/sec	5,750°/sec
Rate of propagation of the contraction wave	11·1 mm/sec	9·7 mm/sec
Time for one cycle of beat (approx.)	0·1 sec	0·085 sec
Metachronal wave velocity	72·9 mm/sec	40·6 mm/sec
Number of comb plates excited per sec	197	185
Separation of comb plates	0·37 mm	0·22 mm
Overall length of animal	6·3 mm	4·2 mm

Table 2. Parameters of the Metachronism of Four Animals

	Paramecium[3]	*Stentor*[3]	*Beroe*	*Pleurobrachia*
Metachronal wave velocity (μ/sec)	712	657	40,600	72,900
Cilia excited per sec	228	173	185	197
Interciliary distance (μ)	3·1	3·8	220	370
Time interval between excitation of adjacent cilia (msec)	4·4	5·8	5·4	5·1

comb row of *Pleurobrachia*, and are shown by the present investigation to be quite different from values measured on an intact animal. Data obtained from cinematograph films taken at 64 frames/sec are shown in Table 1; it is not appropriate to use a stroboscope or quote the frequency of beat since the cilia rest frequently between beats.

The rate of movement of these compound cilia in the effective stroke is not abnormally fast (the angular velocity is little more than half that in *Stentor*), probably because of the large viscous resistance offered to the extensive surface area of the comb plates. However, the propagation of the contraction wave up the cilium in the recovery part of the beat is much faster than in any other example described. In simple cilia the rate of propagation is usually below ½ mm/sec, and this rate rises as the number of cilia in each compound cilium is increased, so that it approaches 1 mm/sec in *Stentor* membranelles containing 60–75 component cilia, and rises further to around 10 mm/sec in these ctenophores where many thousands of cilia are present. It seems reasonable to suggest a correlation between the contractile force that can be exerted and the rate of propagation of the contraction.

The antiplectic metachronism of comb plates appears from preliminary experiments on the effects of viscosity to be of the neuroid type found in *Stentor*[4]. The rates of movement of the metachronal waves in these two types of ctenophore differ considerably from one another and are both much greater than the fastest recorded from other types of animal (0·6–0·7 mm/sec). However, the comb plates are very much farther apart than normal cilia, and the number of cilia excited per second is of the same order in both ctenophores as it is in *Stentor* membranelles and *Paramecium* vestibular cilia at similar temperatures (Table 2). The interval between the excitation of adjacent cilia in ciliary tracts from these four animals appears to be fairly constant at about 4–6 msec, although the distances between the cilia may differ by a factor of 100. This is in agreement with the suggestion[4] that the neuroid type of conduction of metachronal waves involves two steps, a rapid interciliary conduction and a slower build-up of excitation within the cilium.

It is clear from the cinematograph records that the form of beat of ctenophore comb plates resembles that of *Stentor* membranelles[5] in that the cycle of beat cannot be rigidly separated into two parts. The contraction wave responsible for the recovery phase of the beat has clearly started to move up the shaft of the compound cilium before the effective stroke has been completed.

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ENTOMOLOGY

Central Nervous Control of the Mechanical Properties of the Cuticle in *Rhodnius prolixus*

In the course of experiments carried out to examine the mechanism of neurohumoral regulation of water economy, tonus modifications in the cuticle of the abdominal wall conditioned by the nervous system were observed. Such modifications in the mechanical properties of the cuticle were reported in a previous communication[1] as "peripheral inhibition of central origin". Cottrell[2] describes similar changes in the blowfly, *Calliphora erythrocephala*, and interprets them on the basis of the sclerotization process. Certain evidence obtained in the fifth-stage larvæ of *Rhodnius prolixus* during feeding leads to the postulation of a nervous control of these properties of the cuticle.

Fifth-stage larvæ of *Rhodnius prolixus* during feeding may take an amount of blood 10 times higher than their fasting weight (35 mg). This enormous increase in volume is accompanied by a distension of the abdominal cuticle. The distended cuticle may even become transparent, thus allowing details of tracheæ and internal organs to be observed.

If the nerves corresponding to one side of the abdomen (for example, the left side) have been previously severed, during feeding the distension of the abdominal wall occurs asymmetrically, being larger on the intact side (Fig. 1). This asymmetry in distension is permanent and manifests itself whether feeding occurs immediately after neurotomy or one month later.

As abdominal distension progresses during intake of blood in these animals with partial lateral neurotomy, the asymmetry of abdominal distension disappears gradually, and on reaching maximum distension no differences are apparent with normal animals, except for slight changes in the shape of the abdomen. However, the cuticle of the operated animals shows, when observed in polarized light under the microscope, differences in the orientation of the birefringent micelles between the left and the right side. If total abdominal neurotomy is performed at the mesothoracic fused ganglionic mass, there is no asymmetry of distension at feeding, but the distended abdomen has a more nearly spherical shape than in normal animals. Furthermore, the cuticle so distended shows distinct modifications of its physical properties: (1) it has nacreous reflexions and the surface is smoother and more brilliant; (2) it loses elasticity and becomes friable (it tears upon slight external pressure).

The same result is obtained in animals which have suffered neurotomy of the connectives between the prothoracic ganglion and the mesothoracic fused ganglionic mass.

Preliminary observations show that the internal pressure in the abdomen immediately after feeding is not

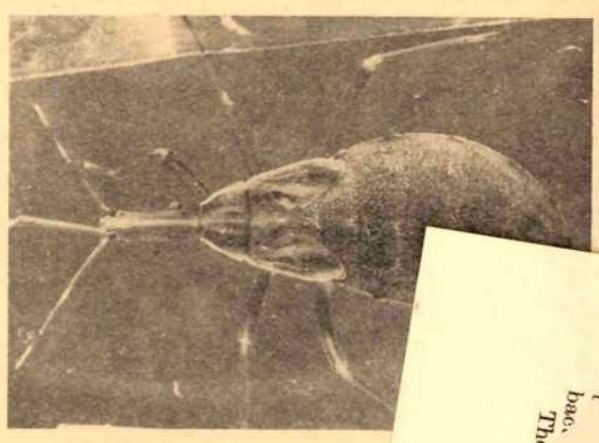


Fig. 1

appreciable and does not differ between normal and operated animals, but that there are differences in the elastic properties which depend on a certain 'cuticular tonus' (as measured by the variation in lymph flow through a cut leg).

Careful observation of an asymmetrical animal (Fig. 1) shows also that the asymmetry results from a different distension of the sclerite cuticle; it may be measured by relating the distance between cuticular setæ or the width of the sternite of the left and right sides.

In normal animals the abdominal cuticle offers a strong resistance to distension when Ringer solution is injected in the hæmolymphatic cavity. The cuticle and the shape of the abdomen of artificially distended animals are similar to those of operated animals. When artificial distension is performed on operated animals, the cuticle breaks between sclerites, even when the volume of injected solution never reaches 50 per cent of the volume normally incorporated through ingestion of blood.

From the foregoing results it may be inferred that in the fifth-stage larvæ of *Rhodnius prolixus* during ingestion there occurs a central nervous controlled modification of the 'cuticular tonus' in the sclerites of the abdominal wall and perhaps in the cuticle of the whole body.

This nervous controlled cuticular modification would consist in a change (decrease) during feeding in the cohesion among chitin micelles accomplished at the level of the structural proteins of the cuticle. Under polarized light modifications are observed in the orientation of chitin micelles which are not related to the process of sclerotization of the cuticular proteins.

On total abdominal disenervation at the level of the mesothoracic fused ganglionic mass or of the connectives between the prothoracic ganglion and the mesothoracic fused ganglionic mass, this cuticular 'antitonus' is eliminated, and when distension occurs profound modifications are produced in the structure and mechanical properties of such unenervated cuticle.

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MICROBIOLOGY

Resistance to Copper of some Oxidizing and Reducing Bacteria

Certain types of bacteria, particularly *Thiobacillus thiooxidans* and *Ferrobacillus ferrooxidans*, are known to assist the oxidation of pyritic ores[1–4]. It has also been shown[5,6] that the rate of natural leaching of uranium from low-grade ores can be significantly increased when the leaching is done in the presence of pyrite that is itself undergoing microbiological oxidation. It is possible that a similar enhanced leaching effect could be obtained for other metals from their low-grade ores. This is of particular interest in the case of copper, since it might be anticipated that cupric ions would have a marked toxic effect on the bacteria, and enhanced leaching would be prevented.

We have therefore examined the resistance to cupric ions of a number of pure strains of sulphur-oxidizing bacteria and of iron-oxidizing bacteria. At the same time, we have also studied the effect of copper on the growth of sulphate-reducing bacteria. At different times various hypotheses of sulphide-ore formation have been put forward by geologists, involving bacteria as the agents responsible for precipitating metals from natural waters. Usually these arguments have been refuted in the belief at copper-bearing solutions would be toxic to any such eria[7].

ollowing pure strains have been examined:

(*a*)	Sulphur-oxidizing bacteria:	
	Thiobacillus thiooxidans, strain Iceland	N.C.I.B. No. 8342
	Thiobacillus concretivorus, strain Parker	N.C.I.B. No. 8345
(*b*)	Iron-oxidizing bacteria:	
	Ferrobacillus ferrooxidans, strain Beck	N.C.I.B. No. 8451
(*c*)	Sulphate-reducing bacteria:	
	Desulfovibrio desulfuricans, strain Hildenborough	N.C.I.B. No. 8303
	Desulfovibrio desulfuricans (*aestuarii*), strain El Agheila C	N.C.I.B. No. 8308
	Desulfovibrio orientis, strain Singapore	N.C.I.B. No. 8382
	Clostridium nigrificans, strain Delft 74*T*	N.C.I.B. No. 8395

Stock cultures were obtained from lyophilized cells using the following media: (*a*) for sulphur-oxidizing bacteria, Waksman's *S*12 mineral salts and elementary sulphur medium[8] incubated aerobically at 30° C; (*b*) for iron-oxidizing bacteria, Beck's mineral salt medium[9] incubated aerobically at room temperature; (*c*) for sulphate-reducing bacteria, Baars' lactate-mineral salts medium[10] supplemented with 0·1 per cent yeast extract and 0·01 per cent thioglycollic acid incubated anaerobically at 30° C under hydrogen with 1 per cent carbon dioxide. (55° C in the case of *C. nigrificans*.)

Subcultures were made into fresh batches of the appropriate media supplemented with a small known concentration of copper ions added as copper sulphate. When growth of the subculture occurred, a second subculture was made into medium containing double the concentration of copper. This procedure was continued until a concentration of copper was reached sufficient to inhibit growth. Six successive subcultures were then made at the next lower copper concentration before a second attempt was made to obtain growth at the high concentration. If growth now occurred the process was continued. If inhibition was still obtained, the lower copper concentration was taken as the limiting concentration.

Final figures obtained for limiting copper concentration (Cu^{2+}) were as follows:

Thiobacillus thiooxidans (Iceland)	20,000 p.p.m.
Thiobacillus concretivorus (Parker)	10,000 p.p.m.
Ferrobacillus ferrooxidans (Beck)	10,000 p.p.m.
Desulfovibrio desulfuricans (Hildenborough)	50 p.p.m.
Desulfovibrio desulfuricans (*aestuarii*) (El Agheila C)	20 p.p.m.
Desulfovibrio orientis (Singapore)	30 p.p.m.
Clostridium nigrificans (Delft 74*T*)	30 p.p.m.

The results for the *Thiobacilli* and the *Ferrobacillus* show a remarkable resistance to copper and there seems to be no reason why they should not be used to advantage to assist in the leaching of copper ores.

The sulphate-reducing bacteria were very much more sensitive. It has been reported earlier that 100 p.p.m. copper sulphate (approx. 40 p.p.m. Cu^{2+}) is ineffective in inhibiting *D. desulfuricans* (Hildenborough) and *C. nigrificans* (Delft 74*T*) (ref. 11).

In those experiments, however, the medium used included 0·1 per cent cysteine hydrochloride (that is, was approximately 0·0064 M w.r.t. –SH compared to 0·0011 M w.r.t. –SH in the present experiments). It is to be expected that a larger fraction of the added copper might be rendered ineffective by complexing in the cysteine medium. It seems unlikely that the sulphate-reducing bacteria play a significant part in the deposition of copper sulphide ores.

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A Selective Medium for the Isolation of Soft Rot Coliforms from Soil

BACTERIAL species which cause soft rot of plant tissue secrete pectic enzymes which destroy the middle lamella of the cell wall and, *in vitro*, liquefy pectate gel. This property has been utilized by Wieringa[1] and Stewart[2] in the preparation of selective media for isolating soft rotting *Erwinia* spp., and by Paton[3] for the isolation of pectin-decomposing *Pseudomonas* spp. The preparation of these media involves a double-layer technique in which a calcium agar medium is overlaid with a sodium polypectate gel in which the formation of shallow pits serves as a diagnostic characteristic.

During investigations of *E. atroseptica* (the causal organism of potato black leg), the selective-diagnostic medium of Stewart[2], which was developed in these laboratories, has been used for the routine isolation of the organism from diseased plants and tubers and has proved to be extremely efficient for this purpose. On this medium which incorporates McConkey agar plus calcium chloride in the basal layer, *E. atroseptica* develops as red colonies in 'cup-shaped' depressions formed in the pectate gel of the upper layer. The bile salts present in McConkey agar inhibit the growth of certain contaminating bacteria.

In examining the survival of *E. atroseptica* in soil where black leg of potato had occurred it was found, by direct soil isolation methods, that as the diseased plant débris rotted there was a marked increase in numbers of saprophytic bacteria, some of which were pectin-decomposing strains of *Pseudomonas* as described by Paton[3]. On Wieringa's medium these latter types formed depressions indistinguishable from those formed by *E. atroseptica*. On Stewart's medium the fewer colonies of *E. atroseptica* were overgrown and quickly masked by the much more numerous colonies of saprophytic bacteria which included *Pseudomonas* spp. Replacement of lactose by salicin in the basal layer as suggested by Stewart[2] was ineffective.

In an attempt to find a more selective substrate the following formula for the basal layer of the medium utilizing sodium citrate as the sole carbon source was devised: Simmon's citrate agar (Oxoid '*C.M.*155'), 23·0 g; calcium chloride, 3·0 g; bile salts (Oxoid '*L.*55'), 5·0 g; crystal violet, 0·001 g; distilled water, 1 l. These ingredients were autoclaved at 15 lb. pressure for 15 min, cooled, adjusted to *p*H 6·8 and 15 ml. amounts poured into sterile Petri dishes. The surface of the medium was dried before the addition of 4 ml. amounts of the upper layer of pectate solution prepared as described by Paton[4] and adjusted to *p*H 7·2. After drying for 12 h at 37° C the pectate gel set firmly and was ready for inoculation.

After incubation for 48 h at 27° C, *E. atroseptica* develops as pale-blue colonies in 'cup-shaped' depressions, 1·0–1·5 mm in diameter, which contrast clearly with the deep-blue colour of the surrounding medium caused by the production of alkali. Other citrate-utilizing coliform types produce pale-blue colonies but cause no liquefaction of the pectate gel. *Pseudomonas* spp. develop slowly and appear as yellowish-green colonies; the pectin-decomposing types form 'pin-point' depressions, not more than 0·5 mm diameter, which are easily distinguished from the deeper and larger 'cup-shaped' depressions formed by *Erwinia*. The latter may be easily counted by holding the plate over a strong light. Although bile salts and crystal violet are necessary when isolating *E. atroseptica* from soils, they may be omitted in its isolation from soft rotted plant or tuber tissues.

Comparisons of this medium with Stewart's shows that it is more selective for *E. atroseptica*. Whether or not this is due to a preference for citrate rather than lactose as a carbon source or to the suppression of the other competitive bacteria is not yet known. The medium has also proved effective for the isolation of *E. carotovora* and *E. aroideae* from soil.

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MISCELLANEOUS

Periodic (Liesegang) Precipitation of Chemicals

LIESEGANG'S periodic precipitation of inorganic chemicals[1] has yet to be explained adequately, possibly because experiments in gathering data to describe it have failed to recognize and control numerous complicating effects influencing it. Some of these are illustrated in the work reported here.

Reagent grade chemicals, single- or triple-distilled water (glass still), Difco 'Bacto' agar and a purified product of this[2] were used for this work. (Ion agar No. 2 and 'BBL' agar for bacteriological media were also used, and gave similar results. A process for de-ionization is described by Crowle[2].) Periodic precipitation was induced in 1-mm layers of 2 per cent agar on 75 × 50 mm microscope slides. When internal reactant was used, it was dissolved in the agar before pouring the latter on to a slide. External reactant was allowed to diffuse from 5 mm disks of Eaton-Dikeman No. 652 filter paper. (By comparison with depot wells punched in agar, this filter paper had no detectable effect of its own on our results, other than that due to absorption of fluid. It was more convenient to use than such wells.) Reactions were allowed to develop in humidified Petri dishes at 2° C; this temperature was permitted to vary ± 2° or ± 0·1° C, depending on the purpose of the experiment. Results reported below are representative of numerous experiments, which among themselves were highly reproducible.

Fig. 1 is a photograph of the numerous Liesegang bands developed overnight at the more constant temperature between 1 per cent silver nitrate and anion contaminants in 'Bacto' agar. This test repeated in de-ionized 'Bacto' agar[2] showed no precipitate, unless appropriate anions purposely were re-added to it. The tendency of the unpurified agar to form Liesegang bands diminished upon prolonged boiling during the process of dissolving it.

Several Liesegang bands will form in de-ionized agar in triple-distilled water between 0·1 per cent silver nitrate external reactant and concentrations of sodium chloride internal reactant 100-fold less than those required to initiate turbidity, detectable by Klett colorimeter, due to silver chloride in liquid, as opposed to gelled, aqueous solution. So sensitive is the agar gel for this effect that brief and light contact with bare fingers, such as occurs when a slide is handled by the edges for a few seconds needed to move it from one place to another, contaminates it with enough chloride ions to form such bands with silver nitrate in the de-ionized system. A similar effect was obtained by making up the de-ionized agar in single- rather than triple-distilled water.

Liesegang bands develop only at certain reactant ratios. This is illustrated in Fig. 1, which shows that until external reactant has diffused a certain distance characteristic for a particular precipitating system the solid disk of precipitate does not begin to break up into concentric rings. But as Fig. 2 shows, such a disk can be broken into similar rings by temperature fluctuations such as occur in the cycling of an ordinary refrigerator (for example, ± 0·5° C in 10 min). Fortunately, by the time the critical reactant ratio favouring true Liesegang precipitation has been reached (for example, immediately outside the solid disk of precipita-

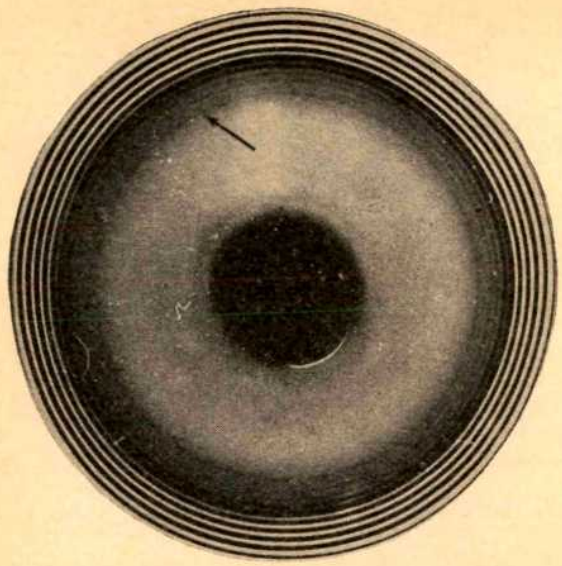


Fig. 1. Heavy precipitation formed by 1 per cent silver nitrate external reactant diffusing from the central dark area and reacting with contaminant anions on unpurified Difco 'Bacto' agar. When silver ions have become sufficiently diluted by diffusion to reach a critical ratio with contaminating anions, silver salts begin to precipitate in the form of numerous Liesegang rings instead of a solid disk (arrow). A control slide set up using de-ionized 'Bacto' agar developed no precipitate of any kind

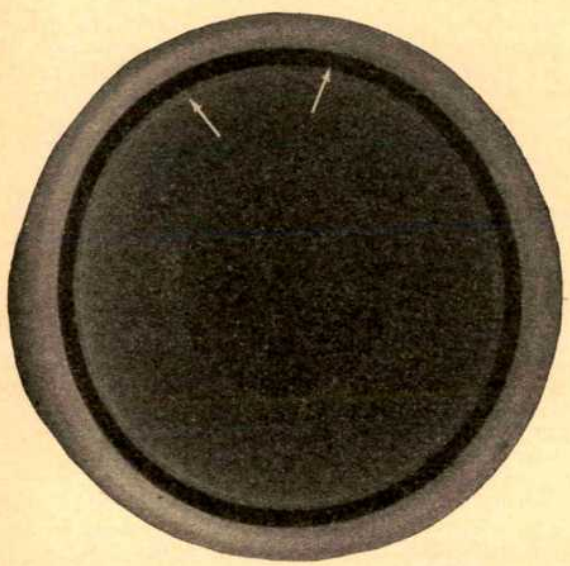


Fig. 2. Effect of temperature variations on the formation of an otherwise uniform disk of precipitate by 2 per cent silver nitrate diffusing through unpurified 'Bacto' agar. The stria pointed out by an arrow was caused by a modest but normal rise in temperature of the refrigerator in which this plate was being held. The gap indicated by a second arrow developed shortly after the plate was held for 1 min at 37° C and then returned to the refrigerator. Control slides set up in the same manner but maintained at a temperature not varying more than ± 0·25° C developed only a uniform disk of precipitate without any striations or gaps

tion in Fig. 1), the sensitivity of these plate tests to temperature fluctuation-induced artefacts has dwindled to the point where fluctuation must be large to produce an artefact (for example, 5° C in 10 min).

The effects observed above are not unique to the silver chloride system. They occur also with other salts as well as with antigen and antibody[3].

These results and other information[3–5] provide some ideas which help explain certain factors affecting and peculiar to Liesegang precipitation. For example, if sodium chloride and silver nitrate are mixed together as very dilute liquid solutions in various proportions, they yield more precipitate at some proportions than at others, even though smaller initial quantities of each may have been used. Furthermore, the lowest concentrations at which sodium chloride and silver nitrate will precipitate in fluid aqueous medium are achieved at unequal molar proportions (for example, 3 : 1), suggesting that the formation of precipitate is a complex phenomenon involving various uncomprehended ion interactions, which occur at some reactant proportions but are suppressed at others.

The piecemeal diffusion and interaction of ions within gel-stabilized aqueous media permits an almost infinite variation in reactant proportions, so that many of the effects of this variation which are missed by tube dilution techniques nevertheless are apparent in gels. For example, precipitation should be expected to occur at much lower reactant concentrations in gel than can be demonstrated practically in liquid, because optimal conditions for precipitation will be achieved far more readily in some plane of a gel-diffusion plate than in any series of concentration variations in liquid media. This effect may also be promoted by the restrictive property of gels upon diffusion of growing molecular aggregates[4].

Another characteristic of gelled medium probably of primary importance in Liesegang precipitation is that because all precipitation in tests using such medium must await arrival by diffusion of reactants at a mutual reaction area, zones of internal reactant depletion can occur immediately ahead of an advancing front of precipitation[3–5]. In time this depletion causes the influx of internal reactant into the reaction plane to drop until it becomes insufficient to precipitate external reactant still persistently diffusing into this area. Consequently, the latter begins to diffuse through this zone of precipitation and the narrow internal reactant-depleted zone immediately to the other side of it without being precipitated, until after passing through the depleted zone it once again begins to encounter sufficient internal reactant to precipitate. This explanation seems to account for the periodicity of Liesegang lines, for their propensity to form progressively and predictably farther apart[6], and for their ability to form only within narrow reactant ratios.

Temperature artefacts differ in several respects from Liesegang banding[3,4]. They may form in the following manner. In a test being held at a given temperature, both reactants are diffusing into a common meeting area where they mix and react in a spectrum of ratios to form a variety of complexes. This dynamic but regular situation is deranged by any temperature shift causing reactant ratio changes. If these are sudden enough for the existing situation, then a temperature artefact will appear. Lowering the temperature causes a gap to appear in the expanding disk of precipitate, while raising the temperature will have the opposite effect and create a stria; both effects are pointed out in Fig. 2. This sensitivity to temperature artefact formation decreases in proportion as the overbalance of external reactant drops, because there is also a proportional drop in the rate at which external reactant feeds into the reaction zone and, consequently, in the events of precipitation.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

DEMONSTRATOR IN THE DEPARTMENT OF GEOLOGY—The Registrar, University College of Wales, Aberystwyth (August 14).

TUTORIAL STUDENT (graduate, or about to graduate) IN GEOGRAPHY—The Registrar, King's College (University of London), Strand, London, W.C.2 (August 16).

ASSISTANT EXPERIMENTAL OFFICER (with a degree in science, horticulture or agriculture, or equivalent), to assist in biochemistry studies on the role of potassium in plant nutrition—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (August 17).

BIOCHEMIST, Basic grade (with a degree in biochemistry or Grad. R.I.C.) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY, for routine and developmental work and special investigations—The House Governor, King's College Hospital, Denmark Hill, London, S.E.5 (August 17).

RESEARCH ASSISTANT (biochemist or physiologist), to assist in metabolic research—The Director, Medical Unit, King's College Hospital Medical School, Denmark Hill, London, S.E.5 (August 17).

ASSISTANT EXPERIMENTAL or EXPERIMENTAL OFFICER (with at least G.C.E. in five subjects, including two scientific subjects at "A" level, or equivalent, or, at age 22, a pass degree, H.N.C., or equivalent, and preferably a knowledge of biochemistry and experience in chromatographic techniques)—The Secretary, Institute of Animal Physiology, A.R.C., Babraham, Cambridge (August 19).

CHIEF ENTOMOLOGIST (with a first- or second-class honours degree, considerable experience in research in applied entomology, and preferably experience in the tropics) at the Gezira Agricultural Research Station, Wad Medani, Republic of the Sudan, to control and conduct research on entomological and associated problems of practical importance—The Sudan Embassy, Appointments Section, 3 Cleveland Row, London, S.W.1, quoting Ref. 4/407 (August 20).

HYDROLOGIST, Principal Scientific Officer (with a first- or second-class honours degree in civil engineering, physics or mathematics, and considerable experience in hydrological or hydrometeorological research) at the Hydraulics Research Station, D.S.I.R., Wallingford, Berks, to take charge of a new and growing Hydrological Research Unit undertaking extensive research on the effects of land management on water balance and other hydrological topics—Civil Service Commission, 23 Savile Row, London, W.1, quoting Ref. S/5640/63 (August 23).

SCIENTIFIC OFFICER (with a university degree in science with chemistry as a major subject) IN THE DIAGNOSTIC SECTION, Agriculture Department, Taieri, New Zealand, for the analysis of animal tissues for trace elements and poisons, and to provide a service in general diagnostic toxicology—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B.13/2/19/2137 (August 23).

ASSISTANT LECTURER or LECTURER IN PSYCHOLOGY—The Secretary, University of Exeter, The Queen's Drive, Exeter (August 26).

LECTURER (graduate in medicine, science or veterinary science, preferably with a Master's degree and experience in research) IN PHYSIOLOGY at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, August 30).

LECTURER (with good academic qualifications in geography, successful teaching experience, and preferably qualifications in some other aspect of education) IN EDUCATION at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, August 30).

RESEARCH FELLOW (with a Ph.D. or equivalent experience in high energy physics or a related field) IN HIGH ENERGY PHYSICS in the Research School of Physical Sciences, Institute of Advanced Studies, Australian National University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 30).

ASSISTANT (experienced in standard biochemical procedures, and preferably some knowledge of chemical pathology) IN THE BIOCHEMISTRY DEPARTMENT—The Secretary, Houghton Poultry Research Station, Houghton, Huntingdon (August 31).

ASSISTANT LECTURER IN PHILOSOPHY with special reference to political philosophy—The Registrar, The University, Manchester 13 (August 31).

ASSISTANT LECTURERS (2) (with a medical or scientific qualification) IN BACTERIOLOGY—The Registrar, The University, Manchester 13 (August 31).

CHAIR OF CIVIL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

LECTURER (with a good honours degree in metallurgy or chemistry or equivalent qualifications, and experience or special interest in one of the following fields: extraction metallurgy, alloy thermodynamics, electrochemistry, high temperature chemistry or structural inorganic chemistry) IN CHEMICAL METALLURGY IN THE DEPARTMENT OF METALLURGY of the Faculty of Science—The Registrar, The University, Manchester 13 (August 31).

PLANT PHYSIOLOGIST OR BIOCHEMIST (with a good honours degree and preferably some research experience), to study growth regulators in the potato plant, especially those affecting tuber dormancy—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (August 31).

SENIOR LECTURER and a LECTURER IN BOTANY; a LECTURER IN GEOGRAPHY; and a LECTURER IN GEOLOGY at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

ZOOLOGIST (Scientific Officer grade) IN THE DEPARTMENT OF INSECTICIDES AND FUNGICIDES, to study the mode of action of insecticides using histochemical and radioactive-tracer techniques—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (August 31).

ASSISTANT LECTURER IN VETERINARY ANATOMY IN THE DEPARTMENT OF PRE-CLINICAL SCIENCES, University of Melbourne, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 1).

RESEARCH ASSISTANTS (2) (honours graduates in chemistry or biochemistry, with research experience, preferably to Ph.D. level) IN CHEMISTRY, for work on peptide and protein chemistry under the supervision of Prof. H. N. Rydon—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devon (September 1).

SENIOR BIOCHEMIST (graduate in chemistry or biochemistry and at least five years postgraduate experience in a hospital laboratory) IN THE DIVISION OF BIOCHEMISTRY, The Royal Women's Hospital, Melbourne, to assist the Head of the Division in the maintenance and development of the clinical chemistry service of the Hospital, to carry out special investigations and to undertake research in some aspect of clinical science—Head of the Division of Biochemistry, D. Watson, Esq., The Royal Women's Hospital, Melbourne, c/o Australia House, Strand, London, W.C.2 (September 1).

RESEARCH FELLOW (graduate in physics, electrical engineering or metallurgy, with experience in the physics of alloys) IN PROPERTIES OF NON-FERROUS ALLOYS IN THE DEPARTMENT OF PRODUCTION TECHNOLOGY AND CONTROL ENGINEERING, to undertake research into, and development of, alloys having optimum electrical characteristics for the manufacture of stable resistors—The Secretary, Northampton College of Advanced Technology, London, E.C.1 (September 7).

TEMPORARY CONTRACT ASSISTANT (with experience of transistorized circuitry) IN THE DEPARTMENT OF PHYSICS, to construct and test electronic equipment for satellite instrumentation—The Secretary and Registrar, The University, Southampton (September 7).

LECTURER IN INORGANIC AND PHYSICAL CHEMISTRY; and a LECTURER IN ORGANIC CHEMISTRY at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 10).

LIBRARIAN (with a university degree, experience in an academic library, and a professional qualification)—Secretary to the Trustees, Magee University College, Londonderry, Northern Ireland (September 11).

LECTURER (honours graduate in architecture, engineering or science, with an interest in building services, and preferably with qualifications in both architecture and science, with research experience) IN ARCHITECTURAL SCIENCE at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 13).

RESEARCH FELLOW (with a degree in science, medicine, or veterinary science, postgraduate experience in research, and a working knowledge of the parasitic arthropods) IN PARASITOLOGY, to be concerned with the establishment of a nucleus of postgraduate education relating to the parasitic insects and arachnids, and the initiation of a programme of research on the feeding mechanisms of parasitic arthropods, in relation to the development of allergic and immunological response—The Registrar, University of Queensland, Brisbane, Australia (September 13).

CHAIR OF ADULT EDUCATION—The Registrar, The University, Manchester 13 (September 20).

LECTURER (medical or non-medical) IN CHEMICAL PATHOLOGY—The Dean, Postgraduate Medical School of London, Ducane Road, London, W.12 (September 20).

CHAIR OF MECHANICAL ENGINEERING—The Registrar, The University, Sheffield (September 30).

CHAIR OF MATHEMATICAL STATISTICS—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (October 15).

SENIOR LECTURER and a LECTURER IN PHYSICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 15).

ABSTRACTOR (with a working knowledge of a Slavonic language, and preferably a second foreign language, a scientific background, preferably biological)—The Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey.

ANIMAL TECHNICIAN (experienced in animal work), to take charge of a well-equipped animal unit concerned with research on the nutritive value of cereals—The Director, Cereals Research Station, Old London Road, St. Albans, Herts.

ASSISTANT LECTURER IN THE DEPARTMENT OF CHEMICAL TECHNOLOGY, to lecture mainly in the field of fuel science and technology—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1.

ASSISTANT LECTURER IN THE DEPARTMENT OF PATHOLOGY at Camden TOWN, with a special reference to morbid anatomy and histopathology—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1.

BIOMETRICIAN (with an interest in human biology and preferably experience in biometrical work and programming) in a department investigating growth and development of children and animals—The Secretary, Institute of Child Health (University of London), Great Ormond Street, London, W.C.1.

BOTANIST, ZOOLOGIST or GENETICIST (graduate, with an interest in cytogenetics) IN THE BIOPHYSICS SECTION, for work concerned with investigation of the effects of ionizing radiations on living cells, particularly the effects on the nucleus and cell death—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks, quoting Ref. GJN/5.

CHEMIST or BIOCHEMIST (graduate, preferably with some research experience) at the Chester Beatty Research Institute, Fulham Road, London, S.W.3, to join a group studying basic mechanisms of damage of mammalian cells by ionizing radiation—The Secretary, Institute of Cancer Research, Royal Cancer Hospital, 34 Sumner Place, London, S.W.7, quoting Ref. 300/G/19.

CHEMIST or BIOCHEMIST (with an honours degree in chemistry or biochemistry, or Grad. R.I.C.) IN THE BIOCHEMISTRY DEPARTMENT, to assist in studies in the clinical chemistry of respiratory disease—Mr. T. P. Whitehead, Queen Elizabeth Hospital, Edgbaston, Birmingham 15.

EDITORIAL ASSISTANT (with scientific qualifications, and preferably a knowledge of French and/or German) FOR METALLURGICAL ABSTRACTS—The Editor, Institute of Metals, 17 Belgrave Square, London, S.W.1.

FIELD ASSISTANT (female, with a university degree in botany, preferably with subsidiary geography)—The Warden, Flatford Mill Field Centre, East Bergholt, near Colchester.

JUNIOR RESEARCH ASSOCIATE (graduate in biological sciences, with interests in histology, electron microscopy and tissue culture) IN THE DEPARTMENT OF PATHOLOGY, for experimental work on pulmonary disorders—The Professor of Pathology, The Medical School, The University, Newcastle upon Tyne.

JUNIOR TECHNICIAN (male, preferably with some experience in basic biochemical methods and a mechanical aptitude), to assist with varied research programmes in the new well-equipped biochemistry section of the Pædiatric Research Unit—The Secretary, Pædiatric Research Unit, Guy's Hospital Medical School, London Bridge, London, S.E.1.

MATHEMATICIAN-PROGRAMMER (with an honours degree in mathematics from a recognized university, and preferably with a higher degree and possessing experience in programming and numerical analysis) IN THE NATIONAL RESEARCH COUNCIL (Canada) COMPUTATION CENTRE, for duties involving the analysis and programming of problems originating in the various scientific and engineering divisions of the Council—The Employment Officer, National Research Council, 100 Sussex Drive, Ottawa 2, Canada, quoting File ER-282.

MEDICAL RESEARCH OFFICER (male/female, with a registrable medical degree and two years postgraduate research experience, preferably in im-

munology, protozoology or tissue culture techniques) AT THE WEST AFRICAN INSTITUTE FOR TRYPANOSOMIASIS RESEARCH, Kaduna, Northern Nigeria, for research on human trypanosomiasis—The Crown Agents, 4 Millbank, London, S.W.1, quoting Ref. M.3/54323/NF.

PHYSICS TECHNICIAN, Senior grade (male or female, with at least three years experience as a technician, passed the Ordinary National Certificate in applied physics, or its equivalent, and previous experience in handling radioactive substances) IN THE PHYSICS DEPARTMENT, for duties which include work in the Radioactive Isotope Unit—The Secretary, General Hospital, Southend-on-Sea, Essex.

POST-DOCTORAL ASSISTANT, to work with Prof. J. S. Anderson, F.R.S., on the study of chemical reactions on metal surfaces by means of the Field Ion Microscope—The Secretary, Inorganic Chemistry Laboratory, The University, South Parks Road, Oxford.

RESEARCH ASSISTANT (organic chemist or biochemist), to work on problems in chemical carcinogenesis—Dr. D. B. Clayson, Department of Experimental Pathology and Cancer Research, School of Medicine, Leeds 2.

RESEARCH ASSISTANT (with a degree in a scientific subject and some knowledge of statistics) IN THE DEPARTMENT OF MEDICAL STATISTICS, for work which will include the analysis of clinical and laboratory data in connexion with research projects carried out over a wide field in the Hospital and Medical College—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. AC/195.

RESEARCH SCHOLAR or ASSISTANT (graduate in biochemistry or chemistry) IN THE DEPARTMENT OF EXPERIMENTAL NEUROPHARMACOLOGY, to work on aspects of the biosynthesis of phospholipids in the nervous system with Dr. G. B. Ansell—The Head of the Department of Experimental Neuropharmacology, The Medical School, The University, Birmingham 15.

RESEARCH STUDENT (graduate holding a good honours degree in pharmacology or other biological subjects) IN PHARMACOLOGY, to work on problems related to the physiological function of histamine—Dr. P. B. Marshall, Department of Pharmacology and Therapeutics, Queen's College (University of St. Andrews), Dundee.

SENIOR LECTURER/LECTURER IN MECHANICAL ENGINEERING; and a LECTURER IN ENGINEERING at the University of New South Wales, Broken Hill Division—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia.

SENIOR TECHNICIAN, to be in charge of two newly equipped Chemistry Teaching Laboratories—The Administrator, Department of Zoology, The University, Oxford.

TECHNICAL ASSISTANT (with a degree in biological sciences, Higher National Certificate or equivalent qualification, and previous training in microbiology) IN THE DEPARTMENTS OF BOTANY AND ORGANIC CHEMISTRY, to join a group investigating microbiological aspects of fungal biosynthesis—The Registrar, The University, Liverpool 3, quoting Ref. CV/374/N.

TECHNICIAN (male) IN THE DEPARTMENT OF PATHOLOGY, for routine research and work with an electron microscope—Prof. R. C. Curran, Department of Pathology (West Wing), St. Thomas's Hospital Medical School, London, S.E.1.

VETERINARY RESEARCH OFFICER (male/female, member of the Royal College of Veterinary Surgeons (or its equivalent), and at least two years experience in research) AT THE WEST AFRICAN INSTITUTE FOR TRYPANOSOMIASIS RESEARCH, Northern Nigeria, for research work, etc., in the Veterinary Section—The Crown Agents, 4 Millbank, London, S.W.1, quoting Ref. M.3/54322/NF.

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TECHNOLOGY IN HIGHER EDUCATION

IN his Handley Page Memorial Lecture at Cranfield on May 21, the Duke of Edinburgh combined an apt tribute to Sir Frederick Handley Page with a discussion of education for technology in which the needs of both the technician and craftsman and of the technologist were equally recognized. The Duke quoted repeatedly from Handley Page's own writings on technical education to illustrate some of the defects of our present system, and particularly the irrelevant division which is customarily made between general and vocational education. This, he suggested, was largely responsible for weaknesses in our existing system and for its failure to provide men and women of the qualities required in sufficient numbers.

Handley Page himself, as the Duke showed, was fully convinced that it was impossible to divide education into vocational and non-vocational. Competence in a particular craft or profession must be accompanied by a cultural, intellectual and spiritual life if men and women were to develop full personalities. After some shrewd comments on some particular weaknesses in our present system and a trenchant repudiation of the social differentiation between certain jobs and professions, the Duke strongly endorsed Handley Page's plea in 1955 that our technological training should be directed towards producing the ability to remain contemporary in thought and to develop the habit of thinking in terms of technical matters of to-day and to-morrow and not in terms of what was learnt five, ten or even twenty years earlier.

"Unless we can develop in the technologist that ability to remain contemporary in thought and stimulate in him an intellectual curiosity he will have little chance of holding his own in an era in which the progress of technology makes heavier demands on his intellectual capacity and adaptability than at any time in our long history". With this quotation from Handley Page's address on *The Art of Training*, the Duke closed his own address, remarking that Handley Page possessed this ability to remain contemporary in thought to an unusual degree. This emphatic repudiation of the false dichotomy between two cultures of the humanities and the sciences, and between vocational and non-vocational education is reflected in an inaugural lecture by Prof. H. Irving which has been published by the Leeds University Press under the title *The Three Cultures**. Like the Duke of Edinburgh, Prof. Irving pays a tribute to the Colleges of Advanced Technology, and the title of his lecture, in fact, derives from his belief that in these colleges, in which general studies are introduced to familiarize the student with problems of value in contemporary society, we may have the makings of a distinctive third culture—of a group of people who, unlike their contemporaries in the universities, will have been closely in contact during their professional training with the industries into which the majority will ultimately be assimilated.

Prof. Irving does not accept uncritically Sir Charles Snow's thesis of the two cultures, but he welcomes its insight and, like Sir Charles, is doubtful whether we are choosing the right people to be our leaders. Further, he believes that acceptance of the concept of experiment, and the full acceptance of the implications of an experiment, has produced a most significant change in intellectual outlook in the world. Moreover, he suggests that if trends in the recruitment of students were to follow entirely different patterns, for example, from grammar school to university, and from modern secondary school to college of technology, differences between the scientific and the technological cultures could well be exacerbated. Intercommunication on purely technical subjects might present no serious difficulties, but this might not apply to contacts on more humanistic planes.

For this reason, Prof. Irving suggests that the diversification of interests and ideals which the colleges of technology could offer should be welcomed and encouraged, and he points out that the colleges of advanced technology in particular may often be able to offer conditions in laboratories and equipment far superior to what many university departments can offer. Whatever views may be held as to the soundness of Prof. Irving's diagnosis of a third culture, it is undeniable that the colleges of technology offer opportunities which are fully comparable with those of many university institutions. It would be a national disaster if the social distinctions which the Duke of Edinburgh condemned so firmly were allowed to hinder the colleges from making their full contribution to the expansion of higher education, and beyond this it is manifest they have a contribution to make in their own right. It is not simply a question of whether they can fill the gap between the available university places and the huge excess of applicants: they should be receiving many of those applications in the first instance.

Prof. Irving's stout championship of the claims of technology would be welcomed merely for its contribution to the public understanding of what these colleges have already done and are able to do, and, like some recent addresses of the Duke of Edinburgh, it should help to dispel any idea of intellectual inferiority to a university, at least so far as the colleges of advanced technology are concerned. It is indeed of vital importance that the idea that a post in a university carries a social prestige greater than that of a similar post at a college of technology should be destroyed as soon as possible, and Prof. Irving frankly welcomes a social climate in which there ceases to be any distinction between the man who has received his higher education in the university and in the college of technology. Nor is he concerned at the outcome of competition between the scientific and technological cultures he postulates for students as well as staff. Indeed, he deliberately postulates the question whether the survival of a purely scientific culture such as the universities provide is desirable.

A negative answer to that question is implicit in some of the criticism of the products of the present system such as Mr. R. F. Tuckett voiced in a challenging broadcast, "Down with Ph.D.s", printed in *The Listener* of March 28. Mr. Tuckett not only indicted the science graduate as almost illiterate and disclaimed any confidence in a British Ph.D. course as an educative influence; he questioned whether the two- or three-year course of research for a higher degree as organized by the Department of Scientific and Industrial Research or some similar body, for example, favoured truly original thinking, though he admitted that the quality of research training in Britain and

* *The Three Cultures*. By Prof. H. Irving. Pp. i+26. (Leeds: The University Press, 1963.) 2*s*. 6*d*.

elsewhere is extremely variable. He pleaded for more attention to the broader aspects of research training and education, for an attempt to re-think and streamline the content of a first degree and for recognition that the specialized honours course might be ill-adapted to the needs of many of the increasing numbers of students who would be coming forward in the next ten years.

Mr. Tuckett recognized that the opportunity to re-think the whole purpose of a university provided by the present expansion was, in fact, being grasped and that the idea of a new model of the old concept of a broadly educated man was already being discussed, though he had nothing to add about the contribution of even the colleges of advanced technology. Prof. Irving suggests that the success of these colleges, in providing a technological education appropriate to the needs of contemporary society and in effectively combining professional training with a measure of general studies, is largely due to the fact that they are in the enviable position of being able to work from foundations of their own creation and are not handicapped by any pre-existing organization or curricula. To some extent the new universities have the same freedom of manœuvre, but Prof. Irving is much concerned about the extracurricular education of the student who goes straight into lodgings and remains in lodgings for the whole of his academic career, especially if those lodgings are at some distance from the centre of the university.

This is a problem which could arise in the new universities as well as in the older Redbrick universities if measures are not taken to ensure that every student has the opportunities of assimilating what we believe to be the ethos of a university. Prof. Irving does not suggest that halls of residence are the ideal and the only solution to problems of student accommodation and their extra-curricular education. He does suggest that universities should be more concerned with the training of character and that residential experience has a great part to play here because character can be conspicuously developed by the exercise of leadership in every branch of communal activities, nor does he believe that such development of character is incompatible with academic specialization.

What is noteworthy is not so much that the views on the importance of character training expressed last year by Lord Fleck in his presidential address to the Society of Chemical Industry should find some strong support on academic quarters as that narrow specialization should be attacked in such a forthright manner. Here is further evidence of fresh thinking about teaching methods and of a readiness to reassess curricula and examination procedures such as Prof. T. L. Cottrell, for example, has already given us, which shows that the United States is not alone in working out new methods of teaching science. Prof. Irving is explicitly prepared to see that while specialist chemists are trained to the increasingly high standards demanded to-day, sufficient leisure time is left for the equally important aspects of their extra-curricular education.

It might have been hoped that the Federation of British Industries, in its recent report on management education and the training needs of industry†, would have shown something of the interest if not concern displayed by Lord Fleck towards the broader aspects of a university education. However, although the working party in this report distinguishes between three levels of management for which training is desirable it has little or nothing to say about the basic education which a manager of any type should receive. That the working party advocates a clear distinction between management and supervision and the restriction of the term management to courses which are not concerned largely with supervisory aspects makes it the more unfortunate that so little is said about the educational foundations. A new approach is indeed suggested, but while the working party suggests that the success of many graduates in industry or commerce is due rather to their innate ability than to the suitability of their education, it is content to observe that it would be encouraging to think that those taking a first degree who will not be staying in the academic world could study a modern version of the humanities in the future, along the lines being developed in the new universities.

Nor does the pamphlet *The Case for Advance*‡ put out by the 1963 Campaign for Education quite measure up to the opportunity. Sir Geoffrey Crowther, it is true, challenges the hierarchical structure of our education, as does Mr. Allan Bullock, who refers specifically to the intellectual snobbery which still prevents sufficient attention being paid to the development of applied studies, not only in technology and management but also the whole range of the application of economics and social studies to our problems. Mr. Bullock himself recognizes that the expansion of higher education should include the colleges of technology, but although he comments on the existence of class barriers in education, neither he nor the other contributors to the pamphlet appears to sense the opportunity which such a campaign for education affords of removing the misunderstanding and prejudices or even sheer ignorance which leave places unfilled in the colleges of technology and the technical colleges while universities may be turning half their applicants for places away.

Clearly the nation cannot afford such waste even at the present level of expenditure let alone the much higher level for which Mr. Bullock, Sir Geoffrey and their colleagues, Lady Albemarle, the Archbishop of Canterbury and Sir Ronald Gould argue so cogently. It is reasonable enough, as Mr. Bullock admits, to await the Robbins Report before adopting many new measures affecting the pattern of the development of higher education, but there are many ways in which the effectiveness of the present system could be improved meanwhile, and to secure the full use of the colleges of technology and the technical colleges is clearly one. To bring the colleges of advanced technology at the least to the clearing-house for university admissions is an obvious step, and although it might be at least a year before it becomes effective, the psychological effect in breaking down prejudice could be immediate and considerable.

Apart from such practical steps, the more existing practices are re-examined and subject to critical constructive thinking the better the ground will be prepared for action on the recommendations of the Robbins Committee. The value of Prof. Irving's address lies largely in its contribution to that, and this is also the merit of a contribution from Prof. B. R. Williams in the current issue of the *Manchester School of Economic and Social Studies*, in which the problems of university capacity and expansion are subjected to economic analysis. As Sir Geoffrey Crowther points out, an immense increase in higher education is inevitable, and this will involve a very large increase in national expenditure.

† *Management Education and Training Needs of Industry: a Report by an F.B.I. Working Party.* Pp. v+27. (London: Federation of British Industries, 1963.) 6*s*.

‡ *The Case for Advance.* By the Archbishop of Canterbury, Lady Albemarle, Alan Bullock, Sir Geoffrey Crowther, and Sir Ronald Gould. Pp. 16. (London: 1963 Campaign for Education, c/o Hamilton House, Mabledon Place, W.C.1, 1963.) 1*s*. 6*d*.

That will be secured the more readily if assurance is forthcoming that the institutions on and through which that expenditure is incurred are most efficiently and wisely administered. If proposals such as Prof. Williams advances, on the ground of making the most efficient use of universities and their staffs, for giving two courses in one year and doubling the academic staff, are to be rejected, it must be for convincing and valid reasons and not merely on grounds of tradition. Here again is room for much critical thinking, though it is only fair to note that both the University Grants Committee and the Advisory Council for Scientific Policy are already fully aware that much more effective use could be made of academic staff both in teaching and in research if they were provided with more ancillary assistance.

Here again the problem is largely one of finance in which the Government's start–stop policy is liable to prove penny-wise pound-foolish. It is reasonable to expect of university institutions that they should, as Prof. Williams urges, make the best possible use of their equipment and facilities, that they should be efficiently managed and that they should see that their most precious and expensive asset—their highly trained staff—should be provided with whatever assistance it needs to give of its best and that it should not be employed on duties for which much less highly trained labour is competent. But this is not the only test of an institution of university rank. If it is to exercise its vital creative function, whether in teaching or research, if it is really to father discovery, to use Dr. Magnus Pyke's apt phrase, it must first ensure that it provides the fundamental conditions in which creative work is possible.

LAW, HISTORY AND LAND USE

The Common Lands of England and Wales

By Dr. W. G. Hoskins and Prof. L. Dudley Stamp. (The New Naturalist: a Survey of British Natural History.) Pp. xvii + 366 + 28 plates. (London: William Collins, Sons and Co., Ltd., 1963.) 42*s*. net.

COMMON land is probably the most misunderstood part of our national heritage, and therefore a volume giving a clear account of the issue is welcome. The joint authors were members of the Royal Commission on Common Land, and each contributed an appendix to the report. They now present their findings in a readable and telling form.

The term 'common land' has no precise meaning in England and Wales (it does not exist as such in Scotland) and the nearest estimate of the area is about 1½ m. acres—a formidable 4 per cent of the land surface. The importance of common land lies in the number of interested parties of whom, apart from those concerned with agriculture, recreation and building development, there is a succession of historians, geographers, archæologists, ecologists, conservationists and Nature lovers. The legal tangles concerning doubtful ownership, the uncertain rights of the commoners and questions concerning public access frequently defy the understanding of even the initiated, and have been responsible for limiting, and even prohibiting, the purposeful use of certain commons, and encouraging the dereliction of others in both upland and lowland areas.

Dr. Hoskins traces the history of common land, and although the origin is obscure the common rights antedate the very conception of private property. Since the imposition of the manorial organization all such land has been, and still is, private property, owned by the lord of the manor, or his descendant or his legal representative. Common land is unique in that a large number of people have legal rights over the area which they exercise in common. The main use to which it is put is grazing and the gathering of wood for fuel, but there are other customs, some long in disuse, which made common land essential for peasant economy up to the end of the eighteenth century. The public by custom, with or without rights, wander more or less freely over the common land areas.

The history is interesting and often exciting. The vast reservoir of land that existed at the time of the Domesday Book has been drawn on heavily by an increasing population from the eleventh century onwards, leading eventually to stinting on common land, and inevitably to bitter disputes which reached a climax during the period of Parliamentary Inclosure. A movement to protect common land was bound to follow, but it left in its wake a system of laws and rules far from beneficial to present-day needs.

Prof. Dudley Stamp deals with the general state and usage of commons, features of special interest and geographical distribution; the geographical distribution depends largely on soil type, terrain and geological factors. The picture is presented on a regional basis, starting with the lowland heart of England where commons are few, moving progressively to the upland areas where commons are more widespread—the north of England and Wales (30 per cent of Breconshire is common land).

Research workers and those professionally concerned will find a wealth of information in this part of the book, also in the appendix that follows extending to more than 100 pages and providing for the first time a county-by-county list of the known commons, village greens, common gravel pits and fuel allotments of England (Wales presented an impossible confusion!). Other readers will doubtless concentrate on the areas with which they are most familiar—their native heath or favourite fell. Readers who delve deeper than this will find that no two commons are alike and they will be rewarded by fascinating reading of history, anachronism, human fallibility as well as fact. One is often surprised by the wide range of activities and opportunities that exist on common land.

The authors maintain a balanced view and a sympathetic appreciation of the demands of all concerned.

ROY HUGHES

IMMUNOLOGICAL PHENOMENA AND CLINICAL ALLERGY

Allergology

Proceedings of the IVth International Congress of Allergology, New York City, October 15–20, 1961. Edited by Ethan Allan Brown. Pp. xii + 455. (London and New York: Pergamon Press, 1962.) 105*s*.

IN this volume some of the promise of the rapidly increasing understanding of immunological phenomena for clinical allergy is foreshadowed, although the gap between scientific and clinical aspects is still only too obvious. The rate of progress is shown by Dr. J. M. Andrews's comments on the meteoric increase, from £30,000 to £2 million devoted to this subject by the United States in the past six years, and in the number of projects from 9 to 277. One wonders how the figures for the United Kingdom compare with this.

Of the 43 papers by leading workers, at least half are well worth reading. Part 1, the most impressive of seven parts, commences with a review by M. Heidelberger on the "Unity and Heterogeneity of Antibodies", showing how the introduction of precise quantitative chemical micro-methods, established, for example, the identity of agglutinins and precipitins, and the predictability of cross- and homologous-specific reactions, based on the chemical structure of antigens and particularly of poly-

saccharides. S. Cohen and R. R. Porter describe the chemistry and biological function of 19*S* and 7*S* γ-globulin and of the three pieces obtainable from 7*S* γ-globulin by enzymatic digestion: piece 3 with its capacity to sensitize skin and promote placental transfer, and pieces 1 and 2 with their antibody combining sites and allotypic specificity. These findings add to the interest of B. N. Halpern's work on "Le processus de Sensibilization", showing the remarkable competition for cellular attachment between normal γ-globulin and antibody γ-globulin. D. H. Campbell discusses the importance of antigen–antibody ratios for the tissue reactions produced by their complexes. J. Humphrey discusses biochemical mediators of antigen–antibody reactions, with reference to two performed mediators, histamine and serotonin, two produced mediators, *SRS*-A and bradykinin, and complement-fixing esterase activation in anaphylaxis.

Immunological and clinical aspects of drug hypersensitivity are discussed with an introduction by M. W. Chase on chemical considerations. In reviewing the immune response of hypersensitivity, S. Raffel concludes that the early appearing Jones–Mote type of sensitivity to protein antigens is probably of the delayed tuberculin type. L. E. Thomas argues that the teleological significance of delayed-type hypersensitivity is protective, by destroying potentially neoplastic cells. S. B. Salvin and H. N. Eisen discuss delayed-type sensitivity to protein and simple chemicals respectively.

In the section on auto-allergy, homograft rejection is discussed by E. Billingham, hypogammaglobulinæmia and immunological responses by C. A. Janeway, and autosensitization to thyroid and to vascular antigens by E. Witebsky and D. Pressman respectively, with a paper on the inhibition of immune reactions by J. Sterzl.

Useful reviews of techniques are given, on the measurement of antibody by D. W. Talmage, and on hæmagglutinating factors in allergic sera by A. H. Sehon. Agar-gel, immunoelectrophoretic and fluorescent antibody techniques are well covered by J. Oudin, P. Burtin and M. H. Kaplan respectively. The uses and interpretations of the passive cutaneous test for anaphylaxis are discussed by Z. Ovary, and O. G. Bier concludes with a critical discussion of the rôle of complement-fixation in immediate hypersensitivity reactions.

The discussions on the clinical use of steroids, psychotherapy, general methods and the use of mineral oil emulsions for 'desensitization' show the need for more precise investigations in these fields. J. Pepys

DISCUSSIONS OF STATISTICAL INFERENCE

The Foundations of Statistical Inference

A Discussion. Opened by Prof. L. J. Savage. (Methuen's Monographs on Applied Probability and Statistics.) Pp. 112. (London: Methuen and Co., Ltd., New York: John Wiley and Sons, Inc., 1962.) 15*s*. net.

THIS monograph is divided into three parts. In the first, Prof. L. J. Savage expounds his views on statistical inference, arguing that all statistical practice should be based on Bayes's theorem, that there is no dichotomy of statistical problems into inference or decision, and that all probabilities are "subjective". In the second, five eminent British statisticians (Profs. Bartlett, Barnard, Cox, Pearson and Dr. C. A. B. Smith) present their views on this topic and then, in the third part, the discussion at the seminar at which these lectures were given is recorded.

The dust jacket proclaims "most of the monograph is non-technical and should interest anyone concerned with statistical inference". Both these statements are true! There are very informative discussions of such purely "objectivist" problems as testing a simple hypothesis without specifying an alternative. On this topic, for example, Prof. Cox points out that in a great many "testing situations" any test-statistic will itself generate a family of alternative hypotheses against which it is "optimum"; one is left wondering whether it would not always be better to make some attempt to specify alternatives rather than have it done, so to speak, for one. The major part of the monograph is, however, taken up with the discussion of points of disagreement between the subjectivists and the objectivists, and here the non-technical nature of the monograph has, I think, been taken too far.

There is no statement of the basic axioms used and of the major results derived by Prof. Savage in his book *The Foundations of Statistics*. This makes the outline of the area of disagreement unnecessarily vague, which means that certain parts of the discussion will not appear fruitful to a reader unacquainted with a formal statement of Savage's position. Indeed an explicit statement of the axioms and the main result in *The Foundation of Statistics* would, I think, have helped in the discussion itself. For example, a direct attack on the axioms or the derivation of the main result would then have been called for, which might have cleared the way to a better understanding of where the points of disagreement lie.

The discussion is, however, always interesting and the book is well worth buying on a number of counts. For example, for the discussion on the difference between the precision expected from an experiment before it is performed and the precision actually yielded by it when it is performed, and for Prof. Barnard's discussion on likelihood functions and their interpretation. The reader will find the monograph more rewarding, however, if the first few chapters of Prof. Savage's book and the paper of Prof. A. Birnbaum are read first[1]. The book deals with a "decision" or "potential decision" situation and the subjective probability distributions derived are really shorthand descriptions of "betting" behaviour. An understanding of this should help one to decide, for example, whether in fact Prof. Savage's discussion on inference derives backing from his book, and whether Prof. Barnard is in fact at cross-purposes with Prof. Savage when he states (p. 41) "Whoever manages to guess the prior distribution most accurately will obtain the best decision rule". Finally, Prof. Birnbaum's paper based on purely "objectivist" ideas, proves, on the basis of some apparently compelling axioms, that "objectivist inference" should be based on the likelihood function alone, and it is interesting to follow the discussion with this latter result in mind. M. C. Pike

[1] Birnbaum, A., *On the Foundations of Statistical Inference*. *J. Amer. Stat. Assoc.*, **57**, 269 (1962).

SYMPOSIUM ON SPACE SCIENCE

Space Research III

Proceedings of the Third International Space Science Symposium, Washington, D.C., May 2–8, 1962. (Organized by the Committee on Space Research—Cospar, and the U.S. National Academy of Sciences.) Edited by Prof. Wolfgang Priester. Pp. xvi+1275. (Amsterdam: North-Holland Publishing Company, 1963.) 220*s*.

THIS volume contains most of the papers presented at the third International Space Science Symposium at Washington in May 1962. It does not include the papers on life sciences or the contributions to the two related international conferences at Washington which immediately preceded the Symposium, namely, those on "Rocket and Satellite Meteorology" and on the "Use of Artificial Satellites for Geodesy". The papers on these topics are being published in three separate volumes.

Even with these omissions, the book has a wide range, covering the Earth's atmosphere from a height of 80 km up to its outermost limits, the Sun and the interplanetary plasma, the Moon and planets, galactic astronomy and the technology of space research. There are more than a hundred papers, almost all of them in English, and each is preceded by a summary in English and Russian. The Committee for Space Research (COSPAR), which organized the conference, has always been genuinely international, and so are the papers: though a large proportion comes from the United States or the U.S.S.R., there are also many excellent contributions from other countries.

The first group of papers, totalling 170 pages, is devoted to the Earth's upper atmosphere from heights of 80 to about 2,000 km, and includes elegant analyses of variations in atmospheric density by L. G. Jacchia, H. K. Paetzold and others, and a long review of upper-atmosphere chemistry by V. I. Krassovsky. The second group of papers (150 pages), dealing with the charged particles in the same region of the atmosphere, is dominated by the many reports on measurements of electron-density profiles. The third group of papers (170 pages) is on the magnetosphere, that is, the atmosphere from a height of approximately 2,000 km out to the farthest limits of influence of the Earth's magnetic field, at about 10–15 Earth radii on the sunlit side. This group includes accounts of various measurements of the Earth's magnetic field and the zones of trapped radiation.

An excellent review by C. de Jager, on "Emission of Gas from the Sun", sets the scene for the next two groups of papers (250 pages), which are concerned mainly with the outflow of charged particles from the Sun, the 'solar wind', and its interaction with the Earth's atmosphere. This complex, fascinating and rapidly developing subject has in the past few years completely altered our view of the Earth's environment: what was an absolute blank not long ago now stands revealed as the scene of a rich and fluctuating hydromagnetic struggle. Notable among the papers on this subject are those giving results from the *Explorer* 10 satellite, which probed the fluctuating boundary of the magnetosphere.

The next group of papers (110 pages) covers solar radiation, ultra-violet and X-ray, and records an impressive number of detailed measurements of the solar spectrum between 5 and 3000 Å by rockets and satellites. There follow three papers on the astronomical unit, including a useful review by A. A. Mikhailov, who recommends a value of 149,550,000 km. Then come four papers on meteorites and tektites, including an excellent survey of interplanetary dust measurements, and ten papers on some larger solid bodies of the solar system, namely, the Moon, Mars and Venus.

"Space Astronomy" is the subject of the next group of papers (70 pages), which includes reports on the first southern-hemisphere observations of stellar ultra-violet radiation, made by a *Skylark* rocket in 1961, and the γ-ray measurements by *Explorer* 11.

The final section of the book (110 pages) is concerned with the technology of space research and consists chiefly of papers describing instruments used, plasma probes, mass spectrometers, photo-detectors, and others.

The editor, Prof. W. Priester, and the North-Holland Publishing Co. are to be congratulated on producing, within a year of the Symposium, such a handsome, well-edited and well-printed volume, which provides an authoritative survey of most aspects of space science. One of the book's most remarkable features—which reflects particular credit on the translators of the Russian papers—is the excellent literary style of the contributions. When one considers that the papers are in English, which is not the native tongue of many of the authors, that the editor comes from Germany and the publishers from Holland, the stylistic felicity of the book is quite admirable.

D. G. KING-HELE

SNOW CRYSTALS

Snow Crystals

By W. A. Bentley and W. J. Humphreys. (Dover Edition *T*287). Pp. 226. (New York: Dover Publications Inc.; London: Constable & Co., Ltd., 1962.) 24*s*.; 2.95 dollars.

THIS is a paperback re-issue of the book originally published by McGraw-Hill in 1931. It represents the collected work of W. A. Bentley, who spent nearly fifty years photographing snow crystals at Jericho, Vermont. The appearance of this new issue is superb; it is a masterpiece of paperback book production. The contents are reproduced exactly as they appeared in the earlier edition.

From the scientific point of view it is a pity that there is no record of the magnification or amount of retouching on the prints, particularly since some of them show unusual and interesting forms. Dobrowolski, in his treatise *Historja Naturalna Lodu* (1923), made extensive use of Bentley's pictures, but Nakaya in *Snow Crystals* (1954) was critical of his technique of cutting out the snow crystal negative and printing to produce a black background. This gives a very impressive picture but tends to diminish the scientific value.

The pictures could contribute much to the study of snow crystal form if it were known that they possessed the original outlines and features. This is illustrated by two photographs in the collection, on p. 205 and p. 207; these show an apparent overgrowth anomaly. A plate crystal has formed round a columnar crystal but the hexad axis of the plate is perpendicular to the hexad axis of the column.

It appears that Bentley was concerned with the scientific aspects of his work and he published several papers in *Monthly Weather Review* during 1901–27. The introduction to the collection was added by Humphreys for the publication after Bentley's death and he presents the pictures as true representations of the snow crystals with the exception of a few plates which have had arms and details trimmed off. The more elaborate and unusual crystals are accurately portrayed.

I. J. SMALLEY

VEGETATION AND SOILS OF THE WORLD

Vegetation and Soils

A World Picture. By Dr. S. R. Eyre. Pp. xvi + 324 + 32 plates. (London: Edward Arnold (Publishers), Ltd., 1963.) 36*s*. net.

AS the Freedom from Hunger Campaign gains momentum and the public at large begins to give thought to the problems associated with food shortage, any book on soil will catch the eye. In other words, there is already a wide readership for any publication which will outline fairly and factually the scope of the world's potential resources in this medium of plant growth. Accepting this premise, one may expect from authors on this topic texts which are explicit and discernible, which inform and educate. Dr. Eyre's work measures up to some of these requirements, but by no means all.

The subject is a complex one and integrates basic knowledge from many not-so-kindred sciences. Indeed, it is a project that might well have been undertaken by a botanist, an agriculturist or a soil scientist. But the author, a geographer, tackles it from his own particular point of view.

As a generalization on the world distribution of vegetation and soils it is quite valid. Both on established vegetation (climatic climax) and transient vegetation (ecotones) an adequate picture is given. The relating of the Krakatoa

explosion to the field of vegetation and soil history certainly shows this catastrophe in a fascinating soil-forming perspective.

In places the author is distinctly speculative—an admirable trait in a subject that is at times beleaguered by hesitancy and procrastination (*vide* the lack of agreement among soil scientists on systems of soil classification). His explanation of the uneven terrain in the tundra is simple but entirely acceptable, although his declamations on the role of fire in vegetation ecology require more statistical evidence than is provided.

In terms of balance, the text leans very heavily on the side of vegetation. An outstanding account is given of the principal species to be found in the various climatic zones with cogent reasons for their being there. This will make excellent reading for foresters and decorative horticulturists in their pursuit of soil studies. Incidentally, the bracketing of tree names with Latin binomials is not as pedantic as would at first seem. The vegetation maps and tables of climatic climax correlations help to illuminate the script and do credit to the publishers.

When considered as a reference book on soils, its limitations are soon apparent. Pedologically, the author does not appreciate, or fails to stress, that vegetation is only one facet of a single soil-forming factor. Dokuchaev's concept of soil being the product of five soil-forming factors (namely, parent material, organic matter—including vegetation, climate, relief and time) each of equal importance if differing in content and quantity, is universally agreed by soil scientists.

The mechanisms of physical and chemical actions are clearly enough explained for the type of reader referred to in the cover although chemical terms could be used more exactly, and a number of interesting and intriguing statements are made without due qualification, for example, on the formation of raised bogs and on the absence of ice sheet in Asia. Finally, there could have been at least a hint at the agricultural possibilities of the various soils.

Notwithstanding the criticisms, this is a book which had to be written, and it will certainly find a place in recommended if not essential reading for students of many faculties and others interested in botanical geography and its relation to soils. W. S. Wilson

ANIMAL BEHAVIOUR

Roots of Behavior

Genetics, Instinct and Socialization in Animal Behavior. Edited by Prof. Eugene L. Bliss. Pp. xi + 339. (New York: Harper and Brothers. A. Hoeber Medical Book, 1962.) 16 dollars.

TITLES of books on behaviour have almost exhausted the possible permutations of a small population of words. We have had the organization, principles, elements, foundations, structural basis of behaviour, the behaviour of organisms. One recent work bore the unadorned title, *Behaviour*. The work under review has found a new word—"Roots". It is a symposium of twenty-three papers by thirty-one authors (S. Altmann, Bruell, Calhoun, Carpenter, Clark, Collias, D. E. Davis, Denenberg, Dethier, Dilger, Fuller, Goy, Harlow, Eckhard Hess, J. Hirsch, Jakway, Lehrman, S. Levine, McClearn, D. A. Rodgers, Rosenblatt, Ross, Sawin, Schneirla, Scott, Shaw, Tobach, Turkewitz, Waller, W. C. Young, Zarrow). Their contributions are grouped under four headings: "Genetics of Behavior", "Instinctual Behavior", "Early Experience", and "Social Behavior". From the groupings it can be inferred that the various roots penetrate into different soils, and indeed some of the roots seem to be the crop as well. The crop is quite definitely spelled -ior and not -iour; it is a pity that no non-American participants were included, since the views of leading authorities such as Tinbergen, Hinde, Thorpe, Lorenz, and others are represented only indirectly.

Be this as it may, the volume will be a useful addition to many departmental libraries, although it is unlikely that many individuals will think it worth 16 dollars. It is useful because of its breadth—very few of us can keep up with detailed knowledge in all four areas of interest: it contains a number of articles of authority and substance. I suspect the book will be of greater interest to experimental psychologists than ethologists, who will tend already to be familiar with much of it.

It is obviously impossible to give a detailed review of 23 papers. I found the genetics papers by Bruell and by Rodgers and McClearn quite helpful both as general reviews and as accounts of current experimentation. The section on instinctual behaviour is the longest and meatiest. Young's paper on sexual behaviour is a brief but clear and provocative review by one of the pioneers in that area of research; Lehrman follows on from it quite naturally and with a sensible analysis. Harlow's account of his important developmental work with monkeys will be familiar, by now, to many readers, but as always it makes for entertaining reading.

Fuller and Waller's paper on early experience is useful for its analysis of the methodological aspects of the problem and for its discussion of experimental design. Again, many readers will be familiar already with Levine's experiments on infantile stimulation, but some new facts are presented which seem to require a modification of his earlier conclusions. Levine appears to combine, in this paper, a reluctance to speculate with polemics, depending on the facts under review, and one of his main conclusions, that "stimulated animals are more reactive to distinctly noxious situations, but the nonstimulated animal appears to react to a greater variety of environmental changes" (p. 250) seems plausible but is based on very little evidence. Hess and Collias both deal usefully with imprinting and following responses, Collias taking a broader and somewhat more cautious approach than Hess, whose paper is principally concerned with his admirably quantitative research in an area that is apt to be rather anecdotal. His distinction between imprinting and ordinary discrimination learning, however, does not appear to me to be carefully reasoned.

Finally, the section on social behaviour is flimsy, perhaps necessarily so. Only Carpenter's paper combines substance with clarity. His account of the long-term observations of howler monkey populations over thirty years is surely unique, but the analogy between animal groups and homeostatic mechanisms is very rough, and there is a sense of anti-climax by the end of the paper.

The editor admits that "the volume, like any collection of papers by different authors, lacks the evenness of style to be found in a textbook by one author". But there is little evidence of the editor's red pencil having been much exercised. The book often gives the appearance of having been hurried and first-draftish, and sometimes authors are quite unnecessarily ponderous and verbose, for example, ". . . the food hopper [in the diagram] may be identified by its cone-shaped dorsal aspect"—nothing else in the diagram is cone-shaped except the food hopper. "Unfortunately, no measures of disruption of this behavior were made. I say unfortunately because as discussed below, alteration of the duration of this behavior—that is, shortening it—should be the first behavior disrupted." ". . . studies in our laboratory indicate that the behavioral bond between the female and her offspring begins at partuition." In Hess's paper we find, "We make use of the Hess Pecking Preference Apparatus (Hess, 1956)". And so on. But those who can tolerate a bit of fertilizer with the roots will find most of these plants healthy and worth cultivating. L. Weiskrantz

STRUCTURE OF HÆMOGLOBIN

A THREE-DIMENSIONAL FOURIER SYNTHESIS OF REDUCED HUMAN HAEMOGLOBIN AT 5·5 Å RESOLUTION

By HILARY MUIRHEAD and DR. M. F. PERUTZ, C.B.E., F.R.S.

Medical Research Council Laboratory of Molecular Biology, Cambridge

MAMMALIAN hæmoglobin is a protein of molecular weight 64,500. It consists of four polypeptide chains, which are identical in pairs, and of four hæm groups. In horse oxyhæmoglobin each of the chains has a configuration closely resembling that of sperm whale myoglobin[1,2]. The four chains form a tetrahedral array with the hæm groups facing outwards, each hæm group lying in a separate pocket or fold of its own globin chain. The wide separation of the four hæm groups is a puzzling feature of the structure, since it rules out any direct kinetic interaction between them, of the kind postulated to explain the sigmoid shape of the oxygen dissociation curve.

Since Haurowitz's discovery that a change in crystal form follows the reaction of horse hæmoglobin with oxygen[3], a mounting body of evidence has accumulated suggesting that this reaction is accompanied by some major change in the structure of hæmoglobin[4]. In myoglobin, which consists of only a single chain, no such evidence has been found, which would seem to indicate that the structural change in hæmoglobin is somehow dependent on the interaction between the four chains.

After the structure analysis of the oxyhæmoglobin of the horse[1], the obvious next step would have been an attack on its oxygen-free or 'reduced' form. Unfortunately, this crystallizes with a unit cell and space-group unsuitable for detailed X-ray analysis[5], so that we had to turn to a different species. Reduced hæmoglobin of man crystallizes in three different forms[6,7], one of which was chosen as suitable for X-ray analysis. We have now obtained a three-dimensional electron density map at 5·5 Å resolution which is sufficient to reveal the general course of the polypeptide chains and the positions of the hæm groups. As in horse oxyhæmoglobin, the molecule is divided into four sub-units which correspond to the pairs of α- and β-chains of the chemical sequence[8–11]. At the present resolution no significant differences can be detected between the structures of corresponding globin chains in horse oxy- and human-reduced hæmoglobin, nor in the relative arrangement of the two α-chains. However, the two β-chains in human reduced have moved apart, increasing the distance between their two hæm groups by 7 Å compared with that in horse oxyhæmoglobin. On the atomic scale this represents a striking change of structure.

It would have been more satisfying if this discovery could have been accompanied by a demonstration that the oxyhæmoglobins of horse and man have the same structure; but in this we have not yet succeeded, because our crystals of human oxyhæmoglobin have failed to form isomorphous heavy atom compounds. However, a close similarity between the hæmoglobins of the two species is likely on chemical grounds; indirect X-ray evidence suggesting that the structural change observed by us is due to the oxygenation reaction, rather than to the difference of species, is presented in the accompanying paper on hæmoglobin H (ref. 12).

Method of X-ray Analysis

X-ray pictures were taken of crystals grown from solutions containing 2·6 M ammonium sulphate and 0·01 M ferrous citrate, buffered to *p*H 6·5; the crystals were mounted in an atmosphere of nitrogen. The crystals belong to the monoclinic space group $P2_1$. The unit cell has the dimensions $a = 63{\cdot}4$ Å, $b = 83{\cdot}6$ Å, $c = 53{\cdot}9$ Å, $\beta = 99° 15'$, and contains two molecules. Thus, unlike the molecules of human and horse oxyhæmoglobin which lie on two-fold symmetry axes, those of the reduced form lie in general positions, which meant that the number of reflexions contained in one-quarter of the limiting sphere of $(5{\cdot}5\ \text{Å})^{-1}$ was 2,000, as compared with 1,200 in horse oxyhæmoglobin. hkl and $\bar{h}kl$ reflexions were recorded photographically on Buerger Precession Cameras, using a Broad rotating anode tube[13] and a Joyce–Loebl microdensitometer. Intensities were put on an absolute scale by comparison with horse oxyhæmoglobin. All calculations were made on *Edsac II* at the Mathematical Laboratory, University of Cambridge.

The phase angles were determined by the method of isomorphous replacements with heavy atoms. Three heavy atom compounds were used: one in which each of the two reactive sulphydryl groups is combined with parachloromercuribenzoate (PCMB); another in which the same groups are combined with dimercuriacetic acid (DMA); and a third in which two of the four 'unreactive' sulphydryl groups[14] are combined with mercuric acetate ($HgAc_2$).

The parameters of the mercury atoms in the first two compounds were found from three-dimensional difference Patterson syntheses using $[|F_{\text{protein + mercury}}| - |F_{\text{protein}}|]^2$ as coefficients[15]. Fig. 1 shows a section through the three-dimensional difference Patterson of the PCMB compound in which the vector peaks due to interactions between the mercury atoms stand out above the background. No such clear-cut results could be obtained from difference Pattersons of the $HgAc_2$ compound, partly due, as we now know, to its incomplete substitution. Its mercury atoms were eventually located in a three-dimensional difference Fourier synthesis using $[|F_{\text{protein + mercury}}| - |F_{\text{protein}}|]$, modified by a weighting factor m, as coefficients, and α_{protein} as the phase

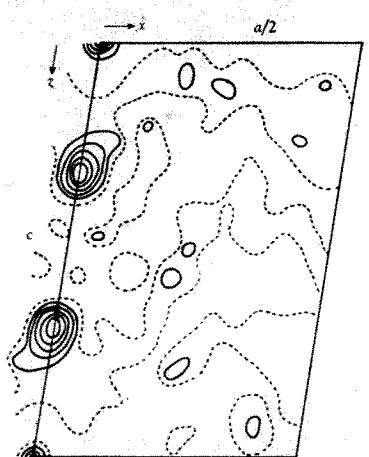


Fig. 1. Section at $y = 2/40$ through the three-dimensional difference Patterson synthesis of PCMB versus native hæmoglobin. The two prominent peaks occur at 18 and 37 Å from the origin, and mark vectors between pairs of mercury atoms in the structure. The peak at $x = z = 0$ is part of the maximum at the origin. The zero contour is marked by a broken line. Negative contours are omitted

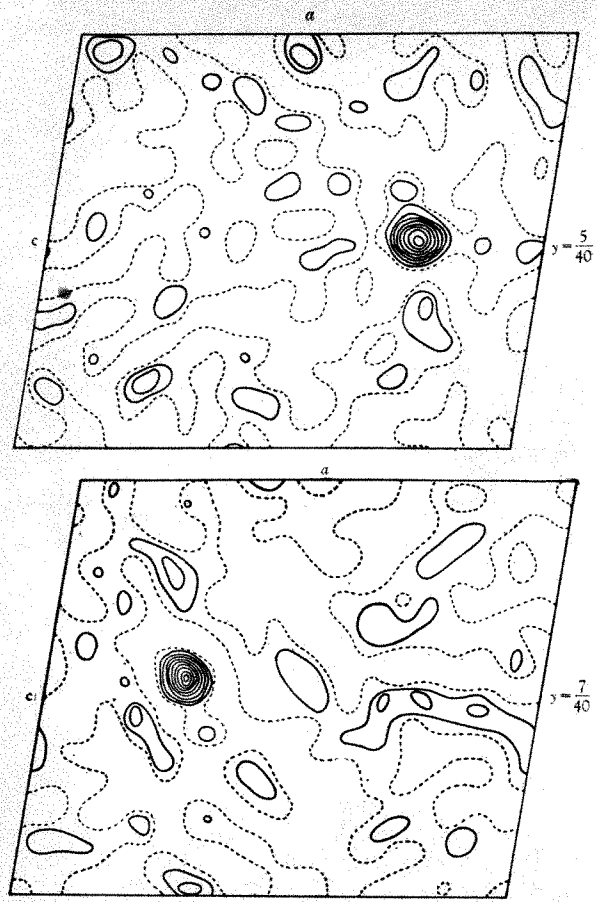


Fig. 2. Sections through the three-dimensional difference Fourier synthesis of the $HgAc_2$ compound versus native hæmoglobin. The high peaks mark the positions of mercury atoms. Contours are at arbitrary intervals; those below the broken line are omitted

angle; the values of α and m were calculated by the isomorphous replacement method using only the PCMB and DMA compounds. Owing to the close proximity of the mercury atoms in these two compounds, the phase angles were not much better than if they had been calculated from a single isomorphous replacement[16]. Nevertheless, the difference Fourier showed two prominent peaks on a low background (Fig. 2), corresponding to the two moles of mercuric acetate per mole of hæmoglobin added to the crystallization mixtures. The peaks were later found to lie close to the known positions of non-reactive sulphydryl groups[14,26].

Phase information derived from the isomorphous substitution and anomalous scattering of the three compounds was now combined, and each structure amplitude was multiplied with a weighting factor proportional to the accuracy of the particular phase angle[17,18]. The average error in phase angle was estimated as 45°, but may in fact be somewhat greater, compared with 37° in horse oxyhæmoglobin. The electron density was calculated at intervals of about 2 Å and plotted in sections normal to *b*.

Results

(*a*) *Structure of the sub-units.* As in the electron-density maps of myoglobin and hæmoglobin calculated earlier, long, straight, cylindrical regions of high density are among the prominent features (Fig. 3); these regions tend to join up to form the same kinds of three-dimensional figures[1]. However, in some regions the density is not continuously high along what would be the course of the polypeptide chains in horse oxyhæmoglobin, and the breaks which occur might have made the chain unrecognizable without previous knowledge. In other regions the course of the chains is clearly defined. Prominent peaks appear in positions corresponding to those of the hæm groups in myoglobin and oxyhæmoglobin; but again they would not easily have been recognized as such, because they do not stand out above the density of the chains as they did in the other two proteins. However, despite these imperfections, which are probably due to the larger error in phase angle, the main features of the structure had emerged clearly from the maps even before any models had been built.

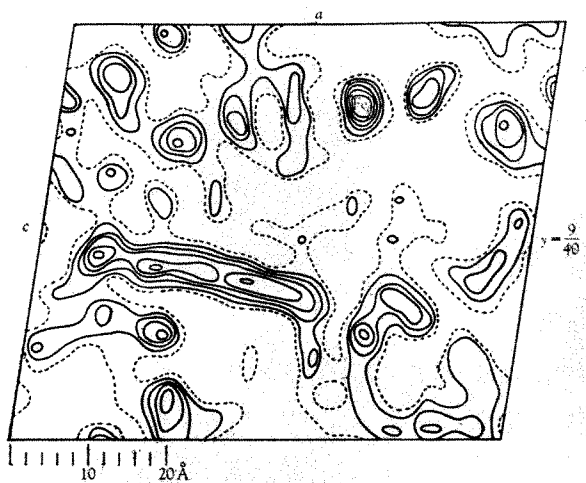


Fig. 3. Section through the three-dimensional Fourier synthesis of reduced human hæmoglobin showing one of the straight cylindrical regions of high density, later identified as helix *G* of one of the α-chains, and a spheroidal peak (Fe_4) representing the hæm group of its symmetrically related partner. Contours are drawn at intervals of approximately 0·075 electrons/Å^3, and the broken line is at 0·42 electrons/Å^3, which is the average density of the hydrated protein. The second plain contour was taken as a reference density for building the models. Contours below 0·42 electrons/Å^3 are omitted

Models of the electron density distribution were constructed by cutting from a sheet of plastic the shape of each peak where the density exceeds 0·57 electrons/Å^3, and then assembling the pieces according to their positions in the different sections. Figs. 4 and 5 show the α- and β-chains next to the corresponding chains of oxyhæmoglobin. (The β-chains were recognized by the mercury atoms attached to their reactive sulphydryl groups.) The figures illustrate both the similarities in structure and some of the imperfections of the new Fourier. A more quantitative comparison was obtained by measuring the directions of the straight segments of chain which are known to correspond to α-helical regions[2], and calculating the angles between them (Table 1). The α-helical segments have been given the labels *A*–*H*, starting at the amino-terminal end[19], but only the directions of the longer segments (*A*, *B*, *E*, *F*, *G* and *H*) can be measured with reasonable accuracy (~ ± 5°). The angles between segments within any one sub-unit of reduced hæmoglobin were found to be the same, within the limits of error, as in the corresponding sub-units of oxyhæmoglobin. If any change has occurred in the folding of any of the chains, it is too small to be detected at the present resolution and accuracy.

Table 1. Angles between Helical Segments of the Polypeptide Chains (See also Fig. 6)

Segments	Horse oxyhæmoglobin	Human reduced hæmoglobin	
	α-Chains		
		α_1	α_2
AB	94°	92°	93°
BE	117°	123°	124°
GE	97°	87°	83°
GH	156°	158°	151°
	β-Chains		
		β_1	β_2
AB	90°	86°	88°
BE	117°	116°	122°
EF	151°	155°	156°
FG	113°	108°	108°
GE	91°	86°	85°
GH	158°	159°	159°

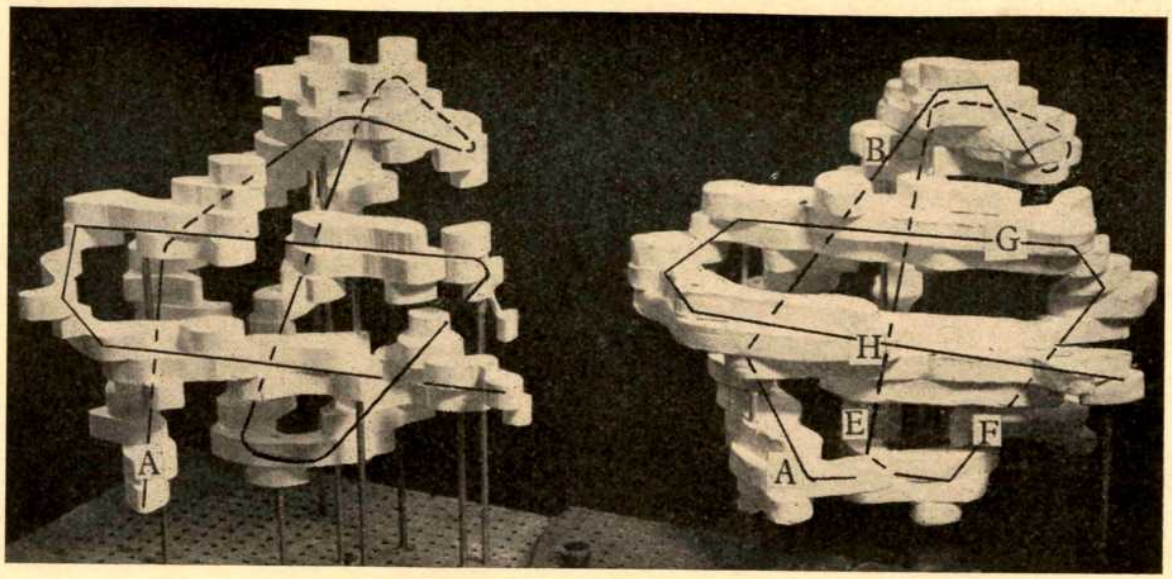


Fig. 4. Models of α-chains in human reduced (left) and horse oxyhæmoglobin (right)

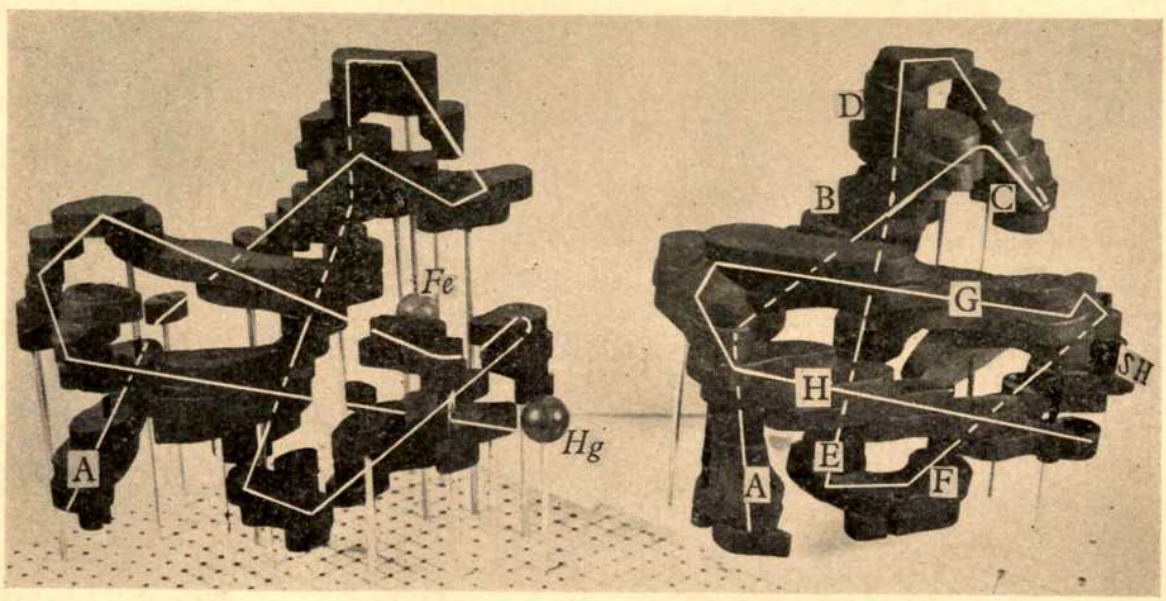


Fig. 5. Models of the β-chain in human reduced (left) and horse oxyhæmoglobin (right). Figs. 4 and 5 show the models of the two hæmoglobins with the a and b axes of the two lattices in parallel, because the orientation of the molecules relative to these axes happens to be approximately the same in the two structures. However, the correspondence is not exact, and the chains of the human and equine forms are seen here from slightly different angles. A comparison with the idealized model pictured on the left of Fig. 6 may help the reader to visualize their three-dimensional structure

So far as can be judged, the positions of the hæm groups relative to the globin chain are also the same as in oxyhæmoglobin. A quantitative estimate is possible in the β-chain, where the iron atoms are linked to the mercury atoms of PCMB through the intermediary of only two residues, the hæm-linked histidine and the cysteine immediately following it. The distance from the hæm to the mercury peaks is 14·4 Å in reduced, as compared with 13·6 Å in oxyhæmoglobin; the difference of 0·8 Å is within the limits of error of the present Fourier. In horse hæmoglobin the orientation of the hæm groups had been determined by electron spin resonance of the ferric or *met* form[20], which is paramagnetic, but forms isomorphous crystals with the diamagnetic oxyhæmoglobin used for the X-ray work. Reduced hæmoglobin is also paramagnetic, but its crystals failed to give sharp electron spin resonance lines in preliminary tests, so that the orientation of the hæm groups has not yet been determined. In the models their positions have therefore been indicated by balls rather than disks.

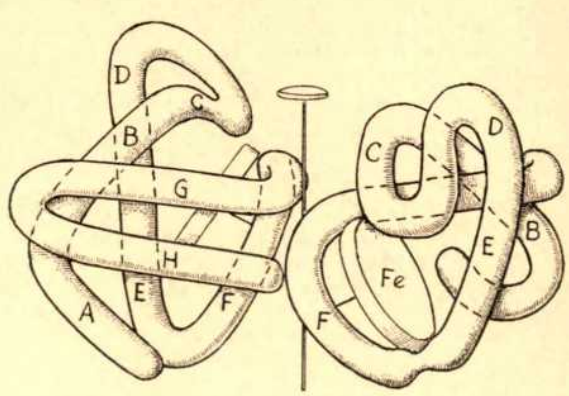


Fig. 6. Idealized drawing of the two β-chains in horse oxyhæmoglobin. The sign at the centre marks the two-fold symmetry axis. The α-chains form a similar pair (see Fig. 6 of ref. 1)

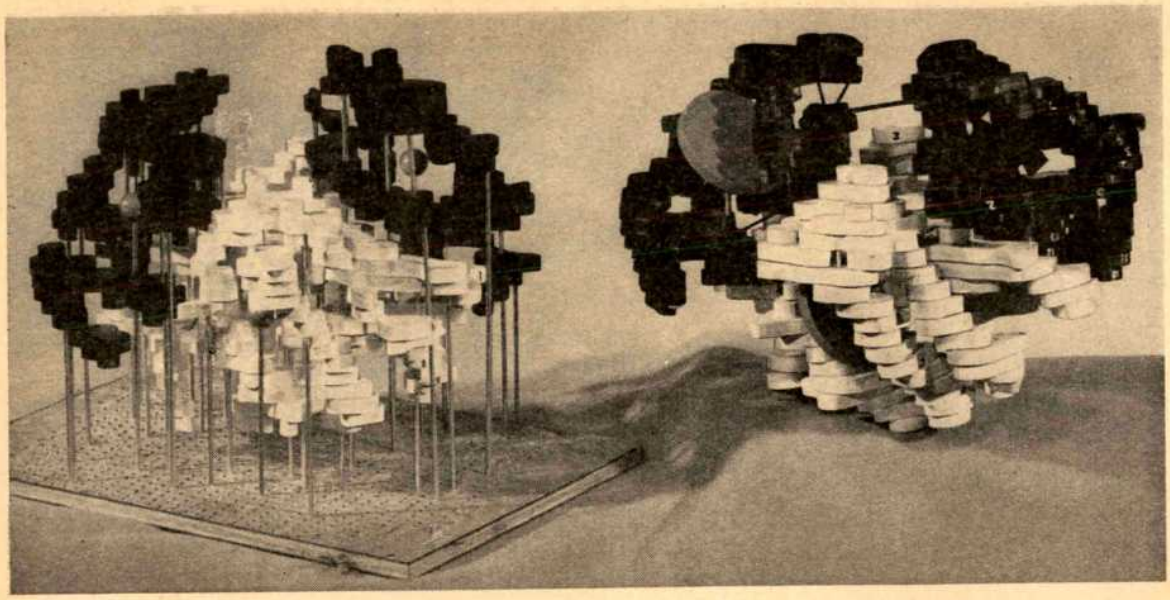


Fig. 7. Complete models of human reduced (left) and horse oxyhæmoglobin (right). The inclination of the hæm groups in the reduced forms is not known; their positions are indicated by balls instead of the grey disks used in the oxyhæmoglobin model

(*b*) *Arrangement of the sub-units.* The model of the complete molecule, like that of oxyhæmoglobin, is assembled by first arranging the sub-units in symmetrically related pairs (Fig. 6); one pair, the black, say, is then inverted and placed over the top of the white so as to form a tetrahedral array (Fig. 7). To examine the arrangement of the sub-units more accurately, sections of the Fouriers containing the hæm groups and the long C-terminal helices *G* and *H* were projected on a plane perpendicular to the crystallographic *b* axes. Figs. 8 and 9, showing sections of the α- and β-chains of the two proteins side by side, illustrate once more the similarity in structure of the individual sub-units. In Fig. 10 the outlines of the dense projected regions of the reduced and oxygenated forms are superimposed. The α-chains show no significant differences, the apparent shift of the hæm groups being due to the different tilt of the two molecules relative to the *b* axis. The two β-chains, on the other hand, cannot be superimposed at the same time; if one chain of the reduced is superimposed on one of the oxygenated form, their symmetrically related partners are displaced relative to each other by 7 Å. The displacement opens a gap between the two β-chains in the reduced form (Fig. 11).

The distance between the two iron atoms attached to the β-chains is 40·3 Å in the reduced compared with 33·4 Å in the oxygenated form, and the distances between the two mercury atoms attached to the β-chains differ by the same amount (Table 2). On the other hand, the distances between the iron atoms attached to the α-chains of the two forms are identical within the limits of error, and the same is true, surprisingly, of the distances between the iron atoms which lie in adjacent pockets of the α- and β-chains ($Fe_1 - Fe_3$; these belong to the two hæm groups in closest proximity, shown in Fig. 8*a* of ref. 1).

Table 2. Distances between Hæms and Mercury Atoms

	Horse oxyhæmoglobin	Human reduced hæmoglobin
Fe_1–Fe_2	33·4	40·3
Fe_3–Fe_4	36·0	35·0
Fe_1–Fe_3	25·2	25·0
Fe_1–Fe_4	30·4	37·4
Fe_1–Hg_1	13·6	14·4
Hg_1–Hg_2	30·0	37·7

Fe_1 and Fe_2 refer to the hæms in the β-chains, Fe_3 and Fe_4 to those in the α-chains, Hg_1 and Hg_2 are the mercury atoms of PCMB attached to cysteine 93 of the β-chains. The location of the groups is shown in Fig. 4 of ref. 1.

So far as can be ascertained, no measurable change in the relative orientation of the four sub-units accompanies the shift of the β-chains.

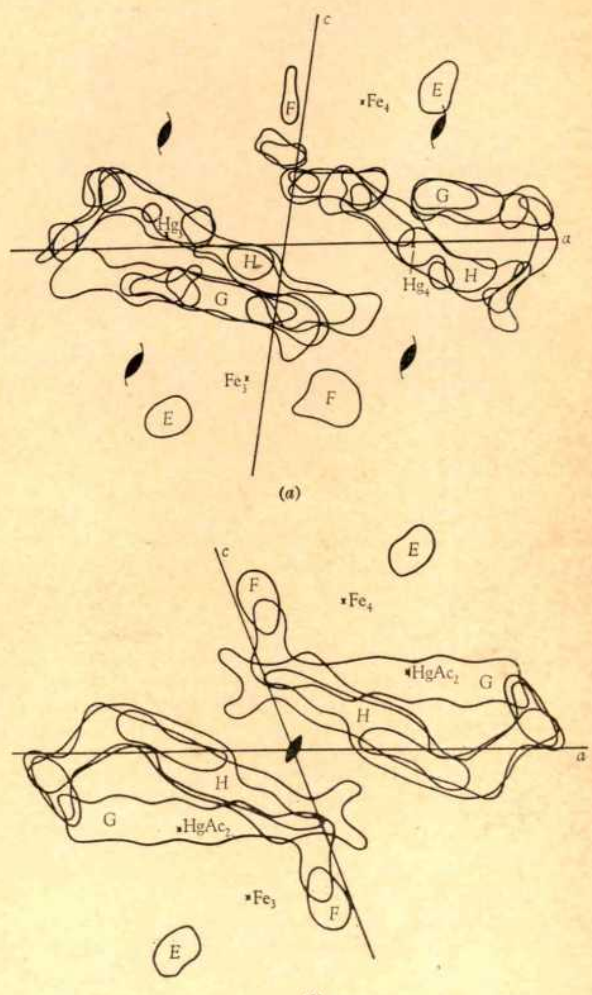


Fig. 8. α-Chains. The helical regions *G* and *H*, the hæm groups and sections through the helical regions *E* and *F*, projected along the *b*-axis in (*a*) human reduced and (*b*) horse oxyhæmoglobin. All contours are drawn at the reference-levels used for the construction of the models (0·57 electrons/Å^3 in human and 0·54 in horse hæmoglobin). Contours at several levels of *y* are superimposed to produce the projected images. Fe marks the hæm positions. In (*a*) the screw dyads are indicated; in (*b*) the dyad is shown at the centre

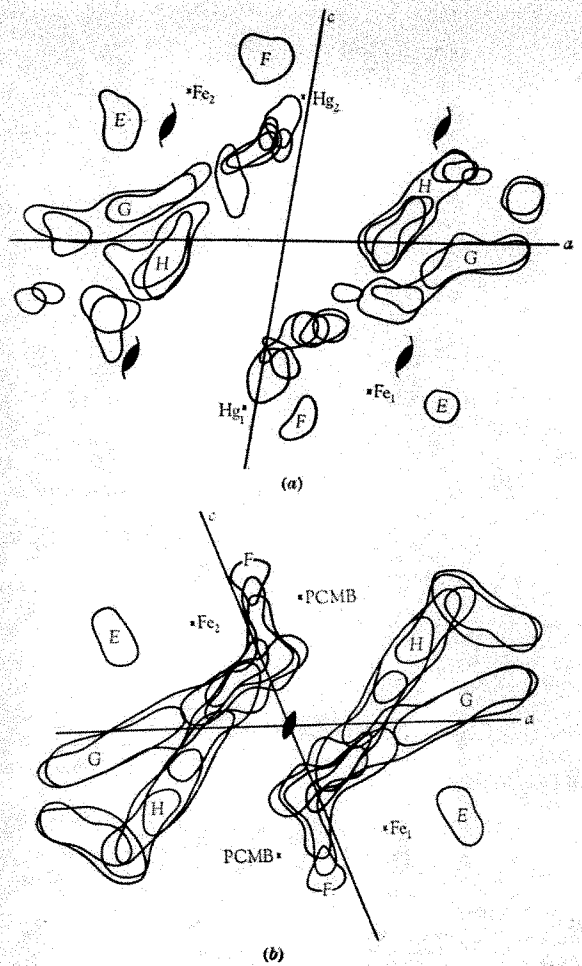


Fig. 9. Same as Fig. 8 for the β-chains. The electron density in the β-chains of the human reduced form is lower, and the number of gaps interrupting the chains is larger than in the α-chains, due probably to oxidation of the hæmoglobin during exposure to X-rays, which would displace the β- but not the α-chains (see Fig. 11)

(c) *Molecular symmetry, shape and packing.* In oxyhæmoglobin, two-fold symmetry of the molecule follows from the crystal symmetry; but this is not true of the reduced form in which the molecules lie in general positions. However, failure of the molecular symmetry to be expressed by space-group symmetry is a situation frequently encountered in crystal structure analysis and does not make molecular symmetry less probable. The difficulty lies in checking molecular symmetry at the present limited resolution and in the face of errors which are liable to affect symmetry-related parts of the molecule unequally. A comparison of distances between symmetry-related iron and mercury atoms, shown in the two columns of figures in Table 3, reveals no significant differences. From the co-ordinates of these atoms, the orientation and position of the probable molecular two-fold axis can be deduced: if this axis is denoted as d, the angles it encloses with the crystal axes are $ad = 79°$, $bd = 11°$, $cd = 96°$ and $c^*d = 94°$. It passes through the point $x = 0{\cdot}23$, $y = 0$, $z = 0{\cdot}77$, where $x = z = 0$ is the locus of the crystallographic screw dyad and $y = 0$ is at the level of one of the mercury atoms in the PCMB compound (Hg_2).

Table 3. Interatomic Distances Related by the Molecular Dyad Axis

Fe_1–Fe_3	25·2	Fe_2–Fe_4	24·7
Fe_1–Fe_4	36·9	Fe_2–Fe_3	37·8
Fe_1–Hg_1	14·4	Fe_2–Hg_2	14·4
Fe_3–Hg_2	20·3	Fe_4–Hg_1	19·6
Hg_1–Hg_2	25·6	Hg_2–Hg_1	24·8
Hg_1–Hg_1	27·8	Hg_2–Hg_2	27·8

Hg_2 and Hg_1 are the mercury atoms of $HgAc_2$ attached to the non-reactive SH groups, probably cysteine 104 of the α-chains (see Fig. 1*a* of ref. 26).

Large differences in the electron-density distribution of symmetry-related parts of the globin chains are noticeable in certain regions, for example, in the non-helical region *CD*, but these are probably due to error, because differences of this kind diminished markedly when going from the first, tentative, Fourier which had been calculated on the basis of only two heavy atom compounds, to the present one.

Like oxyhæmoglobin, the molecule is a spheroid with a height of 50 Å parallel to the two-fold symmetry axis and a width of 55 Å perpendicular to it. Its length is 69 Å, compared with 64 Å in the oxygenated form, and it is cut across by a groove separating the two β-chains (Fig. 7). The packing of the molecules in the crystal is body-centred as predicted by Bragg and Perutz[5]. Their orientation had also been forecast correctly from the early X-ray data[5,7].

Discussion

The first question which comes to mind is whether the structural differences between horse oxy- and human reduced hæmoglobin are really due to the oxygenation reaction, or whether they merely represent a species difference. The amino-acid sequences of the human and equine α-chains differ in only 17 places, and six of the replacements are trivial ones, such as alanine taking the place of glycine[6–11,21]. The sequence of the β-chain of equine hæmoglobin is known only in part; preliminary results indicate that it also is very similar to that of the corresponding chain of the human form[22], which makes large differences in molecular structure unlikely. The massive body of chemical and kinetic evidence in favour of a major structural change accompanying the oxygenation reaction has already been referred to[4]. An interesting piece of indirect evidence is reported in the accompanying

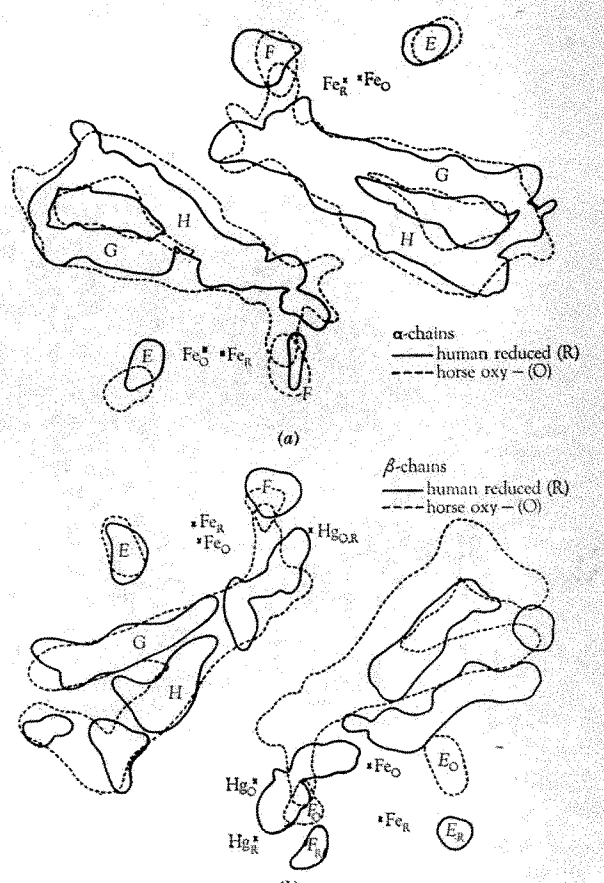


Fig. 10. *a*, Superposition of the outlines of Figs. 8*a* and *b*; *b*, superposition of the outlines of Figs. 9*a* and *b*

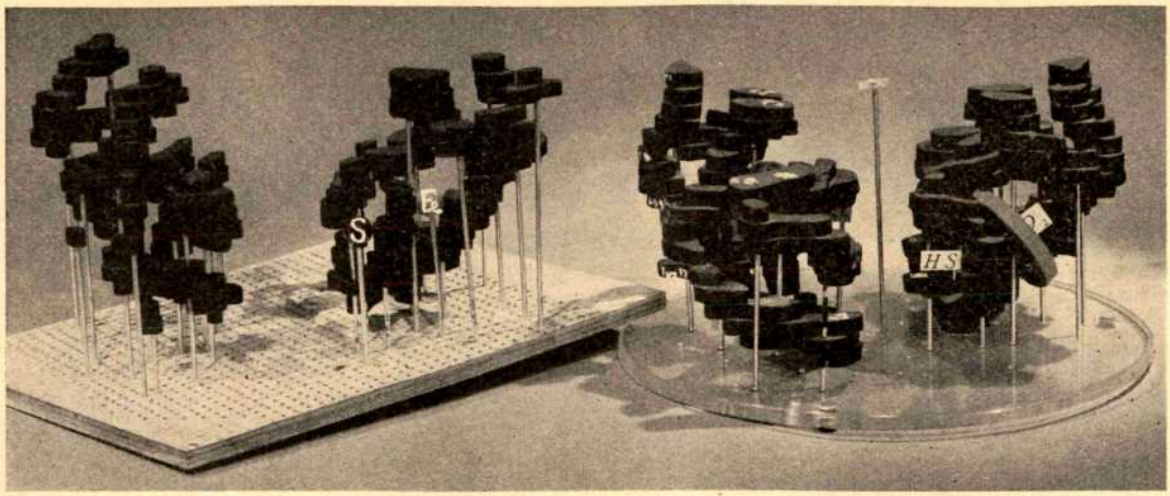


Fig. 11. View of the two pairs of β-chains, showing the widening of the gap between them in the human reduced form (left), as compared with horse oxyhæmoglobin (right). The hæm groups in human reduced hæmoglobin are indicated by balls, those in horse oxyhæmoglobin by grey disks. *S* marks one of the mercury atoms of PCMB

paper on hæmoglobin H (see p. 639), which shows no hæm–hæm interaction and appears to have the same crystal structure in the oxygenated and reduced states[12]. This suggests that the occurrence of a structural change in normal hæmoglobin is linked to hæm–hæm interaction; but it still does not prove, of course, that this change corresponds to the difference between horse oxy- and human reduced hæmoglobin, a point which can be settled only by solving the structure of the oxygenated and reduced forms in the same species.

On the whole, it seems likely that the structural differences are genuinely due to the oxygenation reaction; but it is not at all clear what they mean. The rearrangement which takes place cannot be caused by interaction between the two β-chains by themselves, since in the reduced form these are no longer in contact. The results on hæmoglobin H also preclude that possibility. If the rearrangement is brought about by interaction between the α- and β-chains, as seems likely, it may be triggered off by structural changes within both these chains, and there is as yet nothing to indicate what these are. Nor is it clear how the rearrangement of the β-chains can affect the oxygen affinity of the hæm groups, which lie near the surface, seemingly unaware of what goes on inside the molecule. The Bohr effect may be easier to explain, since the rearrangement of the β-chains would affect the environment and hence the dissociation constants of many polar groups. The increased uptake of bromthymol blue by the reduced as compared with the oxygenated form[23] would be consistent with the greater free surface of the former. A clearer picture of the stereochemical factors involved in the structural rearrangement and of its meaning in terms of reaction kinetics might emerge if the crystal structure could be solved at higher resolution; this would make it possible to find the atomic positions of the iron atoms and of the amino-acid side-chains which surround then, as well as the exact orientation of the hæm groups.

Hæmoglobin may be regarded as an enzyme with oxygen as one of its substrates. The structural change which accompanies the reaction of hæmoglobin with oxygen suggests that there may be other enzymes which undergo a major change of structure on combination with their substrate. In recent years a class of enzymes has been discovered which have been called allosteric[24]. These are concerned with the biosynthesis of essential metabolites, such as amino-acids, and usually the terminal metabolite synthesized in any given pathway inhibits the rate of its own synthesis by means of a specific action on one enzyme, generally the first in the pathway. The kinetics of inhibition suggest that these enzymes possess at least two different active sites, one for combining with the substrate and another for combining with the inhibitor, and that the interaction between two sites resembles that between the oxygen-combining sites in hæmoglobin. There are indications of structural changes in these and other enzyme systems[25].

We thank Mr. D. A. G. Broad and Mr. L. G. Hayward for designing and constructing the nitrogen box in which we mounted the crystals, and Mr. Michael Scott, Miss Sadie Nicholson and Miss Joanna Wheatley for their assistance with computing and model building. One of us (H. M.) thanks the Rockefeller Foundation for supporting her during her first year of research and the Medical Research Council for a scholarship during subsequent years. We also thank the Director and Staff of the Mathematical Laboratory, University of Cambridge, for their help and co-operation in the many long calculations involved in this work.

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A PRELIMINARY X-RAY ANALYSIS OF HAEMOGLOBIN H

By Dr. M. F. PERUTZ, C.B.E., F.R.S., and L. MAZZARELLA

HÆMOGLOBIN H is an abnormal human hæmoglobin which consists of four β-chains[1,2]. Its oxygen equilibrium curve is similar in both shape and position to that of myoglobin, showing that the four hæm groups react with oxygen independently of each other and that their oxygen affinity is higher than normal. Like myoglobin, it exhibits only a negligibly small Bohr effect[3].

Crystals of oxyhæmoglobin H, suspended in 2·3 M phosphate buffer of *p*H 6·7, were given to us by Dr. Helen M. Ranney of the Albert Einstein College of Medicine in New York. They were strongly birefringent monoclinic prisms. Birefringence was negative, with the optic axial plane approximately normal to the prism axis. The crystals were only just large enough to be mounted in glass capillaries for X-ray analysis. Their unit cell dimensions and space group are shown in Table 1. The space group is the same, and the unit cell dimensions are closely similar to those of normal human reduced hæmoglobin, and quite different from those of any of the forms of human oxyhæmoglobin[4]. The habit of the hæmoglobin H crystals is different from those of normal human reduced hæmoglobin, the former being prisms elongated along [001], while the latter are elongated along [100].

Table 1. UNIT CELL DIMENSIONS OF NORMAL HUMAN REDUCED HÆMOGLOBIN AND OXYHÆMOGLOBIN H (β_4)
Space group $P2_1$; $n = 2$

	Reduced human hæmoglobin	Oxyhæmoglobin H
a	63·39	63·29
b	83·63	82·40
c	53·91	54·07
β	99·25°	91°

However, crystallization of oxyhæmoglobin H in a lattice similar to the normal reduced form suggests that the oxygenated and reduced forms of hæmoglobin H both have the same, or very similar, crystal structures, and that no major change in molecular structure accompanies its reaction with oxygen. We have not yet prepared crystals of reduced hæmoglobin H, but have tested the point by performing the following experiment.

Crystals of oxyhæmoglobin H were immersed in a solution containing 2·8 M phosphate buffer of *p*H 6·7 and 0·01 M ferrous citrate until their absorption spectrum had changed to that of reduced hæmoglobin. No other change was observed in the appearance of the crystals. They were then mounted in glass capillaries in an atmosphere of nitrogen, and X-ray diffraction pictures were taken. These showed sharp reflexions extending to a spacing of 3 Å. The unit cell dimensions were the same as those of oxyhæmoglobin H. The distribution of intensities in 9° precession photographs of the *h0l* and *0kl* zones was very similar, though small alterations in the intensities of certain reflexions were noticeable.

If crystals of normal oxyhæmoglobin (human or horse) are reduced, or crystals of normal reduced hæmoglobin are oxygenated, the crystals develop numerous cracks and become opaque. Reflexions of more than 8 Å spacing are much weakened and those of less than 8 Å spacing fade out altogether. In these crystals the reaction with oxygen evidently introduces stresses which break up the regularity of the lattice. There is no sign of any such stresses in crystals of hæmoglobin H, where structural changes, if present, must be very slight.

The absence of structural changes accompanying the reaction with oxygen in hæmoglobin H was predicted from the absence of hæm–hæm interaction, and with the idea in mind that in normal hæmoglobin this interaction is linked to structural changes[3,5]. It is interesting to find this prediction confirmed by X-ray analysis.

However, there are two puzzling points arising from our observations. It is not clear why a hæmoglobin consisting of four β-chains should have the same structure as the normal reduced, since one might expect both pairs of β-chains to be equivalent and each pair to take up the relative position shown in Fig. 11 (left) of the preceding article, with a corresponding increase in width in the region of the molecule normally taken up by the α-chains. The second point concerns the kinetic properties. If the oxygen affinity of hæmoglobin H is as high as that of myoglobin, then its configuration should be that of normal oxyhæmoglobin, which is the fast-reacting, rather than that of reduced, which is the slowly reacting modification. These difficulties will need further study.

We thank Dr. Ranney for giving us the crystals and one of us (L. M.) thanks the Fondazione Alberto Beneduce of Naples for a fellowship.

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THE NATIONAL PHYSICAL LABORATORY

MORE than 150 items from the research programme of the National Physical Laboratory were on display during Open Week, May 13–17, which was attended by several thousand visitors, including many from overseas. Lectures were given by Dr. W. P. Jones (superintendent of the Aerodynamics Division) on "The Work of Aerodynamics Division", and by Mr. A. Silverleaf (superintendent of Ship Division) on "Experiments with Ship Models".

In Standards Division, much interest was shown in an exhibit describing the method used to relate clocks over transatlantic distances by means of an active communication satellite. The inherent symmetry of the arrangement enables the classical method of comparing clocks to be applied, in which signals are transmitted from remotely situated clocks, at the speed of light, and the time differences between the received and local clock signals are observed. If these differences are equal then the clocks are regarded as synchronized; if not, the discrepancy indicates the magnitude and sense of the relative clock adjustment.

An experiment of this form was carried out in August 1962 by the National Physical Laboratory in co-operation with the U.S. Naval Observatory, making use of the *Telstar I* satellite to relate quartz clocks at the satellite ground station at Andover, Maine and Goonhilly Downs, Cornwall. It was determined that the clocks differed in their setting by 72·6 μsec. Due partly to the wide band-width of the radio circuit and its relatively high signal-to-noise ratio, the overall accuracy of the comparison was 1 μsec, an improvement of some three orders over existing methods of synchronization. The path via the satellite is continuously changing and means have to be provided for recording the reception time of the

signals, which may vary at rates of several μsec/sec. This is now done automatically by a servo-controlled 'tracking' system which indicates continuously the arrival time of the signal relative to the local clock, to an accuracy of 0·1 μsec.

The narrow band-width and coherence of the light emitted by a gaseous optical maser make it particularly suitable for use in the measurement of length by interferometry over much greater distances than is possible using the light given out by normal sources. Unfortunately, in spite of the remarkably narrow band-width of its radiation, the wave-length of the laser emission is not by itself sufficiently stable to form a good standard. Variations in the distance between the mirrors of the maser can change the wave-length by about one part in a million. In the laser on display in Standards Division, such variations of wave-length are considerably reduced by a feed-back system which maintains the wave-length near the peak of the spontaneous emission spectral profile. The stability is thus brought to the same level as the usual secondary wave-length standards.

The application of the gaseous optical maser to the measurement of linear movements is simplified by an effect discovered at the Services Electronics Research Laboratory at Baldock. If the light from the laser is reflected back along its path so as to re-enter the laser, then interference occurs with the effect of either strengthening or weakening laser action, depending on the phase relationship. The result is that a mirror moving towards, or away from, the laser can be made to turn the laser on and off at intervals of half a wave-length.

At the National Physical Laboratory, a machine utilizing a helium–neon optical maser is being developed and applied to the measurement of errors in a metrological grating of the type used for automatic machine tool control. The grating is mounted on a moving carriage which also carries part of the optical system in the laser beam. As the carriage moves, the laser gives out pulses of light and signals are obtained from the grating reading head. An automatic electronic system counts the number of laser pulses between each hundredth pulse from the grating and determines on a time scale the position of the grating pulse between two laser pulses. With the optical system used, the laser pulse separation corresponds to about 5 microinches and this is sub-divided electronically into 50 parts. The final accuracy achieved will be limited by mechanical considerations and temperature uncertainties, but is expected to be better than 5 microinches in the measurement of a 36-in. grating.

Research on optical masers in the the Light Division is being concentrated on pulsed systems using ruby or neodymium glass. A programme of optical and spectroscopic assessment of these materials is now well under way, and both high-power and low-power applications are being investigated. Especial attention has been given to the problem of resonator design and the control of optical feed-back. Studies of the light patterns emitted have confirmed that the performance of these devices is very sensitive to minor variations in refractive index or errors in the optical alignment of the resonating crystal. Given sufficient optical homogeneity, recognizable mode patterns are produced, and with neodymium glass there is a good possibility of obtaining a high-power output the directionality of which is limited only by diffraction effects. With ruby operated at room temperature, all the output can be concentrated into a single wave-length, but neodymium sources are less satisfactory in this respect.

A continuous gas laser provided by Services Electronics Research Laboratory is now being used for testing, by multiple beam interferometry, some of the passive resonators which have been constructed, in order to stabilize against frequency drift. Giant single pulses with a peak power of 1 MW have been produced by operating a ruby in conjunction with an external reflector rotating at 6,000 r.p.m. The duration of such a pulse is less than 1 μsec, the beam-width less than 0·2°, and the spectral line-width less than 0·01 Å, so that it is an ideal source for many experiments in high-speed interferometry and non-linear optics. Interferograms have, for example, been obtained showing the instantaneous variations of thickness of a freely falling sheet of water. A more conventional laser flash with an energy of 3 joules and duration of 1 msec has also been used for drilling holes 0·3 mm deep in diamond. The durability of various types of optical element is being investigated with the view of avoiding breakdown under high light intensity.

Also on display in the Light Division was the newly developed method of time ratio photometry. With this technique, the attenuation of the light beam by the sample being measured is matched with the attenuation produced by a continuously variable repetitive shutter, using a detector that gives the same response to a periodically varying flux $\Phi(t)$ as to a steady radiation of the value $\int_0^T \frac{\Phi(t)dt}{T}$, where T is the repetition period (Talbot's law). The novelty and higher accuracy of the National Physical Laboratory's technique derive from the use of a very accurate method of timing the 'open' and 'shut' times O and S of the variable shutter at the desired setting. The transmission or reflexion factor required is then given by $O/(O + S)$. The shutter only needs short-term stability so that the adjusted value is held for a few seconds at a time. Various types of shutter are being examined, and experiments have shown that vacuum photocells and photomultipliers obey Talbot's law to within 1 part in 10^5 for shutter frequencies greater than 30 c/s.

The Optics Section exhibited an improved version of their recording goniometer utilizing radial diffraction gratings and the moiré fringe technique. In the present goniometer, a grating of finer pitch (60 sec of arc) is carried on an adjustable kinematic mounting which enables it to be centred without difficulty to better than 5×10^{-5} in. The spindle carrying gratings and specimen is mounted in a semi-kinematic arrangement of two radial bearings of nylon impregnated with molybdenum disulphide and a 'wobble-pin' thrust bearing—a system which provides negative radial clearance but no detectable slip-stick effects.

Visitors whose field of work involves the measurement of a.c. currents and voltages were interested in a new development in instrumentation by the Applied Physics Division. The electro-thermal converters commonly used as transfer instruments from a.c. to d.c. have a single temperature-detecting element, although in the U.S.S.R. a system having twenty elements in series has been used. At the National Physical Laboratory a multi-junction converter has been developed in which the alternate (hot) junctions are located at intervals of 0·006 in. along a heater about 1 in. in length, giving a total of approximately 160 thermo-elements. The cold junctions are either embedded in a copper block or the whole system is arranged so that they hang freely in a stout copper housing which is evacuated. The principle of the multi-junction is similar to that of the single-junction system; but in the new design a precision of 0·001 per cent is represented by 1 μV when the output from the thermo-electric system is about 100 mV and the heater is only a few tens of degrees centigrade above the surroundings. There are obvious advantages over the single-element converter, which has a maximum available output of the order of 5 mV, and where it is necessary to measure less than 0·5 μV with certainty to obtain a precision of 0·01 per cent.

If the multi-junction thermal converter was perhaps the smallest piece of apparatus on display, the largest new equipment was certainly the mobile audiometric labora-



Fig. 1. Application of an optical maser as a source of light in high-speed interferometry. The interferogram depicts variations in the thickness of a freely falling sheet of water. At the centre of the picture the water impinges on a thin wire obstacle and divides so that none passes into the lower quadrant. The duration of the laser flash was 0·2 μsec and the peak power about 1 MW. The fringes were formed in transmission by a scatter fringe interferometer

tory which will be used by a team from the Acoustics Section of the Applied Physics Division in a series of experiments on occupational hearing loss, which will be conducted jointly with the Medical Research Council in a programme sponsored by the Ministry of Pensions and National Insurance. Although some progress has been made on the problem of impairment of hearing due to exposures to noise of high intensity, there is insufficient evidence to say whether or not industrial deafness should be prescribed as an industral disease in Great Britain. Factors contributing to the complexity of the problem include the variety of noise exposures encountered, variation in susceptibility to noise between individuals, the effects of non-occupational factors such as age and disease on hearing, and the difficulty in agreeing on a criterion of deafness. The widely used criterion of "preservation of the ability to hear speech" is not sufficiently strict and is difficult to interpret. Increasing use is being made of temporary shift in the threshold of hearing as an index of acoustic trauma, and as a means of predicting permanent hearing loss for continued exposure, but this method has not yet been established scientifically.

The report of the Wilson Committee on Noise has made the general public aware of the difficulties of defining human tolerance to noise, as well as the practical problems of preventing or excluding excessive noise from our environment. A related problem, the measurement of the sound absorption coefficients of materials, has been examined for several years in the reverberation chamber of Acoustics Section. A research programme organized by the International Standards Organization with the co-operation of twenty-seven other laboratories has developed methods of minimizing discrepancies between the results of measurements of different testing laboratories by sound diffusion techniques (Fig. 2). This work, and further research on effects of diffraction at the edges of the specimens undergoing tests which was on display, has been embodied in the recently published British Standard method of measurement of sound absorption coefficients in a reverberation room. Now that the initial research has been completed and other laboratories are suitably equipped, routine testing of proprietary materials at the National Physical Laboratory has been discontinued, and future work will be limited to materials of particular scientific interest.

The Autonomics Division demonstrated the translation of four simple Russian sentences on the *ACE* computer, looking up vocabulary and idioms from an abbreviated 2,000-entry dictionary, including information about 150 idioms. The complete research procedure for the translation of a sample phrase was explained diagrammatically on a display board showing the various stages from input card punching, card reading into *ACE*, dictionary look-up, idiom search, and syntactic analysis to output card punching by *ACE* and the print-out of a final legible form.

In the large water tunnel of the Ship Division, fundamental investigations with a cavitating disk were demonstrated. The disk of 5 in. diam. is supported from downstream on the shaft normally used to drive model propellers in the 44 in. diam. working section of the tunnel. Air can be fed to a point just downstream of the disk and, at a sufficiently low tunnel pressure, a remarkably large air-filled bubble is formed behind the disk. This cavity is probably larger than any previously investigated. Preliminary measurements of the pressure inside the cavity suggest that buoyancy forces have an important effect on it at relatively low tunnel speeds, in the region of 20 ft./sec, and thus the cavitation number appears to be exceptionally large. Further work is needed to confirm this observation and to investigate the effect of factors such as the air content of the water. The ultimate aim of the work is to understand better the large cavities which may be encountered with hydrofoils and new types of fully cavitating propellers.

Among the projects immediately related to ship design demonstrated by the Ship Division, were experiments in irregular head waves representative of actual sea conditions, to evaluate the sea-keeping performance of a model. One of the objects of this work is to determine the pitching and heaving behaviour of the model and to measure the propulsion characteristics. From the point of view of design, comparative experiments with different models can be made in the same wave conditions so that critical factors such as vertical acceleration at a particular point,



Fig. 2. An experimental arrangement in the National Physical Laboratory reverberation chamber: sheets of plywood hung in random orientation to promote sound diffusion

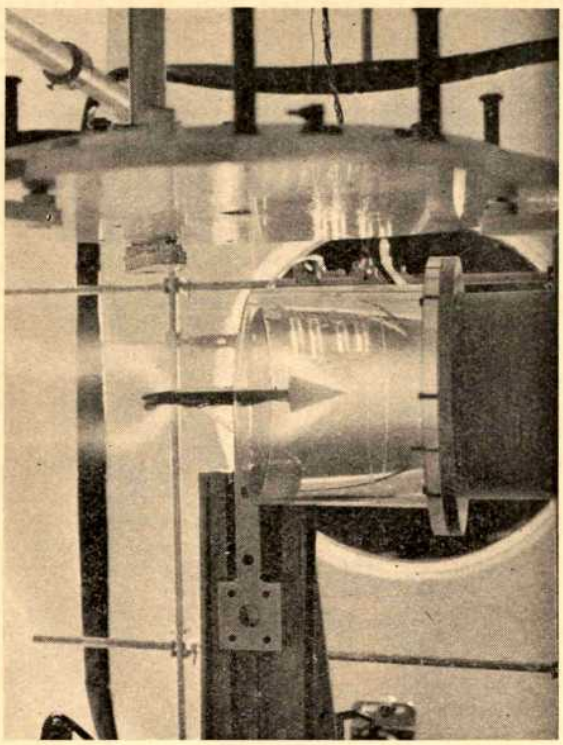


Fig. 3. Flow visualization by means of argon afterglow in the National Physical Laboratory low-density wind-tunnel. The flow is about a cone at twice the speed of sound, and the free stream pressure is 25μ mercury

occurrence of slamming, propeller emergence and shipping of water can be observed or measured. Correlation is being established between full-scale ship motion results and model behaviour in equivalent wave conditions.

The Metal Physics Section of the Metallurgy Division exhibited work which has led to an explanation of the greater increase in hardness during ageing of iron–carbon alloys compared with iron–nitrogen alloys. An important factor in the hardening of metals by precipitates is the degree to which the slip plane in the metal matrix is continued in the precipitate particle. Precipitates which are isomorphous with the matrix are easily sheared by slip dislocations, whereas precipitates with an atomic structure different from the matrix act as obstacles, thus leading to increases in strength and rate of work-hardening. Thin foil electron microscopy has provided a means of estimating the degree of mismatch between the precipitates and surrounding matrix by allowing detection of the magnitude and direction of the strains in the neighbourhood of the precipitates while *in situ* in the metal. The visibility of small precipitates in the electron microscope (in common with other crystal defects), appears to be mainly due to the altered conditions of Bragg diffraction which result from the atomic displacements near the defect. If the diffraction conditions are deliberately changed by altering the angle between the specimen and the incident beam, the image contrast will change and it is possible to analyse the nature of the local atomic displacements around any selected defect. Metallurgy Division have used these techniques to study the strain fields associated with the disk-shaped ε-iron carbide and iron nitride precipitates formed during the ageing of iron–carbon and iron–nitrogen alloys, and their observations show that the slip planes through the nitride precipitates match those through the surrounding iron but that those through the carbide precipitates do not. Nitride precipitates would therefore be expected to be sheared easily by slip dislocations, whereas carbide particles should act as barriers. Thin foil observations of aged and strained iron–nitrogen and iron–carbon alloys containing about the same volume of precipitate have confirmed these ideas.

New facilities for research at high Mach numbers were on display in the Aerodynamics Division. A 15 in. by 10 in. direct discharge wind-tunnel which has just been installed will provide a wide range of flow conditions for Mach numbers of 2–7—an important speed-range for vehicles designed for continuous flight in the atmosphere at very high speeds, and for others during certain phases of their re-entry from space. High stagnation pressures are used to compensate for the reduced scale of the models so that the flow over a 50-ft. long aircraft, for example, can be simulated for 2,000 m.p.h. and the appropriate altitudes between 80,000 and 140,000 ft. A hypersonic wind-tunnel using helium as the working fluid has recently been constructed, for research on flow problems at speeds between 5 and 25 times the speed of sound. The use of helium as the test gas at room temperature, in place of air, allows expansion to very high speeds without liquefaction taking place. Similar conditions would require pre-heating of air as the test gas to about 3,000° K—a formidable engineering problem. This tunnel will reproduce the Mach numbers of sub-orbital flight, but not the high-heating characteristic of hypersonic flight, which is studied separately in high-temperature facilities such as the 'Hotshot', which allows a flow duration of about 30 msec at Mach numbers of 10–20 through its 8 in. diam. working-section. This is the only wind tunnel of its kind in the United Kingdom. An additional electromagnetic shock tube which produces a shock-wave travelling at Mach numbers between 30 and 40 is providing useful experimental information in the field of magneto-gasdynamic.

At 40–50 miles above the Earth's surface, the air may no longer be regarded as a continuous fluid since the mean free path of the molecules is of the same order as some dimensions of the flying object. However, free molecular flow does not occur until the mean free path is of the order of five times the dimensions of the flying object—about 75 miles altitude in the case of a 3-ft. sphere. To study aerodynamics in the lower transitional-flow region, a low-density tunnel is used, having a $7\frac{1}{2}$ in. diameter working-section and operating at a Mach number of 2 and at pressures of 10–100μ of mercury. One of the problems encountered at pressures as low as this is that conventional optical methods, such as shadowgraph, schlieren and interferometry, cannot be used to visualize the flow about the model. Instead, the gas flow is partly ionized by an intense radio-frequency discharge and the recombination process occurring during the flow down the nozzle and over the model is accompanied by a glow discharge, the intensity of the glow being proportional to the local density. Shock waves show up, therefore, as discontinuous increases in brightness. Argon is the most suitable gas to use for this process: an alternative method enriches air with a small quantity of nitric oxide to stimulate bright after-glow. The low-density tunnel is being used to investigate the fundamental nature of flow about simple wings and bodies in the transition region.

The staff of the Mathematics Division were available during the open day to discuss their research with visitors, but no exhibits were arranged because of the inherent difficulty of displaying theoretical work. The Basic Physics Division did not exhibit their work as they were preparing to move into their new building, which has just been completed.

For the staff of the Laboratory the open days provide a welcome opportunity to show the latest developments in their work to other research workers and to technical staff from industry. A warm invitation is extended to all who are interested in attending the open days in 1964 which will be held during May 27–28. Requests for invitations should be addressed to the Director, National Physical Laboratory, Teddington, Middlesex.

NEWS and VIEWS

Botany at New South Wales: Prof. H. N. Barber, F.R.S.

Prof. H. Newton Barber has been appointed to the Foundation chair of botany at the University of New South Wales. Prof. Barber has been professor of botany at the University of Tasmania since 1948. In March of this year, he was elected a Fellow of the Royal Society, "distinguished for his work in experimental cytology, population genetics and the genetics of flowering". He is also a Fellow of the Australian Academy of Science and a Member of the Advisory Council of the Commonwealth Scientific and Industrial Research Organization, being chairman of the Tasmanian State Committee. A graduate of the University of Cambridge, he gained first-class honours in both of the Natural Sciences Tripos in 1935 and 1936, and was awarded a Ph.D. degree by the University of London after spending four years as a research cytologist at the John Innes Horticultural Institute. During the Second World War he was a scientific officer at the Telecommunications Research Establishment, Ministry of Aircraft Production, and in 1946 accepted appointment as lecturer in botany at the University of Sydney. Prof. Barber spent the years 1953 and 1954 at the California Institute of Technology on a Rockefeller special fellowship, and was visiting professor at the Australian National University and at University College, Townsville, during 1961. Prof. Barber will become head of the Department of Botany, which is one of the four Departments which form the School of Biological Sciences.

Plant Physiology and Biochemistry at Newcastle: Prof. S. L. Ranson

The appointment of Dr. S. L. Ranson to a chair in plant physiology and biochemistry, personal to him from July 1, 1963, at Newcastle upon Tyne (University of Durham), will be welcomed by his many friends and colleagues. Dr. Ranson was trained in the Department of Botany in King's College, and commenced research under Prof. (now emeritus professor) Meirion Thomas, on Crassulacean acid metabolism. He was appointed lecturer in 1946, senior lecturer in 1958, and reader in 1960. During the academic session 1949–50 he held an Earl Grey memorial fellowship at Washington University, St. Louis, where he worked with Prof. M. D. Kamen. He has extended the work on Crassulacean acid metabolism initiated by Prof. Thomas by the application of isotopic methods and he has investigated the enzyme systems involved. All his work has been characterized by meticulous attention to detail and a very critical appraisal of the results. Dr. Ranson and his group are now extending their studies on the relation of acid metabolism to the more general problems of carbon dioxide fixation and intermediary metabolism. He has collaborated with Prof. Thomas in recent editions of the latter's well-known textbook and he has also provided an important contribution on techniques for the separation and estimation of plant acids in *Modern Methods of Plant Analysis*. This appointment recognizes not only Dr. Ranson's considerable personal achievement but also the importance and notable part played by plant physiology and biochemistry in the Department of Botany at Newcastle.

Physics at Manchester : Prof. S. F. Edwards

Dr. S. F. Edwards, reader in theoretical physics in the University of Manchester, has been appointed to a newly established additional chair of physics, in the field of theoretical physics, as from October 1. Dr. Edwards received his school education at Swansea Grammar School. In 1945 he entered Gonville and Caius College, Cambridge, to read for the Mathematical Tripos. During 1949–51 he was a research student at Cambridge and in 1951 was awarded the J. H. Cheate Memorial fellowship at the University of Harvard, where he worked under Prof. J. Schwinger. He then spent a year at the Institute of Advanced Study, Princeton, returning to Britain in 1953. In that year he was awarded the degree of Ph.D. (Cambridge) and was appointed Imperial Chemical Industries Research Fellow in the University of Birmingham. Two years later, he was appointed senior lecturer in theoretical physics, and in October 1961 promoted to a readership in theoretical physics in the University of Manchester.

Royal Society Research Professorships

The following have been appointed to Royal Society research professorships: Dr. R. A. Hinde, of the Sub-Department of Animal Behaviour, Department of Zoology, Cambridge, who is well known for his work in the field of the experimental study of animal behaviour, has been appointed as Royal Society Research Professor at the University of Cambridge as from October 1; Prof. F. Sondheimer, head of the Chemistry Department at the Weizmann Institute, Rehovoth, Israel, who is distinguished for his work in the field of organic chemistry, Royal Society Research Professor at the University of Cambridge in the University Chemical Laboratory—he is expected to take up his appointment during 1964.

British Aid to Developing Countries

The first of a series of pamphlets giving a factual survey of British aid to developing countries, issued by the Overseas Development Institute, deals with educational assistance (*British Aid*—3: *Educational Assistance—a Factual Survey of the Government and Private Contribution to Overseas Development through Education and Training*. Pp. 125. London: Overseas Development Institute, 1963. 7*s*. 6*d*.). Following a short historical survey, it sets forth first the part played by the British Government, describing both the organization of the effort and the scale and character of the official programme. Next the private contribution is described, including that of the missionary societies and churches, the various volunteer schemes, and the part played by the foundations and trusts and the universities. Finally, information on overseas students in Britain is summarized, including their distribution in various types of institutes, the Government contribution, the various technical assistance schemes, etc., and the role of the British Council. The pamphlet provides a most useful and comprehensive picture of an effort that already amounts to a very considerable total and one to which full justice is seldom done, though the account is neither designed nor intended to induce complacency. It points out specifically that Britain's present programmes, valuable as they are, represent only a small part of what needs to be done. The study, and those to follow, have been requested by the Foreign Office. The pamphlet questions whether the present system, in which eight different Government departments control funds for educational assistance, allows priorities to be determined effectively. It suggests that British teachers going abroad may not be staying long enough in their posts and that the possibility of a career service for British teachers overseas should be reconsidered. It considers that there are some gaps in the programme of financial assistance and that greater efforts should be made in teacher and in technical training, the provision of cheap books and

research into the educational problems of developing countries.

Overseas Technical Co-operation

In written answers in the House of Commons on July 16, the Secretary for Technical Co-operation, Mr. R. Carr, stated that of the £16 million provided for Commonwealth education during the five years ending in 1965, about £2 million was being made available for the Commonwealth Scholarship scheme, and about £1·7 million for teacher-training bursaries. Actual expenditure up to March 31 was £867,000 on scholarships and £814,000 for bursaries. On the advice of the Committee for University Secondment, he was supplementing existing arrangements by offering a limited number of special awards of £1,500 a year to outstanding men and women in British universities who were willing to serve for at least two years in selected posts in developing countries of the Commonwealth. These would be additional to the total emoluments ordinarily paid to British university staff in the countries concerned, and the first award would be made in 1964. Overseas universities were being consulted about the posts that they would like to be filled in this way, and he was confident that the British universities and academic institutions which he was consulting would co-operate fully in the selection and secondment of people of high distinction. Present commitments under Section 1 (3) of the Commonwealth Teachers Act, 1960, were expected to result in an expenditure of at least £5 million, and it was desirable at this stage to maintain the uncommitted balance of £6 million made available for the quinquennium to meet new requirements which might arise in the next two years. In a written answer in the House of Commons on July 23, the Secretary for Technical Co-operation, stating that he was himself satisfied that Government funds allocated to Voluntary Service Overseas were being effectively used, announced that a target of 500 graduate volunteers had been fixed for 1964 and that the Government had again agreed to meet half the British costs. In addition, Voluntary Service Overseas would send about 300 school-leavers, and the Government would contribute half the cost of this programme instead of a third as this year. The number of volunteers going overseas would thus have risen from about 20 in 1958 to 800, and the number of graduates from 36 to 500 over three years.

Scientists and Civil Research in Britain

In written answers in the House of Commons on July 16, the Parliamentary Secretary for Science stated that 236 British scientists were interviewed by the Joint Board in North America this year, of whom 171 were in the United States, and so far 68 candidates, of whom 51 are working in the United States, had been offered appointments in the United Kingdom. Estimated Government expenditure on civil research in 1963–64 was £171·447 million. This included £2·841 million for the Ministry of Agriculture, Fisheries and Food and £1·957 million for the Department of Agriculture and Fisheries for Scotland, £7·387 for the Agricultural Research Council, £701,000 for the Nature Conservancy and £420,000 for Forestry Commission; £24·8 million for the Department of Scientific and Industrial Research (including capital and current expenditure on buildings by the Ministry of Public Building and Works), £621,000 for the Ministry of Power and £445,000 for the Ministry of Transport; £7·606 million for medical and health research, including £6·908 million for the Medical Research Council; £2·309 million for overseas research; £48 million for the Atomic Energy Authority and £7·87 million for the National Institute for Research in Nuclear Science; £26·2 million for universities and £227,000 for learned societies; £37·778 million for Ministry of Aviation, £631,000 to Air Ministry for meteorology, £606,000 to the Admiralty for observatories, oceanography, etc., and £362,000 to Home Office and Scottish Home Department.

Forestry in Britain

In a statement in the House of Commons on July 24, the Minister of Agriculture, Fisheries and Food, Mr. C. Soames, announced that over the decade 1964–73 the Forestry Commission would aim at planting a further 450,000 acres. It would continue to concentrate on acquiring land in the upland areas, particularly in England and Wales, where the population is declining and expansion of forestry can bring considerable social and employment benefits. The Commission would be able to acquire land in other areas where there are good economic reasons, or where planting can maintain or improve the beauty of the landscape. The planting programme for each year, and its distribution between the three countries, would be determined from time to time by the Forestry Minister. In preparing its future programmes, the Commission would bear in mind the need, where possible, to provide public access and recreation, and would direct more attention to increasing the beauty of the landscape. No change was proposed in the structure of the present grants to private woodland owners. The Government welcomed the increasing acreage of timber planted by private owners, and the tradition of skill and knowledge and the research which had been carried out in private forests had been of great benefit to the nation. This steady expansion in both public and private forestry meant that a growing volume of home-produced timber will be coming on to the market which would benefit Britain's balance of payments already of about £14 million a year. Clearly, it would become more necessary to direct greater attention to the whole problem of the marketing and use of home-grown timber. In answer to questions, Mr. Soames said that with planting by private owners, some 80,000 acres a year would be planted for the next five years and the Commission would, in particular, consult the National Parks Commission in respect of amenity and the beauty of the landscape, employing a landscape architect to assist. Mr. Soames also agreed that the closest co-operation between farming and forestry interests was essential.

Department of Scientific and Industrial Research and NATO Studentships

In a written answer in the House of Commons on July 19, the Parliamentary Secretary for Science, Mr. D. Freeth, gave the number of students holding Department of Scientific and Industrial Research studentships (including NATO studentships) at November 1, 1962, as 3,473, of whom 3,412 held their studentships at universities and colleges in Great Britain and 2 in Northern Ireland. They represented 38·5 per cent of full-time postgraduates in their fields in these institutions. Of 991 such students who entered employment on the termination of their awards in the year ended September 30, 1962, 357 joined the staffs of home universities (201 in teaching and 153 in research posts) and 18 the staffs of colleges of advanced technology. In addition to these 991 students, 141 were continuing their postgraduate training and 48 had taken research fellowships from the Department or NATO. A further 165 students joined the staffs of overseas universities, 97 for teaching posts and 68 for research.

A Blood Typing Service for Cattle

Following discussions with organizations interested in cattle breeding, the Agricultural Research Council is to set up a blood typing service for cattle at its Animal Breeding Research Organization in Edinburgh. The objectives of such a service, which will be available to cattle breeders on a fee basis, are, from the point of view of the farmer, to obtain a description of the blood characteristics of a cattle beast which can be used to determine its relationship to another animal for which similar

information is available, for example, whether it is the progeny of a particular bull. From the point of view of the Council the objective is to obtain data which will be of value in the study of the genetics of cattle. The preliminary organization, equipping of laboratories, and particularly the training of staff, will take some time, and it will not be possible for samples to be received for test for at least a year. The establishment of the service is being facilitated by the action of certain bodies which have guaranteed their support, and priority will be given in the first instance to samples sent in under the auspices of these bodies. When the service is in a position to accept samples, a further announcement giving details of the service will be made.

Political and Economic Planning

THE annual report of Political and Economic Planning for the year ended March 31, 1963, reports briefly on the six projects in the current research programme started in the two previous years and due for completion by 1964 (Pp. 20. London: Political and Economic Planning, 1963). That dealing with community mental health services has concentrated on comparative field studies in four areas where such services are well developed, and in each area a statistical survey, an interview survey of psychiatric patients living at home and of members of their families has been in progress, as well as a survey of mental health work. In that concerned with prisoners and their families, the field-work on the national sample has been completed and a more intensive study commenced of the families of up to 100 prisoners living in the London area. The enquiry into education and training programmes for developing areas is fully under way, and that on Trade Unions in a changing society is due for completion in autumn 1963. The two-year project on attitudes in British industry was started in April 1962, and the main publication during the year of the programme on Britain and European integration was *Atlantic Tariffs and Trade*.

Directory of European Dental Research Workers

THE Commission on Dental Research of the Fédération Dentaire Internationale is in the course of compiling a Directory of European Dental Research Workers. The Commission will be corresponding direct with all dental schools in the United Kingdom requesting information on research personnel. In addition, however, it is desired to include particulars of research workers who are not necessarily dentally qualified and not necessarily working in dental institutions, but who are engaged on studies directly pertaining to dental science. Information concerning institutions where such workers are based should be sent to the Commission on Dental Research, Fédération Dentaire Internationale, 35 Devonshire Place, London, W.1.

Literature in Nuclear Sciences

Publications in the Nuclear Sciences, 1963, is a complete catalogue of 100 pages of the publications of the International Atomic Energy Agency, and is the fifth edition in the series of catalogues, superseding all previous issues and supplements. The titles are listed in inverse order of publication, that is, with the most recent first, under nine general subject headings: biology, medicine and agriculture; health, safety and waste disposal; physics, plasma physics and electronics; chemistry, geology and raw materials; reactor physics and reactors; industrial applications; economics; law; and miscellaneous and documentation. The publications mostly fall into the following groups or series: "The Proceedings Series", being the proceedings of conferences, symposia and seminars under the ægis of the Agency, which are published in their original language together with abstracts in English, French, Russian and Spanish; "The Safety Series", reports on health and safety topics, available in separate English, French, Russian and Spanish versions; "Technical Directories", in English only; "The Technical Report Series", reports on research, mostly in English; "The Bibliographical Series", surveys of world's literature on subjects in the field of nuclear science; and "The Review Series", a completed series of 27 reviews in various languages. Subsequent reviews are now published in the new journal, *Atomic Energy Review*, which appears at irregular intervals. *Nuclear Fusion*, a quarterly journal on plasma physics and thermonuclear fusion, is the other four-language journal, distributed on a subscription basis from Vienna and published by the Agency.

Announcements

ENTRIES for the 1964 Maccabæan Prize and Medal are now invited for an essay of 5,000–7,000 words on some aspect of the history of medicine or pharmacy. Intending candidates, who must be less than thirty years of age on March 15, 1964, can obtain further particulars from the honorary secretary of the Faculty, Dr. F. N. L. Poynter, Wellcome Historical Medical Library, the Wellcome Building, Euston Road, London, N.W.1.

OCEANOGRAPHERS and meteorologists will be interested to know that the well-known 1905 paper by V. W. Ekman, "On the Influence of the Earth's Rotation on Ocean-currents", has been reprinted. Copies are obtainable from the Royal Swedish Academy of Sciences, Stockholm 50, price 3.50 dollars.

THE fourteenth international Astronautical Congress, arranged this year by the French Astronautical Society, will be held in Paris during September 25–October 1. Further information can be obtained from the Société Française d'Astronautique, 6 rue Galilée, Paris 16e.

A ONE-DAY meeting will be held by the British Biophysical Society at the School of Pharmacy, University of London, on September 30. Further information can be obtained from Prof. R. E. Burge, Physics Department, Queen Elizabeth College, Campden Hill Road, London, W.8.

A SYMPOSIUM on "Light Fading and Colour Assessment" will be held by the Society of Dyers and Colourists at St. Anne's on the Sea, Lancashire, during September 11–13. Further information can be obtained from the Society of Dyers and Colourists, P.O. Box No. 244, Dean House, 19 Piccadilly, Bradford 1, Yorks.

A SYMPOSIUM on "Transport and Traffic Noise", organized by the Society of Acoustic Technology and the Institute of Sound and Vibration Research, will be held at the University of Southampton during September 27–28. Further information can be obtained from Dr. G. M. L. Gladwell, Institute of Sound and Vibration Research, the University, Southampton.

THE Fifth International Seaweed Symposium and associated meeting of the International Phycological Society will be held in Halifax, Nova Scotia, during August 25–28, 1965, at Dalhousie University. This meeting has been postponed from 1964 to 1965 due to conflict with other international conferences. Further information can be obtained from the chairman of the local committee, Dr. E. Gordon Young, National Research Council Laboratory, Halifax, Canada.

AN international symposium on the "Thermal Stability of Polymers", sponsored by the Battelle Memorial Institute, Columbus, Ohio, will be held during December 5–6. It will include papers on both pyrolytic and thermal oxidative stabilities of polymeric structures. In addition, papers will be presented on methods for studying thermal stabilities and on stabilizing various types of polymers. Further information can be obtained from Dr. Palmer B. Stickney, Battelle Memorial Institute, 505 King Avenue, Columbus 1, Ohio.

CONDUCTION PROCESSES IN DIELECTRIC LIQUIDS

A CONFERENCE on "Electronic Processes in Dielectric Liquids" was held under the auspices of the Electronics Group of the Institute of Physics and the Physical Society, at Durham, in April 1963. The purpose of the meeting was to discuss electrical conduction and allied phenomena in pure dielectric liquids. The conference lasted three days, and included 43 papers, many of them from abroad.

In the past, a great deal of work has been reported in the literature on conduction and breakdown in liquid dielectrics; a feature of much of this work is that for interpretation it has leaned heavily on the classical studies of Townsend on conduction and breakdown in gases (and, to a lesser extent, on conduction processes in solids) rather than on the study of liquids, *per se*. Perhaps because of this conceptual bias, there has been a tendency to think of conduction in liquid in terms of electronic processes, and at high fields, to talk of ionizing collision, and electron avalanches. Indeed, the title of this conference may be taken as evidence of a preconceived notion that free electrons ought to exist in insulating liquids, whereas the experimental evidence presented at the meeting proved, if anything, rather the contrary.

One important function of the conference was to bring together those concerned with the theory of liquids and with transport properties of liquefied gases, and those who have largely specialized in conduction and breakdown processes in dielectric liquids. The value of the conference from this point of view was enhanced by the invited lecture, given by Stuart A. Rice (University of Chicago), who presented a stimulating survey of the present state of the theory of liquids.

As already implied, one of the most important problems in the examination of conduction processes in liquids is the identification of the charge carrier in the conduction process; a number of papers presented at the conference were concerned with this problem. The methods used depend on the measurement of the mobility of the charge carrier, and all these techniques rely on the production of carriers in the liquid by means of external radiation, by α-particles or X-rays, by the photo-generation of carriers, or by electron injection. (It remains to be seen whether these induced carriers are of the same type as those present under conditions of natural conduction.)

For a number of years now, the workers at the University of Gdansk have been measuring charge carrier mobilities in liquids, using X-rays to create both positive and negative species. These experiments have covered a wide range of liquids, but particular attention has been directed to the hydrocarbons. A large number of measurements of this type were reported by Adamczewski on behalf of the Gdansk group, and in the first paper of this series, mobility measurements in saturated hydrocarbons were described. Positive and negative carrier mobilities have been measured in a number of liquids, including *cyclo*hexane, and the observations extended over a range of temperatures. The mobilities are of the order of magnitude predicted by Stokes's law for molecular ions; moreover, the temperature variation of mobility of the negative carrier shows it to be inversely proportional to viscosity, in agreement with Walden's rule. Jachym, working in the same research group, has made carrier mobility measurements in high viscosity hydrocarbon and silicone liquids, again using the X-ray injection technique. Stokes's law was obeyed by the negative carrier; but the positive carrier mobility varied rather more strongly with temperature, suggesting that the positive species are somewhat unstable.

Two other types of measurement were reported from Gdansk on the X-ray induced charge carriers: Gazda and Adamczewski have measured ion recombination coefficients, and their dependence on liquid viscosity and temperature; for the normal alkanes the recombination coefficients are found to be related to the number of carbon atoms in the molecule, and the results have been interpreted on the basis of Frenkel's hole theory of liquids. Gazda has also measured ion diffusion coefficients in *n*-hexane, using an ionization chamber the geometry of which was chosen to give one-dimensional diffusion in the direction perpendicular to the electrodes; these measurements confirm the validity of the Einstein relationship, for charge carriers in hexane.

A number of workers have used photo-injection techniques to create negative charge carriers in liquids. It has previously been shown that the optical threshold for this process is about the same as for the photo-emission of electrons from metals into vacuum—though more recent observations suggest a major difference between these processes. Inuishi and Chong have used the photo-injection technique up to quite high fields, and they described their measurements of charge carrier mobility in pure hexane and in hexane doped with alcohol. There is a marked decrease of negative carrier mobility with doping, and on this basis it has been suggested that the charge carrier is a polaron; that is, an electron trapped in the polarization field of its surroundings. Ions produced by the injection of photo-electrons from a cathode into the liquid were also investigated by Terlecki. The measurements were made in hexane, octane and decane, up to quite high fields; no change of carrier mobility with field was observed, in agreement with Inuishi and Chong. There is evidence that the carrier mobility increases more rapidly with temperature than predicted by Stoke's law—suggesting that in this case the negative species are somewhat unstable. Watson and Clancy described an electron injection technique for investigating conduction processes in low vapour pressure liquids, including a range of siloxane fluids. Measurements indicate that virtually all the injected electrons are trapped near the point of injection in the liquid, to form stable carriers of low mobility. Currents are space charge limited, and from these measurements values of charge carrier mobility can be inferred.

From these various methods of studying charge carrier mobilities in liquids, a more or less consistent picture emerges; the majority of measurements indicate a negative charge carrier which is presumably a negative molecular ion, though it is possible that in highly polar liquids the polaron model is applicable. In the case of the positive ions, the picture is perhaps not quite so clear; the fact that the mobility of the positive carrier varies more rapidly with temperature than the negative species suggests that the positive ion is not necessarily a stable entity. However, both carriers appear to vary somewhat in behaviour, depending on the experimental conditions.

In view of the increasing body of experimental evidence on the trapping of charges in liquids, there is a need for further theoretical investigations of the nature of the trapping sites; many of the materials chosen for investigation show no tendency to attach electrons and form stable negative ions in the vapour phase, yet they show every indication of trapping electrons in the liquid phase. An adequate understanding of this process is needed. McCubbin reported on some theoretical work on the nature of carrier traps in paraffins in which he suggested that the

density and energy distribution of traps are controlled by the degree of (or lack of) long-range order in the material.

Four papers were presented on electronic and ionic processes in liquefied rare gases. Charge carriers of either sign can be produced by using α-particles to ionize the liquid. With this technique, previous workers have measured very high mobilities (10 cm^2/V sec and greater) for the negative charge carrier in liquid argon, and it has generally been accepted that these carriers are free electrons. New measurements from Chicago, which were presented by Rice, give quite low negative charge carrier mobilities in liquid argon (of the order of 10^{-3} cm^2/V sec), suggesting that the negative carrier is an impurity ion, despite the fact that the liquid used is of high purity. Results presented on behalf of Swan, on the other hand, confirm the high carrier mobilities reported by Williams and others, and suggest that in pure liquid argon the charge carrier is a free electron, but that with oxygen present, negative ions are formed. The attachment coefficient reported for oxygen in liquid argon appears to be of similar magnitude to that observed in oxygen gas, for low-energy electrons. The discrepancy between these two reports suggests that both types of negative carriers are normally present in liquid argon, but that individual experiments may not be capable of resolving both.

The work at the University of Rome on charge carriers in normal and super-fluid helium was described by Careri. The positive charge carrier in normal helium is believed to be He_2^+; the negative carrier may be an electron with a high effective mass caused by electrostriction of its surroundings. In super-fluid helium the nature of the charge carriers is still the subject of controversy. Recent measurements, described by Careri, have revealed that the mobility decreases in a stepwise manner at integral multiples of a certain critical field, and in the case of the positive carrier this has been followed up to six steps in mobility.

Photoconductivity measurements of various organic solutions were described by Ruppel. Solutions of aromatic amines in hexane have a low conductivity in the dark, but ultra-violet irradiation produces short-lived positive ions which increase the conductivity of the solution. Radical ions have been detected by conduction measurements when the solution is excited by a flash of light. Morant described studies of ultra-violet photoconduction in hexane, and reported that illumination of the anode or of the bulk liquid can give currents of almost the same magnitude as illumination of the cathode, implying that the mechanism of charge generation differs from what has been supposed in the past; however, the current is still found to depend on the electrode work function, indicating that it is not simply photo dissociation of the hexane which is involved. Measurements by Terlecki and Gzowski on the photo injection of charge carriers into hexane and decane were described. The currents were proportional to light intensity, and although the measurements were extended up to fields of 200 kV/cm, the current showed no tendency to saturate. In gases the saturation current is a well-established phenomenon; the fact that this is not observed in liquids may be due to space charge effects.

Four papers were presented on the use of liquid-filled ionization chambers. Two of these were by the Polish group, and two were by Blanc *et al.*, all of whom have used ionization chambers filled with dielectric liquids for the detection of nuclear radiation. The best results for fast neutrons, in terms of sensitivity, were obtained using *n*-hexane. Because of the low carrier mobility, however, the response is slow and for the detection of individual particles the best results were obtained with liquid argon.

Nowak has used an ionization chamber to investigate conduction processes in liquid benzene, and to determine the mobility of the charge carriers at different temperatures; preliminary measurements were also made of the recombination coefficients of the carriers. Benzene seems to possess a relatively high natural conductivity, and it has been suggested that this is associated with the π electrons of the molecule. One might therefore expect to find some interesting features in its electrical behaviour. Forster has made d.c. conductivity measurements on the series of compounds, benzene, *cyclo*hexadiene, *cyclo*hexene and *cyclo*hexane, and has noted a systematic decrease of conductivity along the series; a probe technique was used to determine the potential distribution in the conduction cell, and by this means it was possible to avoid errors which usually enter into d.c. conduction measurements as a result of electrode polarization phenomena. On the basis of their measurements, both Nowak and Forster suggested that the conduction mechanism in benzene involves an electron transfer process between molecules.

Conduction in purified polar liquids has been investigated by Felici and by Briere; in the past, highly polar liquids have been rather neglected in both research and engineering, and to a large extent this lack of interest may be ascribed to the sensitivity of these liquids to electrolytic impurities. The use of ion exchange materials and efficient dehydration techniques now makes it possible to purify these liquids to a considerable extent, and measurements were described on ethyl alcohol, for example, for which resistivities greater than 10^9 ohm cm have now been obtained; this probably represents the limit set by dissociation of the liquid itself. Coelho and Belloc described a novel type of technique to study impurity ions in liquids. They used two sets of coaxial electrodes, mounted in a flow system; the liquid (hexane) was subjected to a d.c. field between the upper electrodes and then passed on to the lower electrodes where an electrometer was used to analyse the residual charge in the liquid. Measurements of the net charge have shown that even at very low fields (100 V/cm) some impurity ions are removed. The passage of current through polar liquids is accompanied by an increase in apparent viscosity; Hart has shown that this visco-electric effect is not due to polarization boundary layers at the electrodes, but is probably due to momentum transfer from charge carriers; calculations indicate that the charge carriers are large, and may in fact be sub-microscopic dust particles.

The final day of the conference was devoted to high-field conduction, breakdown and applied phenomena in insulating liquids, and opened with a review paper by Macfadyen. Zaky, Tropper and House reported on their d.c. conduction measurements in hydrocarbon liquids, with fields up to 10^6 V/cm, and using various cathode surfaces. In order to obtain consistent measurements, it was necessary to condition the system by allowing current to flow for a considerable time—before conditioning, severe current fluctuations occurred. This erratic nature of d.c. conduction currents was also stressed by Morant and Kahan; they found that in highly purified hexane, at fields greater than 200 kV/cm, two distinct sets of currents vs. voltage characteristics could be obtained, and transitions between these values appeared to occur spontaneously. At the moment, no satisfactory explanation for this exists; but an adequate understanding of the part played by space charges may give the required answer.

There are a number of techniques for examining pre-breakdown phenomena in dielectric liquids: Murooka, Nagao and Toriyama have made high-speed camera studies using a bubble chamber as the test cell. By this means, pre-breakdown ionization and vaporization processes in the highly stressed liquids can be studied; interesting effects involving growth and movement of bubbles were shown. Toriyama, Sato and Mitsui presented a paper on the mechanism of breakdown of dielectrics, using Lichtenberg figures to investigate the distribution of ionization in the gap. It was concluded that discharges in the gas phase were often present, but that these may not be essential to breakdown.

One of the most interesting pre-breakdown phenomena in liquids is that of light emission at high fields. Gosling has examined the electro-luminescence of carefully purified fractions of mineral oils at high fields, and finds that both light output and conduction current vary in a manner similar to that found in solid phosphors. The position and brightness of the light emitted depends on the gas content of the oil, its chemical constitution, and the degree of electrode oxidation. Darveniza was among the first to observe light emission from liquids at high fields; recent work of his was reported, in which he investigated the phenomena in both liquids and in solid dielectrics doped with fluorescent solutes (these include anthracene and *p*-terphenyl).

The electric strength of liquids, measured with short rectangular pulses, can be changed appreciably by the application of a static stress to the system before the pulses are applied. Ward described measurements of this type in hexane; it was suggested that a static stress sets up charge layers on the electrodes and that these layers modify the emission from the cathode and hence change the pulse strength of the liquid. Gallagher has made breakdown measurements in liquid argon using direct and impulse voltages, applied to uniform and non-uniform gaps. The effect of gaseous impurities was investigated and small quantities of dissolved oxygen were found to increase the strength. It was noted that the breakdown strength depends also on the condition of both cathode and anode.

A thermal mechanism of liquid breakdown was described by Watson and Sharbaugh, based on their conduction current measurements under microsecond pulse conditions. It was suggested that local currents are large enough to be space charge limited, and the energy input needed to cause breakdown is that required to vaporize the liquid. Krasucki has shown that in viscous liquids the first stage of breakdown involves the formation of a region of low density, which has a lower breakdown strength than the surrounding medium. He suggested that this also occurs in non-viscous liquids, and a model was set up for the formation of the bubble involving the hydrodynamic forces of liquid motion adjacent to the cathode. It is likely that under many conditions the breakdown process involves a thermal process, and the formation of a vapour region; it is therefore important to know the breakdown strength of liquid and vapour at high temperatures and pressures. Sharbaugh and Watson described measurements of the electric strength of hexane through the vapour to liquid. No discontinuity in strength was found in going through from vapour to liquid, in the critical region.

A number of conclusions regarding conduction processes in liquids can be drawn from the work presented at the conference. From measurements of charge carrier mobility, it is clear that, in insulating liquids in general, the conduction process is an ionic rather than an electronic process, and that except in the liquefied rare gases free electrons do not normally exist in insulating liquids. An electron would be expected to have a mobility of 1 cm^2/V sec or greater; for lower mobilities one can no longer talk of free electrons—though hybrid species may exist, such as thermally excited electrons, moving from trap to trap (the so-called 'hopping' electron). The low order of magnitude of carrier mobilities measured in organic liquids implies either an ionic carrier, or a hopping electron; moreover, the variation of the mobility with viscosity seems to follow a Stoke's law type of relationship (though the agreement is not perfect) so that an ionic species seems to be the most likely form of charge carrier. At very high fields the situation is less clear, and the fact that light emission has been observed from insulating liquids containing fluorescent additives, at fields approaching 10^6 V/cm, has been taken as evidence for the presence of energetic electrons in the liquid; other mechanisms of excitation are possible, however, so that even this evidence is not unequivocally in favour of a free electron.

There are a number of speculations as to the nature of the trapping site for electrons in liquids; hydrocarbons such as hexane, for example, do not trap electrons in the vapour phase, yet they seem to contain highly efficient electron traps in the liquid phase. It is possible that the traps are associated with the local degree of disorder of the liquid; the resulting traps must be deep ones, however, for a shallow square well (of the type required by the hopping electron model) does not predict the correct field dependence of mobility. The polaron model may be appropriate, particularly for highly polar materials. The mechanism whereby charge carriers are generated is beginning to look more complicated than had been suspected; until recently one had tended to assume that the charge transfer process, taking place at the interface between electrode and liquid, was similar to that between metal and vacuum, but recent results, implying photo-generation of carriers at both anode and cathode, must make us revise many of these views.

Turning finally to high-field conduction and electrical breakdown in liquids, the question of the relative importance of electronic processes in this region still remains; there is virtually no evidence for collision ionization in recently published work, though it was almost taken for granted a few years ago. Indeed, it is becoming increasingly evident that under the experimental conditions in use at present—even in the use of microsecond pulse techniques—electrical breakdown of liquids is probably not an electronic process in the generally accepted sense, but can often be explained in terms of thermal effects.

It has been impossible, in this report, to mention all 43 papers which were presented at this conference: for a complete list of these papers and authors, the reader is referred to a companion review by M. J. Morant, which is to appear in the *British Journal of Applied Physics*.

P. K. Watson

INSTRUMENT DESIGN: MANUFACTURE AND USE

THE new headquarters of S.I.M.A., the Scientific Instrument Manufacturers' Association of Great Britain Ltd., at 20 Peel Street, London, W.8, were officially opened on January 14. The president, Mr. R. E. Burnett (Marconi Instruments Ltd.), in welcoming the guests referred to 1962 as a particularly busy year for the Association in which a number of significant developments in external relations occurred. The association was invited to co-operate with the National Economic Development Office in its initial examinations of certain sectors of industry, and a survey was made by the Association, based on available published statistics and certain forecasts provided by the industry, of the growth potential of the instrument industry. The exercise disclosed the unsatisfactory nature of the official information, particularly in regard to the classification of products and to industrial employment.

Mr. Burnett emphasized the importance of closer collaboration with instrument manufacturers overseas and reported that during 1962 S.I.M.A. became associated with the Comité Européen de l'Optique et de la Précision de Mécanique. The Comité is an informal organization of parallel trade associations of the Common Market countries and is concerned with the exchange of information,

particularly statistical information, and with the discussion of trade problems of mutual interest. Contact was also made with the Instrument Society of America and the Scientific Apparatus Manufacturers' Association of the United States of America.

In co-operation with the Board of Trade and the Central Office of Information, groups of members of S.I.M.A. took part in six exhibitions in Budapest, Sydney, Helsinki, Vienna, New York and Milan. Individual reports on these exhibitions are given in the January issue of *SIMA Review* (3, No. 1; 1963). A special effort was made during 1962 to expand the sales of instruments in Italy and to stimulate the interest of members of Italian universities, technical colleges and research establishments in British instruments.

Output and exports of instruments were again above the record level of 1961. The latest figures, up to November 1962, showed that exports were running at the rate of about 13 per cent greater than those for the similar period during 1961 and that they were expected to reach about 54 million pounds for the full year, representing a rise of 100 per cent since 1958. Particularly good progress was made by the Optical Instrument Section of the industry. Between 1958 and 1961 production of optical instruments rose by 33 per cent and exports during the past three years totalled some 30 per cent of the production, with the United States and Australia as the main markets.

Following a talk given by K. E. Morgan of the Department of Agriculture, University of Reading, at a meeting of the Electronics Section and Nucleonic Group of S.I.M.A., a number of firms interested in the promotion of the use of measurement and instrumentation in agriculture were formed into a new specialized group, the Agricultural Instrument Group, of S.I.M.A. Contact has been made with the Agricultural Research Council and with other Government bodies. In order to collaborate and maintain contact with the various organizations and projects for the development of new syllabuses for the teaching of physics and related equipment, a steering committee has been appointed by the Educational Instrument Group. Dr. D. S. Beard (Griffin and George Ltd.) is honorary secretary of both the Agricultural Instrument and the Educational Instrument Groups.

The eleventh annual convention of S.I.M.A. was held at Eastbourne during November 7–9, 1962. The theme was "Looking Ahead—The Next Five Years". The opening address was delivered by the president. Mr. R. Brech (Unilever Ltd.) spoke on "The Economic Outlook in the U.K. for the next five years", and divided his talk into an impressionist picture in which the opportunities which exist were outlined and a realistic picture in which the extent to which the opportunities could be seized and used effectively were described. He concluded by stating that the coming five years would be tougher than the previous five years, but that with confidence, marketing skills, good designs and good management Britain would be able to hold its own. Mr. M. Seaman (College of Advanced Technology, Loughborough) discussed education and training in his address on "Future Trends in Engineering and Production", and described some highly sophisticated developments in shipbuilding and their place in the shape of things to come. Following these two sessions the delegates split into three discussion groups to consider the implications of the talks from the points of view of administration and training, technology, and marketing. It was agreed that there was a tendency among many foreigners to regard the British as old-fashioned and stodgy, but it was felt that the 'British image' was not wholly bad, since it had a reputation for quality and integrity. There was a great need for a substantial increase in the availability of information about the reliability of components of all types, and attention was directed to the British Scientific Instrument Research Association survey on "Literature on Reliability". General concern was expressed at the form of education in secondary modern schools, where those who stayed for the further two years did not benefit from the extension. It was considered that the standard of teaching in schools needed improvement and that the teachers should be more alive to the needs of industry.

Three other addresses were given to the convention. Mr. E. G. C. Burt (Royal Aircraft Establishment, Farnborough) gave a review of instrumentation in space research, describing in particular the *Aeriel* satellites, the astronomical telescope satellite, and communications satellites. "Science and Everyday Life" was the title of Dr. G. Margerison's talk in which he surveyed some of the new fields in which instruments had started to be applied. He emphasized that great scope for improvement existed in the fields of agriculture, biology, transport and communications. Finally, Dr. E. Eastwood (English Electric Group) gave an instructive and entertaining evening discourse, illustrated by slides and film, on "Radar Observations on Bird Migration". A report of the convention is given in the January issue of the *Review* and a summary of the addresses and discussions forms an insert to the number.

The education and training of personnel for the instrument industry is of especial interest to S.I.M.A., and recently Mr. C. W. Robson, formerly vice-principal and head of the department of electrical engineering and applied physics at South-East London Technical College, was appointed educational adviser to the Association. He has since visited a number of member companies in connexion with their apprentice training schemes. The course on "Leadership in Instrument Design", which was inaugurated by a joint committee of the British Scientific Instrument Research Association and S.I.M.A. and planned by the Northampton College of Advanced Technology to commence in October 1962, attracted only two students. The course had failed because it was too long and necessitated the absence of senior technical personnel from their firms for a period of some twelve months.

The provision of a text-book on instrument design for student use has been met by the publication of *A Guide to Instrument Design**, sponsored by the British Scientific Instrument Research Association and S.I.M.A. The book, which has just been published, consists of a group of nine chapters, on varied topics and written by different authors, but which together form a pattern. The preferred method, sometimes called the project method, of carrying out the development of an instrument through its various stages from the initial idea to design studies, prototypes, costing and final production is the subject of Chapter 1, by Dr. J. Thomson (British Scientific Instrument Research Association). In Chapter 2, Prof. H. F. Trewman describes the fundamental limitations in instrument design. The theory of errors, kinematic design, transducers, noise, masers, computers and ergonomics are all, strange to say, dealt with in this chapter and it is difficult to see the connexion that Prof. Trewman wishes to make between them. However, an attempt is made towards the end of the chapter to emphasize the general principles by brief considerations of a few individual instruments. Mechanical design, the subject of Chapter 3 by G. Birkbeck (Mullard Research Laboratories), will appeal to engineers, as also Chapters 5 and 6, where electronic instrument design and nucleonic instrument technology are treated. These two chapters are remarkable for the almost complete absence of circuitry and for the amount of valuable information, largely omitted from the usual text-books, which they contain. Dr. W. T. Welford (Imperial College of Science and Technology, London) is well known as an able teacher

* *A Guide to Instrument Design. Produced by the Scientific Instrument Manufacturers' Association of Great Britain (SIMA) and the British Scientific Instrument Research Association (BSIRA).* Edited by the Staff of BSIRA. Pp. xii+444. (London: Taylor and Francis, Ltd., 1963.) 63*s*. net.

and writer on optics, and Chapter 4, which he contributes, is an outstandingly lucid and comprehensive manual on optical design. Within 130 pages, he clearly distinguishes between the three different types of optical instrument and lists their design parameters, and then describes in detail how the parameters may be determined for particular optical systems and how systems with given parameters may be designed from available light sources, optical materials and detectors. The chapter concludes with an extensive list of references to which the interested student may refer for additional study.

Two important but somewhat neglected topics are considered in Chapters 7 and 8. These are the reliability of instruments and intrinsic safety. Finally, since an instrument is a piece of equipment to be used, the concluding remarks in the brief six pages of Chapter 9 on integrated design are valuable because they serve to remind the designer that instruments should be such as the user will like to handle and which will give him the information he requires in the most easily assimilated manner.

The Association continues to receive a large number of enquiries from home and overseas, and the Enquiry Service has been dealing with an average of 400–450 a month. A new leaflet directing attention to the service is being prepared for distribution to members. The card index system used to answer enquiries has been brought up to date. The Publicity Committee has been concerned with the improvement of Press relations for the greater dissemination of information about the instrument industry, and arrangements have been made in conjunction with the Central Office of Information to commission a series of articles on new developments and applications in instrumentation. The articles will be offered in the language of the country to technical journals overseas and also to selected journals in the United Kingdom. Mrs. H. Conway was appointed Press relations officer in July 1962.

S. WEINTROUB

THE NEW YORK ACADEMY OF SCIENCES

THE 145th annual meeting of the New York Academy of Sciences was held on December 5, 1962, at 2 East 63 Street, New York. During the year ending October 31, 1962, considerable progress was made in the recovery of the Academy's financial status, both for improvement of its operating budget and for strengthening its investments. The increase in income permitted an extension of the budget for conference and publication costs, an increase from thirty-nine to fifty in the staff, and the establishment of a medical care and group life insurance scheme for the staff. The financial statements, together with the reports of the Chairman of the Board of Trustees, the Treasurer and the Recording Secretary, are published in the January issue of the *Transactions* of the Academy (25, 265; 1963).

The Sciences, the new publication of the Academy, which was established in 1961, has been brought up to date on its publishing schedule. Thirty-one issues of the booklet, totalling 496 pages, were published during the year under review. In the *Annals* thirty-eight monographs, comprising 6,206 pages, and containing 501 papers by 796 authors, were published. As from January 1963, the publication date of any conference monograph will be within fourteen weeks subsequent to a conference, provided the authors submit their manuscripts within two weeks of the conference. Beginning with Volume 25, the *Transactions* will also follow this schedule. The demand for conferences to be held by the Academy continues to grow and the number of papers independently presented for publication in the *Annals* is also increasing regularly each year. Consequently, the Scientific Council has decided that with the current year, 1963, members of the Academy may select up to twenty separate monographs listed each year, with the added privilege of purchase of any publications of the Academy at a 20 per cent discount.

During the year, eighty-five meetings were held by the various sections and divisions of the Academy. The section of Biological and Medical Sciences held a joint meeting during January 1962 with the Columbia University seminar on "Genetics and Evolution" and a joint symposium with the division of Microbiology during April. The division of Instrumentation held a series of eight symposia on "Electronics in the Medical Specialities", and the papers in this series will be published shortly as a monograph. Details of the twenty-two conferences held between November 1961 and October 1962 are listed in the annual report.

The membership had a net increase of 622 during the year, with a record total of 16,249 members in October 1962. A maximum of fifty candidates may now be nominated each year to the fellowship of the Academy. The following were elected officers for 1963: C. H. Mushett, president, Rev. J. J. Lynch, president-elect, and K. Maramorosch and C. C. Stock, vice-presidents of the Scientific Council; C. R. Noback, recording secretary, and R. F. Nigrelli, corresponding secretary.

The Academy consists of the sections of biological and medical sciences, chemical sciences, physical sciences, and planetary sciences, and the divisions of anthropology, instrumentation, microbiology, psychology, biochemistry, biophysics, engineering and mathematics. The sections and divisions meet regularly, one evening a month, during the academic year, October–May, inclusive, in the Academy's premises in New York. The conferences are held at irregular intervals.

UNIVERSITY EDUCATION IN INDIA

THE fourth annual Conference of Vice-Chancellors of the Indian universities and institutions 'deemed as universities' was held at New Delhi during October 11–13, 1962. The report of this Conference* includes the inaugural address of Dr. K. L. Shrimali, Minister of Education, Dr. D. S. Kothari's address on some aspects of university education, the address of the Prime Minister, Sri Jawaharlal Nehru, and the recommendations of the three Committees dealing with admissions, medium of instruction and related matters; service conditions of college and university teachers, and three-year degree courses; and contents of education and co-ordination of research. The most important feature of Dr. Shrimali's address is the stress laid on the medium of instruction. While any university or college will be completely free to continue to use English as a medium of instruction if it wishes, and use of English has been recommended as an alternative or associated medium by the National Integration Council, Dr. Shrimali is firmly convinced that unless

* India. University Grants Commission and Ministry of Education Vice-Chancellors' Conference, 1962. Pp. v+81. (New Delhi: University Grants Commission and Ministry of Education, 1963.) Rs. 2.00; 4*s.* 8*d.*; 0.72 dollars.

the universities adopt and develop their own Indian languages, India will not succeed either in the dissemination of knowledge among the Indian people or in the stimulation of research and the creation of new ideas. There was no desire to minimize the importance of the English language and for keeping abreast of science and technology; where knowledge of a foreign language was essential, English was particularly convenient in India. Dr. Shrimali also stressed the importance of the university's function to seek truth and to advance the frontiers of knowledge. This function of research gave substance to teaching and brought the student into contact with the process of discovery, and he welcomed the recent decision of the Indian University Grants Commission to meet the major part of expenditure on the establishment of schools of advanced studies at universities. He also thought it might be necessary to re-group or federate some existing departments in order to strengthen research.

Dr. D. S. Kothari, chairman of the Indian University Grants Commission, who also referred to the medium of instruction, insisted that it was important that the introduction of regional languages should not exclude the use of English in university life and work. More and more instruction would be through the medium of English as the undergraduate proceeded, but it was also important to produce books and literature, particularly scientific and technical, in the regional languages. Dr. Kothari suggested that the central problem in higher education in India was to raise standards and reduce wastage, and for this energetic steps to improve the quality and strength of the teaching staff, to make good books available at reasonable prices, and to provide reading seats in libraries and hostels were essential. As regards expansion, an increase was needed not so much in pure science as in medicine, engineering and especially in agriculture and veterinary science. Other matters which received attention at the Conference and were discussed briefly in this address included the shortage of teachers, staff appointments, assistance to outstanding teachers, standards of education, the establishment of new universities and centres of advanced study and collaboration between universities and other institutions.

Sri Jawaharlal Nehru also welcomed the use of regional languages as the medium of instruction, but emphasized the desirability of at least one other foreign language besides English if the student was to be in touch with modern thought. He insisted especially on the need to maintain quality, which should never be sacrificed to quantity, and finally referred to the importance of the scientist having some understanding of the humanities and of the non-scientist having some understanding of science.

FREEDOM FROM HUNGER CAMPAIGN: COMBATING DEFICIENCIES OF PROTEIN

MORE than a half the world's population lives in the Far East on a quarter of the total food produced. This is made up of only one-fifth of the world's animal and fish protein and less than one-half of land crops. In contrast to this, 29 per cent of the world's population living in Europe, Oceania and North America has available to it 57 per cent of total supplies, 69 per cent of animal and fish protein and 38 per cent of land crops. The situation in other less-developed regions is also unsatisfactory. These are among the broad conclusions reached in the *Third World Food Survey** recently published by the Food and Agriculture Organization, Rome.

So far as the position in Africa, the Near East and Latin America is concerned, the survey points out that these regions share of the total available food is approximately in proportion to their share of the world's population. It stresses, however, that diets there are low in all-important animal protein and the population is growing at a faster rate than food supplies.

The Food and Agriculture Organization's short-term targets for the developing regions provide for an increase of 5–6 g of animal protein to a total of 15 g per person per day, amounting to 22 per cent of total proteins. The long-term target is 21 g, which is equivalent to 28 per cent of the total.

In the preface to the survey, Mr. B. R. Sen, director-general states "If we are to achieve a reasonable improvement in the level of nutrition, then world food supplies will have to be increased by more than 50 per cent. . . . By the year 2000 it is expected that the world population will be at least double the present figure, while the population in the less-developed countries will have increased by 150 per cent. . . .

"These figures give some indication of the magnitude of the task confronting us, and indicate how timely and urgent is the Freedom from Hunger Campaign".

The annual report for 1961–62 of the Central Food Technological Research Institute of Mysore, India†, refers to a vigorous programme being pursued in the production and development of protein-rich foods. The Institute's work in this field, based on high-protein low-fat oil-seed meals which are plentiful in India, has been recognized by the joint Protein Advisory Group of the World Health Organization, the Food and Agriculture Organization and the United Nations International Children's Emergency Fund. This has led to the offer by the U.S. National Institutes of Health of the *PL* 480 funds for the intensification of work on vegetable protein supplements. The report summarizes the research carried out at the Institute in a large number of fields, including biochemistry and nutrition, engineering, processing and storage, infestation control, microbiology, sanitation and packaging.

* Food and Agriculture Organization of the United Nations. Freedom from Hunger Campaign—Basic Study No. 11: *Third World Food Survey*. Pp. vi+102. (Rome: World Health Organization; London: H.M.S.O., 1963). 7*s*. 6*d*.; 1 dollar.

† Central Food Technological Research Institute, Mysore. Annual Report 1961–62. Pp. ix+153. (Mysore: Central Food Technological Research Institute, 1963).

APICULTURAL RESEARCH

AS research into bees and beekeeping has expanded and become progressively more scientific it has grown increasingly obvious that a new journal in English was needed for research work which was too technical for the popular beekeeping Press. The Bee Research Association has for some years past published *Bee World*, which contained a mixture of scientific and more popular articles, plus news items and the very valuable *Apicultural Abstracts*; but the Association has, however, recognized that a separate journal could usefully be devoted to scientific work alone. As a result it has now commenced the publication of the *Journal of Apicultural Research**.

* *Journal of Apicultural Research*, Vol. 1. (Southall, Middx.: Hon. Treasurer, Bee Research Association, 60 North Road, 1963.) 40*s*. per annum.

The new journal is edited by Dr. Eva Crane, director of the Bee Research Association, with Dr. James Simpson of the Bee Department at Rothamsted Experimental Station acting as associate editor. They are assisted by an international Editorial Advisory Board of nine members well known in different fields of bee research. The stated policy of the journal is to cover all aspects of subjects relating to bees (*Apis* and other bees), and also research on methods and equipment for the management of bees and their products. This is carried out satisfactorily in the first issue.

The general presentation of the journal is acceptable, since it has clear print and tables, and diagrams of a suitable size. All the papers in the first issue are concisely written, which perhaps emphasizes the impression that the proportion of short contributions is rather high. However, this might have been largely remedied if two of them (by W. P. Nye and M. W. Pedersen; and B. Furgala) had been placed in a separate section for preliminary reports, since the first gives rather inconclusive results of two experiments on the relationship between nectar sugar concentration and alfalfa pollination, while the second promises more data and a fuller discussion of the subject—the residual activity of fumagillin in relation to *Nosema* infections—in another paper. It is also a little difficult to see why two short papers by M. V. Smith have not been combined, although the editors may be following a policy of separating descriptions of apparatus from experimental work where possible. In this case, one paper is a report of experiments culminating in the successful transportation of honey-bee brood from Britain to the United States by air, with the object of importing new stock into the United States, and the other describes the portable incubator used for this purpose. Another minor point is that one wishes that some diagrams had been included in the very detailed and clear description of the pre-pupal and pupal ecdyses of the honey-bee by S. Cameron Jay, but no other matters concerning the actual presentation of this journal call for any comment.

As stated earlier, the scope of the first issue is wide, and the remaining papers cover the genetics, behaviour and biology of bees, and the antibactericidal properties of honey. The latter paper is a brief one by D. Adcock on the destructive effect of catalase on the "inhibine" and peroxide values of various honeys. J. Woyke has a paper on the hatchability of "lethal" eggs of honey-bees, in which he reports fairly normal hatching rates in these eggs but rapid removal of the young larvæ by the workers. There is a very useful description of a controlled environment flight room for the study of behaviour inside and outside the hive, together with records for pollen collecting activities in the room at different light intensities (W. P. Nye). J. B. Free shows that geraniol, although it is the principal volatile constituent of honey-bee scent gland secretion, is not nearly so attractive as scent-gland odour itself, and therefore concludes that the attractiveness of the latter depends partly on other constituents.

In a paper describing the events following construction of the queen cell in honey-bee colonies N. E. Gary and R. A. Morse give results demonstrating the extreme variability which exists in the rearing and replacement of queens; it is apparent that swarming or supersedure does not necessarily follow queen rearing. The longest paper is by Cécile Plateaux-Quénu on the biology of *Halictus marginatus*, which she claims to have a type of social organization previously unknown in insects. Workers and queen are morphologically indistinguishable, and both are capable of mating, although for most of the developmental cycle of the colony (5–6 years) they have no contact with the males. Various aspects of social life in this bee are considered, and although more numerical data could have been desired, it is an interesting description.

In conclusion, it can be said that the first issue of the *Journal of Apicultural Research* augurs well for the future, and that, once it has become properly established, it should be a valuable publication.

M. Delia Allen

SPECTROGRAPHIC ANALYSIS AS AN AID TO DIESEL ENGINE MAINTENANCE

THE replacement of the steam engine as the traditional motive power by Diesel locomotives has now become familiar on British Railways, and in fact in many other countries this transition is all but complete. The economic arguments in favour of this change are that the Diesel unit offers to the railways greater efficiency, lower fuel costs and greater availability. Technically these advantages may to some extent be offset by the much greater complexity of the Diesel as compared with the steam locomotive, and problems of maintenance of the complicated machinery involved in the oil-driven engines are constantly arising. The risk of accidental damage to Diesel mechanisms, involving sudden breakdowns with their attendant disruption of operating railway schedules, equally heavy repair costs, is very real. One way of control in prevention of these contingencies has been found by routine spectrographic analysis of Diesel engine crankcase oil. An informative account of this procedure is contained in the well-designed house organ of the British Petroleum Co., *B.P. Magazine* (No. 8; 1963), in an illustrated article by J. K. Lock and P. R. Mason, respectively, of the lubricants and analytical sections of the B.P. Research Centre, Sunbury-on-Thames, where such spectrographic facilities are available as are similarly developed at the Company's Centre at Melbourne, Australia.

As with other internal combustion engines, normal wear in a Diesel engine over a period of time occasions entrance in the crankcase oil charge of minute metal particles. In this case, however, there is little if any sedimentation or sludge as ordinarily understood; the metal particles are so small that they are measured in parts per million; conventional methods of chemical analysis are not applicable to their identification because of their low concentration. In such cases spectrographic technique supplies the answer. "If regular samples of the crankcase oil charge are taken and analysed for these metal contents, a normal pattern of wear can be recognized for any one engine, performing a specified duty. If, however, a fault develops in the engine that causes an increase in wear, this pattern of wear is disturbed and can be recognized at an early date." The authors continue: ". . . the value of the service is that it permits small faults to be recognized and rectified, whereas if these faults were allowed to progress without rectification, serious damage and an expensive repair bill might ensue". Normally, nine elements are determined spectrographically in these crankcase oils: iron, copper, lead, tin, silicon, chromium, boron, aluminium and silver. The significance of the determination of each of these elements in the oil and their interpretation in terms of Diesel engine wear and tear constitute an expert enquiry. This article conveys impressively the substance of the technique involved.

H. B. Milner

EVIDENCE FOR A HUMORAL THYMUS FACTOR RESPONSIBLE FOR THE MATURATION OF IMMUNOLOGICAL FACULTY

By Dr. DAVID OSOBA* and Dr. J. F. A. P. MILLER

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MICE thymectomized within 24 h of birth show a marked deficiency in the lymphoid cell population of their blood and tissues, a diminished capacity to produce circulating antibodies in response to some antigens and a severe impairment of homograft immunity[1,2]. All these effects can be prevented by grafting subcutaneously whole intact neonatal thymus within several days of thymectomy[1]. They cannot, however, be prevented by a single intravenous injection of a suspension of dissociated neonatal thymus cells given within a day of thymectomy[3]. It would appear, therefore, that the whole thymus architecture is an essential part of the restorative system under these conditions. The mechanism by which the thymus ensures the development of the immune apparatus is not clear. The thymus could act either by providing the environment in which differentiation and multiplication of cells with immunological potential can take place, or by providing a non-cellular factor which enables lymphoid precursor cells pre-existing in the peripheral lymphoid organs to attain immunological competence.

Up to the present time, there is no direct, unequivocal evidence to show that cells leave the thymus to populate the lymphoid tissues. There is, however, some indirect evidence which suggests such a migration. The first site of lymphocyte production in the embryo is the thymus and only later do lymphocytes appear in other organs[4]. Mitotic activity of lymphoid cells during embryogenesis is high in the thymus but minimal or absent in peripheral lymphoid tissues. It becomes evident in these tissues only after birth, presumably as a result of antigenic stimulation. Auerbach[5], using elegant tissue culture techniques, showed that a suspension of 14-day-old embryonic spleen cells could aggregate to form follicles and small lymphocytes *in vitro* only in the presence of embryonic thymus cells. When the embryonic spleen cells were separated from the thymus cells by a millipore barrier, follicle formation did not occur.

Experiments using chromosome marker techniques in adult irradiated mice given syngeneic marrow gave results which showed "that the re-formation of lymphoid elements occurs sooner in the thymus than in the lymph nodes" thus suggesting that "the thymus continues to seed the other lymphoid tissues"[6]. Experiments performed in this laboratory showed that the majority of cells multiplying in subcutaneous thymus grafts and lymphoid organs of neonatally thymectomized mice were of host type and could not, therefore, be descendants of donor thymus cells[3]. The cells populating the lymphoid tissues of the thymectomized hosts could have been derived either from cells which differentiated in the thymus graft, or from precursors which differentiated in the lymphoid tissues as a result of a humoral factor elaborated by the graft. In support of the humoral theory, the work of Metcalf can be cited[7]. He isolated a factor from the thymus which, when injected into new-born mice, produced a temporary lymphocytosis in the peripheral blood. In this laboratory, non-cellular saline extracts of neonatal thymus tissue were not effective in restoring lymphocyte counts and homograft immunity in neonatally thymectomized mice[3]. The experiments recorded here were designed to test the hypothesis that a humoral factor from the thymus may play a part in the development of the immune mechanism.

At seven days of age neonatally thymectomized (*CBA* × *T6*)F_1 mice were intraperitoneally implanted with 'Millipore' diffusion chambers (pore diameter of 0·3μ) containing either one lobe of new-born or one to three lobes of 14-day embryonic *CBA* thymus tissue. Control groups consisted of neonatally thymectomized mice which either received no further treatment or which were implanted with empty diffusion chambers, and of mice found to be only partially thymectomized at the end of the experiment. Peripheral lymphocyte counts were done on all mice between the sixth and twelfth weeks of age. The capacity to reject homografts was tested by *AK* skin grafts when the mice were six to eight weeks old. Ten days following skin grafting the protective dressings were removed and the graft site was examined daily for signs of rejection. All mice were autopsied and the retrosternal area examined histologically for thymus remnants. The spleen, lymph nodes, Peyer's patches and diffusion chambers were fixed in Bouin's solution. The lymphoid tissues from some of the mice were subjected to chromosomal analysis.

The results of challenge with homografts are presented in Table 1. The majority of mice (18 out of 26) bearing either neonatal or embryonic thymus tissue rejected homografts within 25 days. In this respect there was no significant difference between mice with neonatal thymus (9 out of 14) and those with embryonic thymus (9 out of 12). None of the 13 thymectomized control mice rejected homografts within 25 days. Whereas only 7 of the 26 mice bearing diffusion chambers with thymus tissue retained homografts for more than 40 days, 12 of the 13 thymectomized control mice retained skin grafts for periods exceeding 40 days. No difference was evident in the inability of thymectomized mice bearing empty diffusion chambers to reject skin grafts (6 out of 7) as compared with mice subjected only to neonatal thymectomy (6 out of 6). Mice which were only partially thymectomized had the capacity to reject homografts and therefore behaved as intact non-thymectomized mice.

Table 1. SURVIVAL OF HOMOGRAFTS IN (*CBA* × *T6*)F_1 MICE THYMECTOMIZED AT BIRTH AND BEARING *CBA* THYMUSES IN 'MILLIPORE' DIFFUSION CHAMBERS (D.C.)

Treatment	No. of mice	No. of mice with following survival time (days) of *AK* skin graft			
		< 25	25–40	40–60	> 60
None	11	11	0	0	0
Partial thymectomy	15	13	2	0	0
Thymectomy at birth	6	0	0	1	5
Thymectomy at birth; empty D.C.	7	0	1	2	4
Thymectomy at birth; embryonic thymus in D.C.	14	9	0	4	1
Thymectomy at birth; neonatal thymus in D.C.	12	9	1	0	2

The peripheral lymphocyte counts are shown in Fig. 1. The findings may be summarized by stating that all mice which were subjected to neonatal thymectomy, regardless of their subsequent treatment, showed in general significantly depressed lymphocyte counts with the exception that some of the mice bearing diffusion chambers containing neonatal thymus tissue had normal counts. A few partially thymectomized mice had sub-normal counts.

Histological examination of the lymph nodes of mice with thymus tissue in diffusion chambers showed in some cases islands rich in lymphocytes scattered in the cortex[8].

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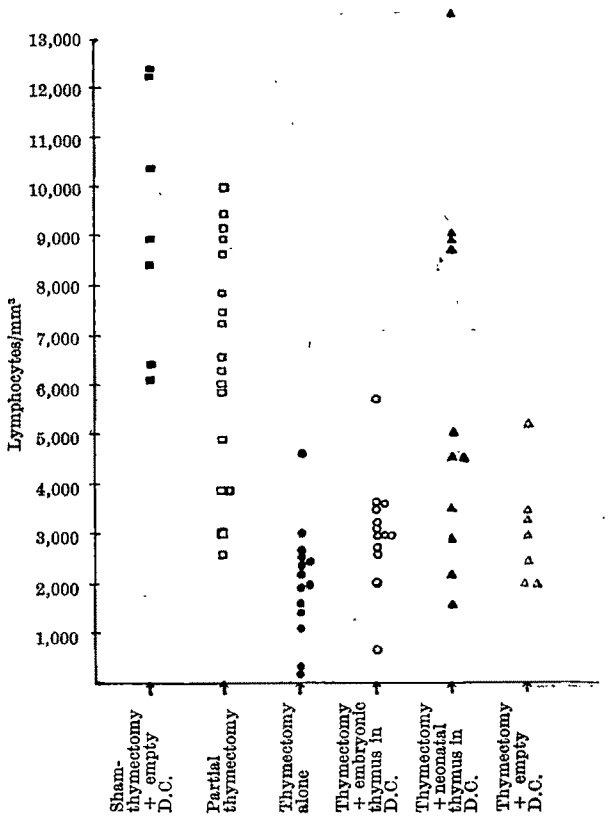
In general, however, there was still evidence of lymphocyte depletion in most lymphoid tissues and therefore no marked difference in comparison with the findings in the control thymectomized mice.

The tissue recovered from the diffusion chambers did not contain any recognizable lymphoid tissue and consisted only of epithelial and reticular cells[8] (Fig. 2) occurring either in clumps or scattered singly in a loose bed of fibrous tissue. In some cases structures resembling acini lined by epithelial cells and containing material which stained positively with periodic acid–Schiff reagent were present. At no time was there any direct histological evidence of cells traversing the chamber walls.

All the host lymphoid cells which were subjected to chromosomal analysis were found to contain the *T*6 chromosome.

The results presented here show that mice bearing embryonic or neonatal thymus tissue enclosed in diffusion chambers have the capacity for rejecting skin homografts. It might be possible to explain these results by: (1) cell leakage from the diffusion chamber; (2) incomplete thymectomy; (3) adjuvant effect of the diffusion chamber; or (4) elaboration of a humoral factor from the enclosed tissue. Passage of cells through the 'Millipore' barrier is unlikely to have taken place because: (1) cytological studies failed to show the presence of any donor cells in the host lymphoid tissues; (2) no lymphoid cells were found in the tissue in the chamber. If cells had gained access to the chamber, one might have expected, by analogy with studies on subcutaneous thymus implants, that these cells would have re-populated the thymus epithelial-reticular framework.

Incomplete thymectomy cannot account for the results obtained in the experimental group since only mice, afterwards found by histological examination to be completely thymectomized, were included in the results in this group. Thymectomized mice bearing empty



(Thymectomy performed at birth; diffusion chamber (D.C.) inserted intraperitoneally at 7 days)

Fig. 1. Absolute lymphocyte levels in 6- to 12-week-old mice thymectomized at birth and bearing thymuses enclosed in 'Millipore' diffusion chambers

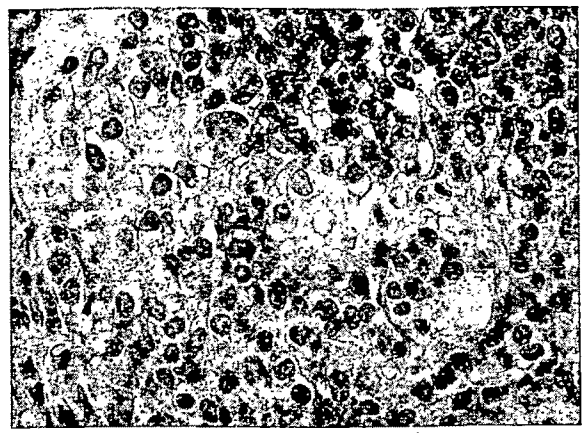


Fig. 2. Fourteen-day-old embryonic thymus enclosed for 6 weeks in diffusion chamber in neonatally thymectomized mouse. Note epithelial-reticular cells and absence of lymphocytes (× *c.* 337)

diffusion chambers were found to behave as mice subjected to neonatal thymectomy alone. This showed that the 'Millipore' material itself had no role in the establishment of homograft immunity.

The lymphocyte population of neonatally thymectomized mice bearing thymus tissue enclosed in diffusion chambers was in most cases not significantly different from that of control thymectomized mice. It seems, therefore, that the capacity of mice to reject homografts is not dependent on the quantity of lymphocytes present in the animal. This is supported by experiments on mice thymectomized in adult life: these mice show permanent depletion of the lymphoid cell population but no impairment of homograft immunity even as late as 100 days after thymectomy[9].

The absence of lymphoid cells in the tissue enclosed in the diffusion chambers[8] suggests that the persisting epithelial-reticular framework of the thymus is the functional factor responsible for equipping the mice with the capacity for rejecting homografts. It would appear therefore that, under the conditions of the present experiments, epithelial-reticular cells elaborate a humoral factor which enables host lymphoid cells to become immunologically competent cells. Whether such a factor plays a part in normal development remains to be determined. Further work is clearly required to characterize this influence and determine its mode of action.

We thank Prof. Alexander Haddow for his interest in this work, and to Miss Barbara Burnett and Miss Winifred House for assistance. The investigations were supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service. They also received special support through a grant from the Tobacco Manufacturers Standing Committee.

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INHIBITORY SYNAPSES ON SOMAS OF PURKINJE CELLS IN THE CEREBELLUM

By Dr. P. ANDERSEN, Sir JOHN ECCLES, F.R.S., and Dr. P. E. VOORHOEVE
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IN the hippocampal cortex a very powerful postsynaptic inhibition is produced by synapses concentrated on the somas of the pyramidal cells; hence it has been concluded that these synapses belong to the basket cells, which have extensive axon ramifications that terminate exclusively on the cell bodies of the pyramidal cells[1]. Optimal conditions for the inhibitory control of cell firing are secured by location of inhibitory synapses on the cell soma, so it was suggested that this localization may be a general feature of synaptic organization in the central nervous system. The basket cells of the cerebellum provide a most remarkable concentration of synapses on the soma and axon hillock of Purkinje cells[2–5]. We have therefore chosen the Purkinje cell (*P*-cell) for our first investigation into the general proposition that the synapses responsible for postsynaptic inhibition are localized on the somas of neurones. Granit and Phillips[6] have referred to the occasional presence of spontaneous hyperpolarizing responses of Purkinje cells.

Fig. 1 is a simplified diagrammatic representation of the most important cellular elements in the cerebellar cortex and of the electrode arrangements used in the present study. The afferent mossy fibres (*mf*) synapse with the granule cells (*G*) which send their axons vertically into the molecular layer, where by a *T*-shaped junction each fibre divides into two branches which are called parallel fibres (*pf*) because they course strictly parallel to the surface of the folium for at least 1·5 mm in each direction; during this traject they penetrate a large number of *P*-cell dendritic trees all of which are oriented in a plane transverse to the long axis of the folium. The *P*-cell axons (*Pa*) represent the efferent link from the cerebellar cortex. Each of them has a number of recurrent collaterals said to end on the soma and initial dendrites of other *P*-cells[2,3,7]. In the lower part of the molecular layer there are a number of special cells, the so-called basket cells (*B*). These have extensive axon ramifications (*Ba*) that run largely transverse to the long axis of the folium and just above the *P*-cell layer. Numerous branches form basket-like plexuses around the somas of the *P*-cells, especially at the axonal poles, hence the name. The vast majority of synapses on the lower part of the somas of *P*-cells do, therefore, belong to the basket cell axons.

The *P*-cells have been excited in two ways, as indicated in Fig. 1, either by local stimulation exciting parallel fibres[8] or by antidromic activation through a concentric needle electrode[6]. Recordings were made by micro-electrodes filled with 4 M sodium chloride or 3 M potassium chloride or 2 M potassium citrate. The cerebellar cortex is extremely sensitive to mechanical or circulatory damage. By covering the surface with 4 per cent solution of agar in Ringer–Locke after the electrodes were positioned, the respiratory and circulatory movements of the cortex were controlled when the agar jelly had set. When agar covered, the cerebellar cortex remained in good condition for many hours. The microelectrode can be withdrawn and re-inserted through the agar gel.

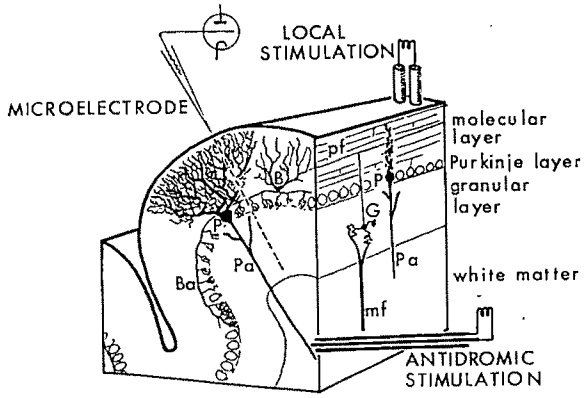


Fig. 1. Diagrammatic representation of cerebellar cortex with the electrode arrangement

When the depth of micro-electrode penetration was 0·3–0·4 mm, it frequently impaled nerve cells as shown by the recording of a resting potential, of repetitive spike potentials in the positive direction and of synaptic potentials. Usually these responses indicated extensive cell damage and there was rapid deterioration. However, resting potentials greater than −40 mV and large synaptic potentials have been recorded from several cells for a sufficiently long time to allow a discriminative investigation. Since the intracellular recording was always at the depth of the somas of the Purkinje cells, and since the intracellular responses conformed to a standard pattern, including antidromic invasion, it may be concluded that in fact our intracellular recording was restricted to Purkinje cells.

As shown in Fig. 2*A*, a weak local stimulation of a folium evoked, after a latency of about 4 msec, a prolonged hyperpolarization that had a rising phase of more than 10 msec and a total duration much in excess of 100 msec. In Fig. 2*B* there are two superimposed traces, the one set up by the stronger stimulation showing an additional depolarization with a latency about 1 msec briefer than for the hyperpolarization. These potentials correspond in every respect to excitatory and inhibitory postsynaptic potentials (EPSP's and IPSP's). The IPSP had the standard properties: reversal to a depolarizing response when a sufficiently strong hyperpolarizing current was passed out of the microelectrode; large increase during the passage of a depolarizing current; reversal to a depolarizing potential when Cl^- ions were injected out of a recording electrode filled with 3 M potassium chloride. Local stimulation produced these large and prolonged IPSP's in every *P*-cell from which we have recorded. In contrast, antidromic activation of *P*-cells by the stimulation as in Fig. 1 did not produce IPSP's, though with strong stimulation IPSP's were produced, presumably in response to the stimulation of the mossy fibres with serial synaptic activation of granule cells and basket cells.

The inhibitory action of the prolonged IPSP is illustrated in Fig. 2*C*, where the spontaneous rhythmic discharges of *P*-cells are inhibited for as long as 800 msec by the same surface stimulation of the folium that evokes the IPSP's. This powerful and prolonged inhibitory action on spontaneous discharges of a *P*-cell has invariably been produced by a surface stimulus that excited the parallel fibres projecting towards that *P*-cell.

The organization of the *P*-cells of the cerebellum gives ideal conditions for investigation of the location of synaptic action on them. The somas are arranged in a single layer parallel to the surface of the folium (Fig. 1) and are essentially bipolar with the dendritic trees projecting from one pole up to the surface and the axon from the other pole into the depths of the folium (Fig. 2*G*). In Fig. 2*D*, *E* are shown typical series of potentials recorded at the indicated depths along a microelectrode track and evoked by a weak (30 V) and a strong (50 V) local stimula-

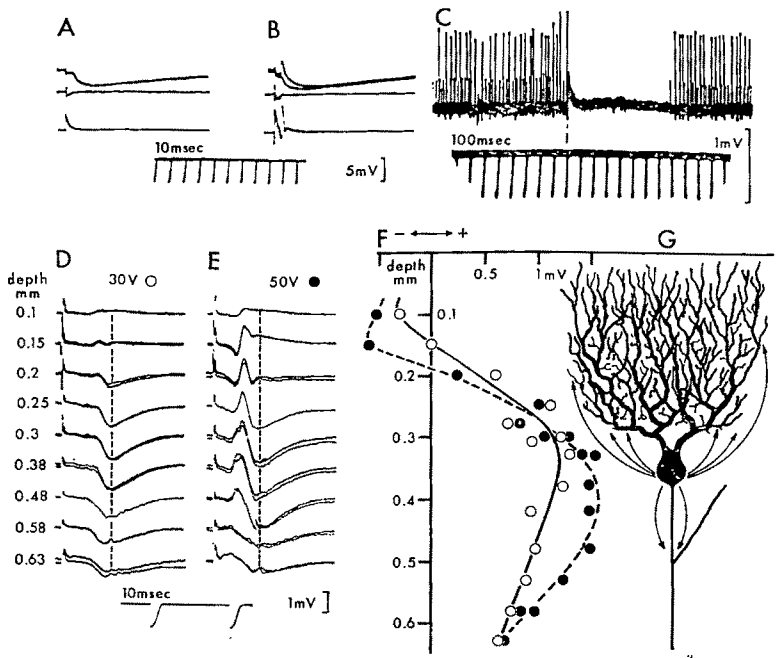


Fig. 2. *A*, *B*, Intracellularly recorded IPSP and EPSP from a Purkinje cell (*P* cell) in response to local stimulation; *C*, inhibition of the spontaneous discharges of two *P*-cells due to a single local stimulus; *D* and *E* show extracellular potentials evoked by local stimulation of 30 and 50 V and recorded at the indicated depths below the surface of the folium; *F*, plotting of measurements from *D* and *E* as described in text; *G*, diagram of a *P*-cell with current flow indicated by arrows

tion. The weak stimulation was remarkable in that it showed the development and the decline of an almost pure positive potential with progressive penetration. With the stronger stimulation there was at depths of 0·1–0·5 mm also an earlier negative response that overlapped with the onset of the positive potential. A trace of this negativity can be seen also with superficial records at the weak stimulation.

In order to obtain measurements of the field potential produced by the positive component relatively uncontaminated by this earlier negative component, measurements have been made at 6·7 and 5·7 msec after the stimulation at the stippled lines in *D* and *E*, respectively, and are plotted in Fig. 2*F*. The positive wave is seen to reach a maximum at a depth of 0·35–0·4 mm with both strengths of stimulation; and more superficially it rapidly diminishes and reverses to a surface negativity, which can be recognized as a distinct component following the initial surface negativity in Fig. 2*D*, *E* at depths of 0·1 and 0·15 mm. Beyond 0·4 mm the positive wave also declines, but much less rapidly, and no reversal is seen. The curves of Fig. 2*F* show that current is flowing outwards from the region of the somas of the *P*-cells very intensely up towards the dendrites all the way to the surface, and very weakly in the reverse direction towards the axon, as shown by the arrows in Fig. 2*G*. This is precisely the extrinsic current that would be generated by an inhibitory synaptic action concentrated on the *P*-cell somas. It cannot be due to depolarizing synaptic action on the *P*-cell dendrites, because in that case intracellular recording from the somas must give a depolarizing potential due to electrotonic spread of the EPSP, and not the hyperpolarization that is invariably observed.

From these various experimental investigations it may be concluded that powerful postsynaptic inhibition is exerted on the somas of the Purkinje cells. Since this is observed in all successfully impaled cells, there must be a concentration of inhibitory synapses on the somas of *P*-cells. Evidently the basket cells are the cells responsible for mediating this inhibitory action. This identification is further supported by the finding that stimulation of the folium may produce an inhibition virtually uncontaminated by a preliminary excitation of *P*-cells if it is off the direct line of parallel fibres to the recording electrode, which was the case with the weaker stimulation in Fig. 2*D*. Under these conditions the inhibition must be produced by mediation of interneurones having axons transverse to the folium, which is the case with basket cells. The parallel fibres traverse the dendritic trees of the basket cells as well as the *P*-cells, and probably synaptically excite both. This would explain the difference in latency of only about 1 msec between the EPSP's and the IPSP's (Fig. 2*B*).

In conclusion, the site of inhibitory synaptic action has been determined for Purkinje cells of the cerebellar cortex. Since the inhibitory synapses are concentrated on the somas, they must belong to the histologically identifiable basket cell, exactly as in the hippocampal cortex. However, this inhibition is not due to recurrent axon collaterals of *P*-cells, since antidromic stimulation of *P*-cell axons failed to give postsynaptic inhibitory potentials in spite of readily demonstrable antidromic invasion into the cell bodies of large numbers of *P*-cells. Taken together with the situation in the hippocampal cortex, the somatic location of inhibitory synapses may well be a generally occurring pattern, as proposed earlier[1]. An additional parallel between the basket cells of the hippocampus and the cerebellum is that synapses of both have the characteristics of synapses that Gray[9] designated Type 2 (refs. 10 and 11). This leads to the further suggestion that all postsynaptic inhibitory synapses may be of Type 2. [See also p. 699 of this issue of *Nature*.]

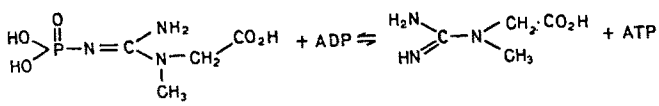
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WHY DO PHOSPHAGENS FUNCTION AS PHOSPHORYL-TRANSFER REAGENTS?

By Dr. V. M. CLARK and S. G. WARREN

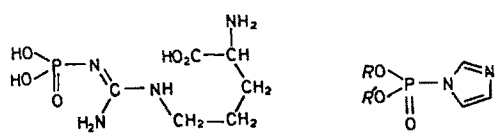
University Chemical Laboratory, Cambridge

N-Phosphoryl-creatine (I) was isolated from muscle by Fiske and SubbaRow[1] in 1927. Measurement of its heat of hydrolysis[2] led Lipmann[3], in 1941, to classify it as an 'energy-rich' phosphate, and its role in the formation of ATP from ADP (equation (1)) in muscle contraction[4] seemed to confirm this view.



(I) ....(1)

At approximately the same time, phosphoryl derivatives of other substituted guanidines, for example, *N*-phosphoroarginine (II), were identified as participants, with the adenine nucleotides, in phosphoryl transfer reactions and the term 'phosphagen' was applied to the group of naturally occurring phosphoroguanidates[5,6].



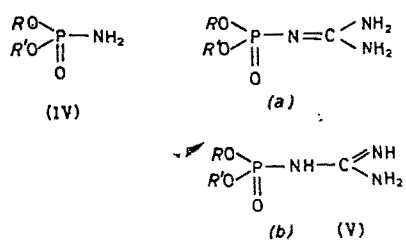
(II) (III)

The transfer of a phosphoryl residue from a phosphoroguanidate (for example, I or II) requires cleavage of the phosphorus-nitrogen bond. Such a cleavage is known to be facile with the phosphoramidates (the simplest P—N system, for example, IV) and using this route ATP has been synthesized from ADP[7]; further development of this reaction has resulted in a highly flexible method of pyrophosphate synthesis[8,9].

A molecular system somewhat closer in structure to that of the phosphoroguanidates is to be found in the phosphoroimidazole (III) and this has been shown to be a powerful phosphorylating agent[10,11].

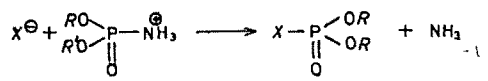
However, *in vitro*, a variety of phosphoroguanidates has failed to function as phosphorylating agents under conditions comparable with those used with the simple phosphoramidates[12].

The marked difference in lability between the phosphoramidate (IV) and the phosphoroguanidate (V: *a* or *b*) can be attributed to the nature of the P—N bond in the two series.



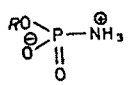
(IV) (a) (b) (V)

In (IV) the P—N bond order is enhanced by overlap of the orbital of nitrogen—occupied by the lone pair of electrons —with the *d* orbitals of the phosphorus atom. Thus, phosphoramidates, unlike carboxylic acid amides, are stable to caustic alkali, and, indeed, diphenyl phosphoramidate (IV : $R=R'=$Ph) is smoothly converted by caustic soda to the sodium salt of the acid (IV : $R=R'=$H) without cleavage of the P—N link[13]. On the other hand, protonation of the nitrogen atom removes this $p\pi-d\pi$ overlap and enhances the possibility of nucleophilic attack at phosphorus[14], providing, concurrently, a good leaving group, namely, the neutral ammonia molecule (equation 2). In consequence, phosphoramidates are



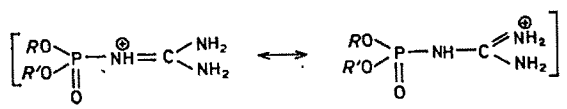
.. (2)

unstable under acidic conditions and can, for example, be converted to phosphorochloridates on treatment with gaseous hydrogen chloride ($X=$Cl)[15]. Moreover, in virtue of tautomerism to the zwitterionic structure (VI), phosphoramidic monoesters (IV : $R=C_6H_5.CH_2$, $R'=$H) are good phosphorylating agents[7,16].



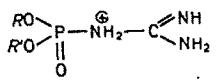
(VI)

Phosphoroguanidates present a more complicated picture, since at least two prototropic forms (V: *a* and *b*) are possible. In (V*a*) each of the two nitrogen atoms not attached to phosphorus has a lone pair of electrons which, on protonation of the nitrogen atom of the P—N bond, can facilitate the delocalization of the positive charge, as expressed in (VII): such delocalization inhibits the displacement reaction indicated in equation (2). On



(VII)

the other hand, protonation of (V*b*) could yield (VIII),

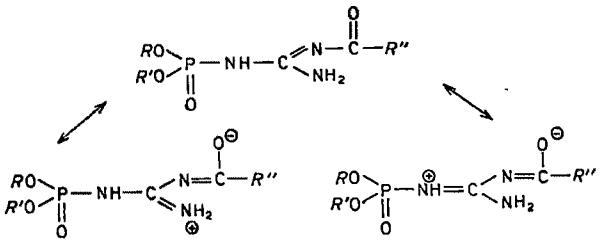


(VIII)

which would undoubtedly behave as a phosphorylating agent in accordance with equation (2).

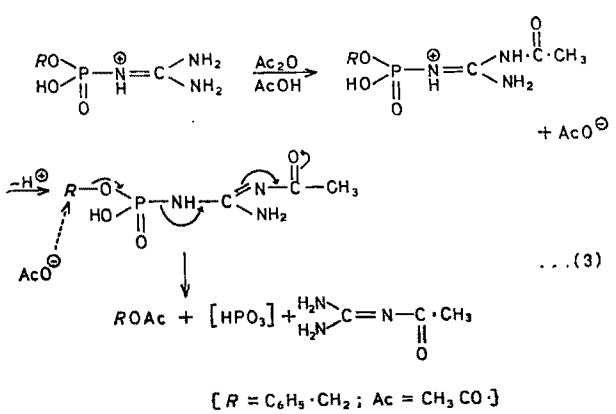
Our infra-red and nuclear magnetic resonance evidence shows that the normal esters of phosphoroguanidic acid have structures consonant with (V*a*). Their stability is not, therefore, unexpected. Structure (V*b*) is related to the phosphoroimidazole (III), which is highly reactive[10]. Thus, it seems likely that a phosphagen might be activated, *in vivo*, by a tautomeric shift of the type (V*a*→V*b*).

The sharp distinction between the chemical and biochemical lability of the phosphagens has led us to search for a structural modification which would convert Va to Vb. Acylation of one of the nitrogen atoms not attached to phosphorus could provide such a mode of activation since delocalization of electrons in the carboxylic amide group, for example (in IX), would militate strongly against the electronic distribution expressed in formula (VI).



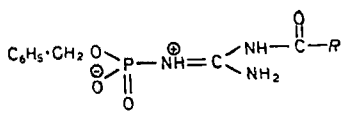
(IX)

Accordingly, a solution of benzyl hydrogen phosphoroguanidate (Va: $R=C_6H_5.CH_2$; $R'=H$) in a (1 : 1) acetic acid/acetic anhydride mixture was heated at 80° C for 2 h. Chromatography of the resultant solution revealed the presence of inorganic ortho-, pyro-, and trimetaphosphate (products of a hypothetical metaphosphate[17]), presumably formed in accordance with equation (3).

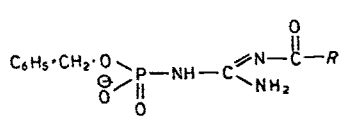


[$R = C_6H_5 \cdot CH_2$; Ac = CH_3CO·]

The dibenzyl ester (Va : $R=R'=C_6H_5.CH_2$) was (i) acetylated, and (ii) benzoylated, and in each case converted to the mono-benzyl ester by debenzylation in hydrochloric acid[12]. Infra-red and nuclear magnetic resonance spectra showed that, in both cases, the product possessed the structure (X : $R=CH_3$, or C_6H_5): however, in basic solution the ultra-violet spectrum[19] indicated that a proton had been removed to give (XI).



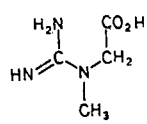
(X)



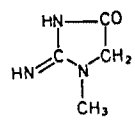
(XI)

In pyridine solution (XI) reacted to give a symmetrical product, namely, the $P^1 : P^2$ dibenzyl pyrophosphate dianion (cf. refs. 7 and 16).

In the phosphagens, a common structural feature is the presence of an additional acidic function attached directly to the carbon skeleton, for example, the carboxyl group in *N*-phosphoryl-creatine (I) and in *N*-phosphoryl arginine (II), the sulphonic acid residue in *N*-phosphorotaurocyamine[6], and the second phosphate residue in *N*-phosphoro-lombricine[6]. The remarkable increase in lability of the P—N link *in vivo* might then be ascribed to the further acylation of the guanidino-residue by this second acidic group. The cyclic form of one of these examples, namely (I), is well known. Creatine (XII) gives rise to creatinine (XIII) under just those conditions in which (I) is labile[20] (under acidic conditions *N*-phosphoryl creatine is hydrolysed 30 times faster than *N*-phosphoryl guanidine[21]), and in 0·1 N hydrochloric acid the



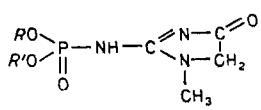
(XII)



(XIII)

cyclic form (XIII) is favoured to the extent of 96–97 per cent at equilibrium[22]. Moreover, *in vitro* hydrolysis of (I) appears[23] to give creatinine.

In order to test this hypothesis *N*-dibenzyl phosphoro-creatinine[24] (XIV : $R=R'=C_6H_5.CH_2$) was synthesized and monodebenzylated with hydrochloric acid to give the ester (XIV : $R=C_6H_5.CH_2$: $R'=H$). Both the mono- and the di-ester were phosphorylating agents undergoing reactions analogous to those of the acyclic acylated phosphoroguanidates (for example, IX and XI).

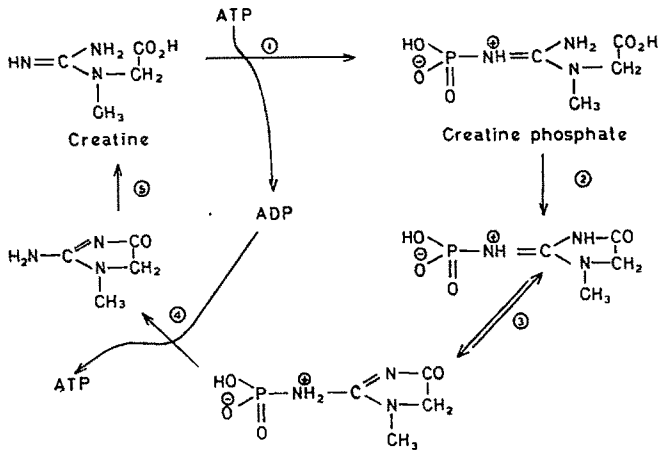


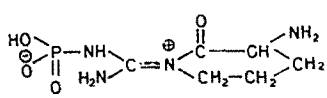
(XIV)

The experiments reported here lead us to propose a pathway for the ATP-creatine phosphotransferase catalysed reaction (Fig. 1) which can serve in a variety of organisms by the replacement of the *N*-phosphoryl creatinine by the cyclic compounds XV–XVII (cf. ref 6). The enzyme must hydrolyse the creatinine (XIII) formed in consequence of the phosphorylation of ADP, since rephosphorylation of the guanidinium moiety cannot be accomplished until the nucleophilicity of the guanidinium residue is restored by deacylation. Such a step would account for the absence of free creatinine in such systems (creatinine formation during *in vivo* phosphorylation has been reported[25] but this evidence may be discounted in the light of later reports[26]), the 'back reaction' being incomplete prior to the final hydrolysis to creatine.

The enzyme catalysing the transfer of a phosphoryl group from (I) to ADP is well known[27] and has been crystallized[28]. The phosphoryl transfer is known to be direct, that is, without any phosphorylated enzyme intermediate[29,30] and it requires at least one metal ion[31] (magnesium, manganese or calcium) bound to a phosphate group or groups in the nucleotide[32].

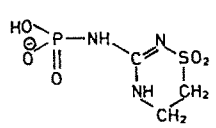
These biochemical data have led to considerable speculation[6,30,33] on the possible rôle (or rôles) of the phosphagens *in vivo*, but, thus far, no consideration has been given to the problem of the chemical stability of the phosphoroguanidates. The mode of action we suggest is in agreement with all the known data (and requires the features of general acid and general base catalysis already observed[34]). It accounts for the fact that the reaction can occur at all and also for the *in vivo* production of creatinine at a rate greater than that which could be accounted for by the cyclization of creatine itself[35].





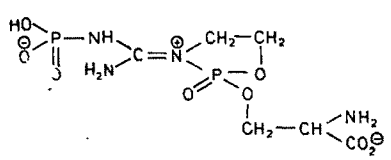
(XV)

'Active' arginine phosphate?



(XVI)

'Active' taurocyamine phosphate?



(XVII)

'Active' lombricine phosphate?

Fig. 1

Biochemical experiments are now required to test the model.

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NON-ENZYMATIC BROWNING: BROWNING OF PHENOLS AND ITS INHIBITION BY SULPHUR DIOXIDE

By H. S. BURTON, Dr. D. J. McWEENY and P. N. PANDHI

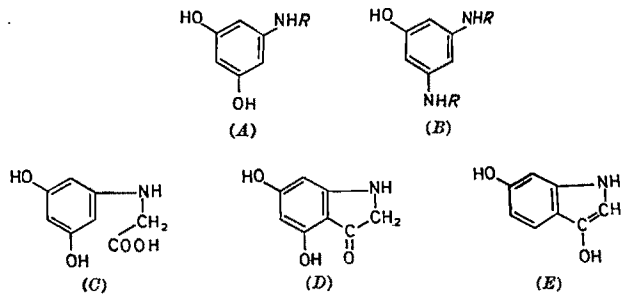
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THE main pathway to non-enzymatic browning seems to begin with the reaction between carbonyl and amino groups to give an unsaturated carbonyl/amino derivative.

Most natural substances contain phenolic compounds, and some polyphenols enter into reactions leading to discoloration and, it is claimed, into melanin formation. It is known that sulphur dioxide and sulphites react with polyphenols; these sulphur compounds also retard the development of colour in sugar/amino compound systems and in phosphatide/amino systems, where the initial reactions are of the carbonyl/amino type.

Rinderknecht and Jurd[1] reported that polyphenols combine with amino-acids in aqueous solution at low temperatures to give a yellow colour. The colour was obtained on heating phloroglucinol with glycine, whereas catechol, resorcinol, eriodictyol and quercetin did not give the colour. It therefore appeared that all three hydroxyls must be free, as in phloroglucinol, for this type of reaction, since both quercetin and eriodictyol contain a combined phloroglucinol nucleus. In support of this contention, resorcinol was found to be inactive in this respect. Phloroglucinol is said to exhibit triketonic properties and is known to react with amines to give products (*A*) and (*B*).

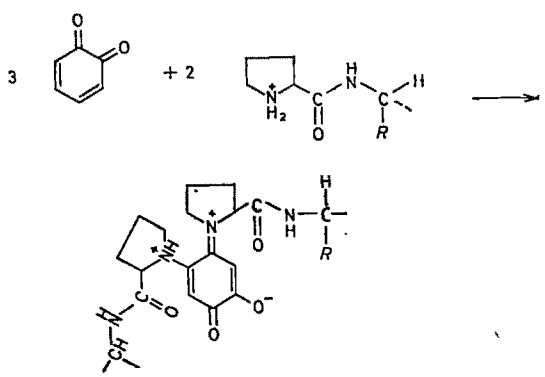
It is possible that cyclization of a condensation product such as (*C*) occurs giving a hydroxyindole. 5,6, Dihydroxyindole-2-carboxylic acid is the product which is said to be an intermediate in melanin formation[2]. Oxidized polyphenolics react, for example, with proline



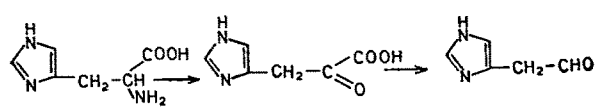
to give purple products[3]. Catechol, when oxidized reacts with glycine[4], and *O*-benzoquinone reacts with glycine ethyl ether to give a black solid.

Cresols, and homocatechol, give brilliant colours with aniline[5]. *O*-dihydroxy acids react via the *O*-quinone with NH_2 groups to give cross-linked structures, the N-function becoming attached directly to the aromatic nucleus[6]. The first step here is believed to be a N-substitution to the quinone nucleus in the 4 or 5, or both positions.

It is noteworthy that the following mechanism has been proposed for the enzymatic *O*-quinine/salmine reaction[7]:



Oxidized polyphenols may also de-aminate amino-acids with the formation of keto acids, which lose carbon dioxide to form aldehydes[8], for example:



Such aldehydes would also react with $-NH_2$ groups, and undergo condensations with substances likely to be present in naturally occurring materials, and in foodstuffs. The reactions of aldehydes in the presence of food substrates has not been fully explored, but they may well undergo condensations in the presence of other substances likely to be present in foodstuffs to give intermediates of high browning potential (cf. Overend and Stacey[9]).

In the presence of sulphites, such carbonyl reactions would be limited[10–12]. The effect of sulphites in retarding non-enzymatic browning and on the carbonyl-amino system has been reported on in a number of publications[11–15] and it was decided to examine its effect on the phloroglucinol/glycine system. The concentration of both reactants was 0·05 M, and the incubation was carried out at 19° C. In the sulphited series the concentration of sulphur dioxide was 2 mg/ml., added to the solutions as sodium metabisulphite, with an initial *p*H 6. The colour of the incubated solutions was measured by means of an EEL colorimeter at 460 mμ. Results are shown in Table 1.

Two things are at once apparent. The first is that even at low concentrations, the effect of the nitrogen function on the development of chromophores was marked, which runs parallel to our recent findings in related browning systems[10,12,13]. The second is that sulphites are effective

Table 1. Browning of Phloroglucinol/Glycine

Both reactants 0·05 M. *p*H 6·0. Incubated at 19° C. Concentration of sulphur dioxide, where present, 2 mg/ml.

Incubation period (days)	Colorimeter readings at 460 mμ: Phloroglucinol/glycine not sulphited	Phloroglucinol/glycine sulphited	Phloroglucinol not sulphited	Phloroglucinol sulphited
4	0·4	0·3	0·4	0·3
6	1·0	0·3	0·8	0·4
12	1·7	0·4	1·0	0·4
19	2·6	0·6	1·2	0·5
26	5·4	0·6	1·3	0·5
33	11·0	1·1	1·5	0·6
41	15·6	1·1	1·5	0·6
47	20·6	1·3	1·6	0·8
54	27·3	1·6	1·7	0·9
61	33·3	1·8	1·8	0·0
68	38·6	2·1	1·9	1·2

in retarding the development of chromophores in the phenol/amino-acid system, where presumably the phenolic groupings become oxidized.

Sodium sulphate had no such effect in retarding browning, and when incubated at 50° C with 0·05 M phloroglucinol/glycine at 0·03 M concentration the readings given in Table 2 were obtained.

Table 2. Browning of 0·05 M Phloroglucinol/Glycine in the Presence of Sodium Sulphate at 50° C

Incubation period (days)	Phloroglucinol/glycine	Phloroglucinol/glycine + Na_2SO_4
5	20·0	22·0
10	48·0	54·0

The effect of the nitrogen function, and of the effect of *p*H on the system is well demonstrated by the results shown in Table 3.

Table 3. Browning of Phloroglucinol and Phloroglucinol/Glycine (0·05 M) over the *p*H-range 3–8

Incubation temperature 50° C

Incubation period (days)	Colorimeter readings at 460 mμ after incubation at *p*H 3·0 Phlor./gly.	3·0 Phlor.	5·0 Phlor./gly.	5·0 Phlor.	7·0 Phlor./gly.	7·0 Phlor.	8·0 Phlor./gly.	8·0 Phlor.
1	0·4	0·3	1·4	0·83	7·0	3·9	45·3	70·0
3	0·7	0·36	3·6	1·0	23·0	5·4	88·0	123·0
6	1·4	0·6	7·3	1·46	46·0	5·8	163·0	166·0
10	2·3	0·9	19·0	1·96	88·6	6·2	270·0	166·0

There was again a great difference between the browning of phloroglucinol in the presence, as compared with the absence, of a nitrogen function at all acid *p*H's. The browning rate rose with increase in *p*H and once the solution became alkaline an enormous increase in browning occurred. A similar effect has already been recorded by us for the carbonyl/amino reactions[16]. It is known that alkaline treatment causes rapid autoxidation of polyphenols to give dark-coloured polymers.

Similar results were obtained with resorcinol in the presence and absence of glycine, as shown in Table 4.

Table 4. Browning of 0·05 M Resorcinol with and without Glycine, and of 0·05 M Phloroglucinol/Glycine in the Presence and Absence of Calcium Chloride ($CaCl_2.6H_2O$ = 0·545 g/l)

Incubation temperature 50° C

Incubation period (days)	Colorimeter readings at 460 mμ: Resorcinol/glycine	Resorcinol	Phloroglucinol/glycine	Phloroglucinol/glycine + $CaCl_2$
1	0·6	0·6	1·83	1·8
3	4·3	0·73	4·7	5·4
6	32·0	0·8	13·0	16·3
10	117·0	0·9	28·0	33·0

The effect of calcium on the browning of sugar systems[13,16–18], and carbonyl systems[10,12,18] ascorbic acid[19] systems and phosphatide systems[15] has already been examined. Calcium appears to retard somewhat the early browning of sugar systems but does not retard the browning of the other components. Calcium chloride is important in the retardation of non-enzymatic browning of some products, for example, White Rose potatoes brown too rapidly in the presence of statutory levels of added sulphite, but respond to conjoint treatment with the calcium salt. It does not appear to retard the browning of aqueous phenol systems. Table 4 shows that in the presence of calcium chloride, the colour developed by

phloroglucinol and glycine on incubation in aqueous solution was, if anything, somewhat enhanced.

Polyphenols containing ortho-dihydroxy and vicinal trihydroxy groupings have the ability to bind metal ions to form coloured complexes. Where such polyphenols occur in foodstuffs, or during the storage of foodstuffs, in the presence of metals, darkening effects may well be produced. Calcium has been known to cause such an effect[20], but it would appear that in some systems one ion may be preferable in the complexes to others due to the lower degree of coloration produced.

In the solid-state, the effect of metabisulphite in retarding colour development was examined by incubation at 50° C of slurries of phloroglucinol and phloroglucinol/glycine, with and without potassium metabisulphite, dried over phosphorus pentoxide *in vacuo* prior to incubation.

While in the dry solid-state the greatest red discoloration as observed visually occurred in the absence of glycine, when the phenol presumably underwent autoxidation and self-condensation. It is well known that polyphenols, particularly those with vicinal hydroxy groups, when absorbed on to a surface and exposed to air or sunlight, will brown rapidly. This was prevented to some extent by the presence of sulphite. When glycine was present the discoloration which occurred was yellower and less red in shade when observed by eye in the dry state, and the discoloration was only very slight in the presence of metabisulphite after five months' incubation. The incubated mixtures were dissolved in water and examined by means of the EEL Colorimeter using a range of filters. Some results are given in Table 5.

Table 5. BROWNING OF PHLOROGLUCINOL AND PHLOROGLUCINOL/GLYCINE (EQUIMOLAR) IN THE SOLID-STATE AT 50° C

Metabisulphite where added to give 1/10 mole SO_2/mole. Dilution 0·2 g of incubate in 10 c.c. water

Incubate	Colorimeter readings after incubation for 150 days (mμ)							
	570	540	520	490	460	450	690	600
Phloroglucinol	1·6	2·3	2·7	3·5	4·2	3·7	0·8	1·2
Phloroglucinol/sulphite	1·0	1·5	2·6	2·0	2·5	2·2	0·6	0·8
Phloroglucinol/glycine	1·5	2·3	2·6	3·6	4·5	3·9	0·9	1·2
Phloroglucinol/glycine/sulphite	0·8	1·2	1·4	1·9	2·45	2·3	0·4	0·6

Over the range examined, the colour produced by the equimolar phloroglucinol/glycine mixture was somewhat greater than that produced by the phloroglucinol alone, but the reduction in colour density effected by the metabisulphite was greater in the glycine series. Some phenolic compounds may, however, cause sulphite loss by a different mechanism to that postulated for carbonyl/amino systems[13], dihydroquercetin, for example, converting bisulphite to thiosulphate, which in turn catalyses the decomposition of bisulphite to thiosulphate and sulphate[21]. When oxidized to the quinone form, many phenolic compounds and phenol acids couple with amino groups of amino-acids to yield darker compounds than those produced by self-condensation[22–24], and even the addition of hydroquinone to dehydrated vegetables produces some increase in degree of browning on storage[14]. Such discoloration was still much reduced in the presence of sulphite, and acid hydrolysable sulphur dioxide still remained in such products after 2½ years storage in nitrogen[14]. In the storage investigations, the sulphitation was carried out at the same time as the blanching, but observations in other fields[25,26] suggest that careful control of *p*H conditions might be necessary for some products depending on the predominance of certain reactive species.

From these observations it is clear that whether in the solid-state or in weakly acid solution, the non-enzymatic browning of phenolic compounds in the presence of a nitrogen function is faster than non-nitrogenous browning, and that sulphite is effective in retarding the onset and development of such browning. Effective browning retarding agents are both carbonyl group reagents and reducing agents, and this is clearly an example of browning associated with carbonyl and amino groupings which sulphites help to prevent developing in some foodstuffs.

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MECHANISM OF SOLANESOL BREAKDOWN DURING PYROLYSIS

By J. D. GROSSMAN, R. M. IKEDA, E. J. DESZYCK and A. BAVLEY

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THE aroma of tobacco smoke has not been fully explained on the basis of the flavour constituents naturally present in tobacco. A number of the steam-volatile constituents in tobacco has been described by Onishi in several publications[1]. Several other workers in this field, particularly Weybrew[2] and Stedman[3], studied the differences in the volatile components of various grades of tobacco. However, tobacco resins, because of their complexity, have been studied only cursorily. As a part of a programme to determine the contribution of tobacco resin constituents to the overall flavour of smoke, we investigated the pyrolysis products of solanesol. Solanesol was selected because it was the first of such tobacco resin constituents to be isolated and described in terms of its chemical structure. The structure of solanesol was established by two groups of investigators: Rowland,

Latimer, and Giles in 1956[4] and Folkers and co-workers[5] in 1959. They proved that it had the following structure:

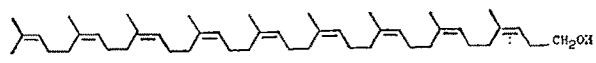


Fig. 1. Solanesol

During our investigation of the pyrolysis of solanesol, we isolated and identified several C_{10}-terpenoid hydrocarbons. We showed that these hydrocarbons are formed partially by dimerization of isoprene, one of the pyrolysis products of solanesol[6]. After establishing the structure of the products present in the C_{10}-hydrocarbon fraction and correlating them with the products formed by the pyrolysis of isoprene and dipentene under identical conditions, it became evident that the products formed during the pyrolysis of solanesol could not be explained fully as being formed by the dimerization of isoprene. Higher yields of dipentene than would be expected led us to believe that other mechanisms of breakdown were involved. By pyrolysing solanesol in atmospheres of hydrogen versus nitrogen, we found quantitative differences in the yields of the C_{10}-hydrocarbons. These differences confirmed our belief that solanesol might be breaking up by other routes.

In a recent publication by Johnstone[7], a mechanism was proposed for the formation of a C_{10}-biradical during the pyrolysis of solanesol with subsequent cyclization to give products such as 1,3-dimethyl-1-vinyl *cyclo*hexene and dipentene. We postulated that this mechanism could account for some of the quantitative differences in the yields of the C_{10}-hydrocarbons. It had been shown in the recent work on the pyrolysis of toluene and of deuterated toluenes[8] that the free radicals which are formed during pyrolysis can be terminated by hydrogen. It occurred to us that, in a hydrogen atmosphere, an intermediate free radical formed during the pyrolysis of solanesol can terminate by capturing hydrogen, giving different yields of C_{10}-hydrocarbons. At this time we want to present evidence that supports our theories that solanesol might be breaking up: (1) through the formation of a C_{10}-biradical which is terminated by capturing hydrogen; (2) through the direct breakdown of solanesol to give dipentene.

First, it was necessary for us to develop a method for isolating solanesol in large quantities from tobacco. Although solanesol has been synthesized[9], it is difficult to produce in large quantities. Instead of using the conventional method of adsorption chromatography[4] for its isolation we developed a simplified procedure by which tobacco was extracted with an appropriate solvent and the solanesol isolated by molecular distillation and crystallization.

Ten pounds of aged tobacco was exhaustively extracted with petroleum ether. The extract (4·5 l.) was washed twice with 1-l. portions of a 10 per cent hydrochloric acid solution, and then with water until it was neutral. All washes were discarded. The same procedure was repeated, using a 5 per cent sodium hydroxide solution. The neutral extract was dried over anhydrous magnesium sulphate, filtered, and the solvent removed under reduced pressure at 40° C. The residue was dissolved in 2 l. of hot acetone, cooled overnight at 4° C, and filtered to remove the solids.

The de-waxed solution was concentrated to a syrup under the conditions previously mentioned, leaving 400 ml. of a dark brown residue. The residue was transferred into a 2-in. molecular still (Arthur K. Smith, Inc.) and, after removal of traces of the solvent, it was subjected to distillation at 300° C and 100–200μ pressure. Two-thirds of the material was distilled under these conditions as a viscous, orange-coloured liquid. Solanesol was crystallized by dissolving the distillate in a minimum amount of ether (200 ml.), covering the solution with methanol (300 ml.) without mixing the two layers, and cooling overnight at 4° C. By recrystallizing three times, we obtained 20 g of solanesol (a crystalline white material, m.p. 33°–34° C) and identified it by its infra-red spectrum. The acetate of the crystalline material melted at 31°–32° C and the 3,5-dinitrobenzoate at 55°–56° C.

All samples of solanesol prepared in this way showed excellent stability, and did not decompose for several weeks on storage under ordinary conditions.

Solanesol was passed under nitrogen through a 30-cm 'Vikor' tube filled with porcelain chips which were heated to the desired temperature. The pyrolysis was carried out in a nitrogen atmosphere, maintaining a constant dropping rate of material into the apparatus. The pyrolysis products were collected in a trap cooled with a dry ice–acetone mixture. The total pyrolysis product was separated into the volatile components by distillation at 40° C. The remainder of the mixture was then separated by fractionation on silica gel into a hydrocarbon and an oxygenated fraction. The hydrocarbon fraction was then separated into various components by gas chromatography.

We established that temperature is a critical factor in the production of the hydrocarbon components; namely, at temperatures up to 550° C the hydrocarbon fraction was composed mainly of monoterpenoids with traces of aromatic products being present. At higher temperatures, aromatization is favoured. This has been previously established with other compounds[10–12]. We observed no formation of terpenoids from solanesol at temperatures above 650° C. Instead, various aromatic compounds were formed. It has been shown[11] that at high temperatures even such compounds as acetylene and butadiene give rise to mixtures of aromatic hydrocarbons.

Our method of isolation of the pyrolysis products is outlined in the following diagram:

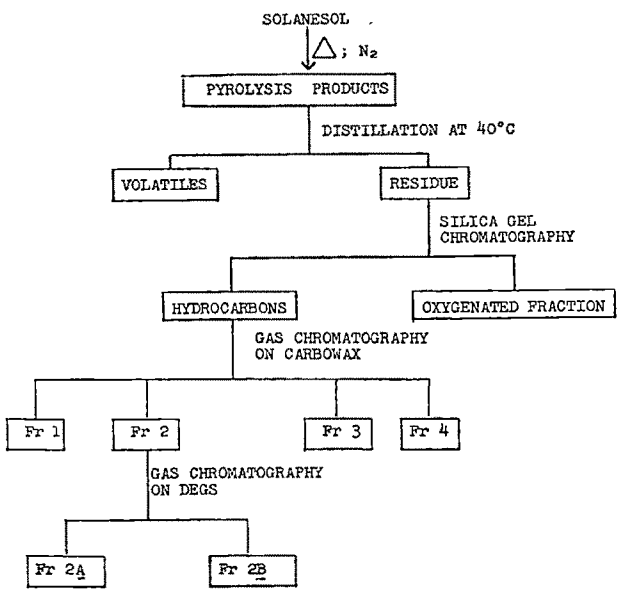


Fig. 2. Scheme of isolation of products from pyrolysis

From work described in the literature, only the mechanisms of pyrolysis of α- and β-pinene[13], *d*-limonene[1], and allo-ocimene[14] have been described. These investigations were significantly helpful to us in the interpretation of solanesol pyrolysis data.

Examination of the volatile pyrolysis constituent by mass spectroscopy, infra-red, and ultra-violet analyses established that the main products in this mixture are isoprene and 2-methyl-2-butene. Several other not yet identified dienes were also present.

The terpenoid fraction was separated by preparative gas chromatography into five fractions.

Fraction No. 1. This fraction was identified by ultra-violet, infra-red, and mass spectroscopy as consisting of a mixture of α- and β-pyronenes. It had been reported previously that these compounds arise from the pyrolysis of terpene hydrocarbons[10,14]. Rechromatography of component No. 2 separated the material into two fractions.

Fraction No. 2A. This material had a molecular weight of 138. Infra-red spectra indicated a tri-substituted double bond. On hydrogenation this material absorbed 2 moles of hydrogen. The resulting product did not have a menthane structure. The hydrogenated product was different from synthetic 2,6-dimethyl-octane prepared by hydrogenation of myrcene and slightly different from synthetic 2,7-dimethyloctane prepared by coupling 3-methyl-1-butene with sodium borohydride-BF_3 and treating it with silver nitrate. Mass spectrometric analysis of the hydrogenated product isolated from the pyrolysis showed that we had obtained a 60–40 per cent mixture of the two dimethyloctanes. Therefore, we conclude that our pyrolysis sample must be a mixture of 2,6-dimethyl and 2,7-dimethyl octadienes. The 2,7-dimethyl octadiene must arise by dimerization of isoprene formed during the pyrolysis process.

Fraction No. 2B. This fraction was characterized by infra-red, nuclear magnetic resonance and mass spectroscopy as being similar to dimethyl-vinyl *cyclo*hexene, which arises from dimerization of isoprene[7]. On hydrogenation, it absorbed 2 moles of hydrogen, giving a product with a different mass spectroscopic pattern of the dimethyl-vinyl *cyclo*hexene hydrogenated product obtained by the dimerization of isoprene. We think that the difference in these two compounds is due to the position of the substituents on the *cyclo*hexene ring. Thus, in the case of isoprene, the compound which is formed is probably the 1,4-dimethyl, 1-vinyl *cyclo*hexene. Our solanesol pyrolysis product, on the other hand, was identical with one formed during the pyrolysis of dipentene and must have been formed through a 2,6-dimethyl-octadiene free radical to give the 1,3-dimethyl, 1-vinyl-isomer.

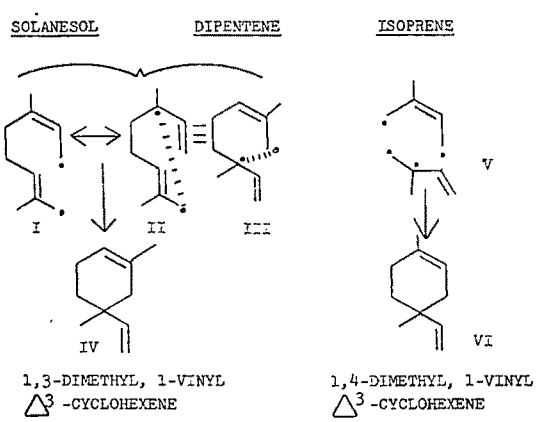


Fig. 3. C_{10} Hydrocarbons (Fr. 2*B*)

Fraction No. 3. Purification of this fraction resulted in the isolation of Δ-1-menthene identical with a reference standard.

Fraction No. 4. This was identified as dipentene, as was reported previously[6].

The pyrolysis of solanesol in a hydrogen atmosphere was accomplished by following essentially the same procedure as that for the pyrolysis in a nitrogen atmosphere. The whole apparatus was flushed thoroughly with nitrogen, and then was flushed thoroughly for half an hour with hydrogen. The pyrolysis was accomplished in a hydrogen atmosphere. The C_{10}-hydrocarbons were isolated, following the same fractionation procedure. Examination of the volatile pyrolysis constituents by mass spectroscopy and gas chromatographic analysis established that they were a mixture consisting of 64 parts isoprene, 8 parts 2-methyl-2-butene, 1 part each of 2-methyl-1-butene and 3-methyl-1-butene, with a trace of isopentane being present. The C_{10}-hydrocarbon fraction obtained during pyrolysis in the hydrogen atmosphere was compared quantitatively with the products obtained during the pyrolysis of solanesol in nitrogen.

The results are given in Table 1.

Table 1

Fraction (pyrolysis of 500° C)	H_2 atm (% of total C_{10}-terpenes)	N_2 (% of total C_{10}-terpenes)
1, Pyronenes	4·17	7·13
2*A*, (Dimethyloctadiene)	19·12	11·82
2*B*, (Dimethyl vinyl *cyclo*hexene)	7·01	12·20
3, Δ^1-Menthene	8·54	12·84
4, Dipentene	52·72	45·31

As can be seen from the results given here, there is a considerable increase in the dimethyloctadiene formed during pyrolysis of solanesol in a hydrogen atmosphere and a decrease in pyronenes, dimethyl-vinyl-*cyclo*hexene and Δ^1-menthene. This confirms one of our suggested mechanisms of breakdown of solanesol; namely, by the formation of a dimethyloctadiene biradical which in a hydrogen atmosphere terminates by capturing two hydrogens, thus forming dimethyloctadiene. This, consequently, caused a lower yield of dimethyl-vinyl-*cyclo*hexene and the other products which are formed through this intermediate biradical.

The increase of dipentene formed during the pyrolysis of solanesol in the hydrogen atmosphere tends to support the third mechanism of its pyrolysis. This mechanism gives dipentene directly through an 'unzipper' type of mechanism illustrated in Fig. 4.

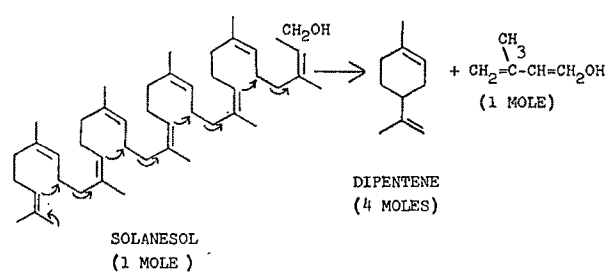


Fig. 4. Direct breakdown of solanesol to dipentene

C_{10}-terpenoid hydrocarbons are formed during the pyrolysis of solanesol by several routes. These pathways of formation have been established: (1) Through the dimerization of isoprene into dipentene and other C_{10}-hydrocarbons. (2) Through the formation of a C_{10}-biradical which is terminated by capturing hydrogen in a hydrogen atmosphere. (3) Through the direct breakdown of solanesol to give dipentene.

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REPLICATIVE FORM OF ENCEPHALOMYOCARDITIS VIRUS RIBONUCLEIC ACID

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INFECTIVE ribonucleic acid (RNA) molecules, extracted from encephalomyocarditis (EMC) virus preparations, each have a size corresponding to the whole RNA content of one virus particle[1,2], as well as physical properties indicating that they exist in solution as single-stranded chains, thus resembling ribosomal and other viral RNA's[3]. Stirred suspensions of Krebs-II ascites tumour cells, infected *in vitro* with EMC virus[4], have been used to investigate the mode of replication of such a single-stranded viral RNA. We now have evidence that an unusual kind of infective RNA, the properties of which are consistent with a double-stranded structure, may be present in Krebs-II cells during infection by EMC virus.

The use of specific inhibitors has led to the conclusion that the replication of small RNA viruses is not dependent on the integrity of host DNA[6,7]; nor does it require DNA synthesis[5,7]. Actinomycin, which suppresses DNA-dependent RNA synthesis[8], also fails to inhibit EMC[7], Mengo[6], or poliovirus[9] multiplication. Moreover, replication of the RNA of bacteriophage *MS*-2 in *E. coli*[10], and Mengo virus RNA[11] in *L* cells, follows the appearance of a specific polymerase able to synthesize RNA directly on an RNA model. Cline, Eason, and Smellie[12] have also observed stimulation of an RNA-primed RNA transferase in our system of Krebs-II ascites cells infected by EMC virus.

These observations suggest that the RNA of the invading virus particle somehow serves directly as a model for the synthesis of new viral RNA. How, then, does this occur? Double-stranded DNA almost certainly replicates by a mechanism involving progressive separation of the two strands, and the formation on each of a further complementary chain[13] in which the sequence of bases is determined by the rules of base pairing laid down by Watson and Crick[14]. The same scheme can be applied to the replication of single-stranded DNA, phage $\varphi X174$ DNA being double-stranded when in its replicative form[15].

Whether viral RNA also first makes a complementary chain, so that the duplex can then replicate like DNA, or whether it utilizes some peculiar mechanism without base pairing[16] is at present unknown. If the first suggestion is correct, an RNA of double-stranded structure should be found at some stage of viral replication. The physical properties of such an RNA in solution should resemble those of DNA, since the structure deduced from X-ray diffraction patterns of the helical hairpin-like portions of transfer RNA does not differ fundamentally from that of a DNA double helix[17]. Moreover, such an RNA duplex should exhibit a sharp thermal transition, and show some resistance to degradation by ribonuclease[18]. We believe that the material we have isolated has a sufficient number of these properties to identify it as a double-stranded RNA of viral origin. No similar double-stranded RNA has as yet been found in uninfected cells, and we suggest that the material present in infected cells may be the replicative form of the viral RNA.

Separation of the bulk of infective RNA extracted from infected Krebs-II cells into two components. Infective RNA can be extracted from infected cells with phenol some time before mature virus particles appear[19]. This RNA sediments just like RNA extracted from purified virus, although it shows up as a broader band on a sucrose gradient[2]. To show that this band contains replicating, in addition to finished, viral RNA, we have first to establish that the replicative form of viral RNA is, in fact, extracted by phenol; in mammalian cells a nuclear RNA fraction associated with DNA, and rapidly labelled by radioactive precursors, is not extractable by phenol alone, but only when a detergent is present[20,21]. Since viral RNA replication is likely to occur in nuclei[22,23], nuclear RNA has to be considered in this context. Thus a technique has been developed for extracting all possible fractions of infective RNA from EMC-infected Krebs-II cells. Two forms of this technique have been used in the experiments to be described.

(1) DNA, as well as all the RNA components, are first extracted from cells without previous separation. Infected, washed, Krebs-II cells are treated with 1 per cent sodium dodecylsulphate in presence of bentonite (0·1 per cent) and 0·05 M EDTA, at room temperature; the viscous homogenate resulting is deproteinized by three phenol extractions, and the mixed nucleic acids treated as described earlier for cellular RNA extracted with aqueous phenol[2]. DNA is then destroyed by treatment with deoxyribonuclease (previously freed from traces of ribonuclease by the technique of Polatnick and Bachrach[24]) (Worthington, 4 μg/ml., 30 min 22° C, in sodium chloride 0·1 M, magnesium chloride 0·01 M). DNase treatment is halted by the addition of EDTA to a concentration of 0·01 M, and after a new cycle of deproteinization by phenol and bentonite, nucleic acids are precipitated twice with ethanol. The precipitate contains all RNA fractions in a natural state, as well as oligodeoxyribonucleotides. (2) Here we make an initial separation between RNA's extractable by aqueous phenol, and that closely associated with DNA. Infected cells are first extracted with phenol, which releases ribosomal and transfer RNA into the aqueous phase. After centrifugation at low speed the aqueous phase is decanted, the phenol layer discarded, and the interface carefully washed and re-extracted 2–4 times with aqueous phenol. Addition of 1 per cent of sodium dodecylsulphate at this stage releases the majority of the DNA and its associated RNA into the aqueous phase. The separate fractions are then further treated as in (1).

To obtain a picture of the appearance of infective RNA, a series of extractions of cells infected with purified virus[25] was carried out at various times during the cycle of virus growth using variant 1 of the technique. The extracted RNA's were then centrifuged on a sucrose gradient[2], and the gradient fractions assayed for infectivity and ultra-violet absorbance, as described by Montagnier and Sanders[2]. As shown in Figure 1*a*, most of the infectivity sediments in front of the 30 *S* ribosomal component, just like RNA extracted from purified virus[2]. As already stated, at all times after infection this infectivity band is broader than that given by RNA from pure virus; sub-fractionation of the sedimentation band shows a definite heterogeneity, some portions sedimenting faster than 37 *S*. A similar heterogeneity was also found in very early stages of infection—immediately after virus adsorption in the cold, when most of the recorded infective RNA comes from virus particles which have adsorbed

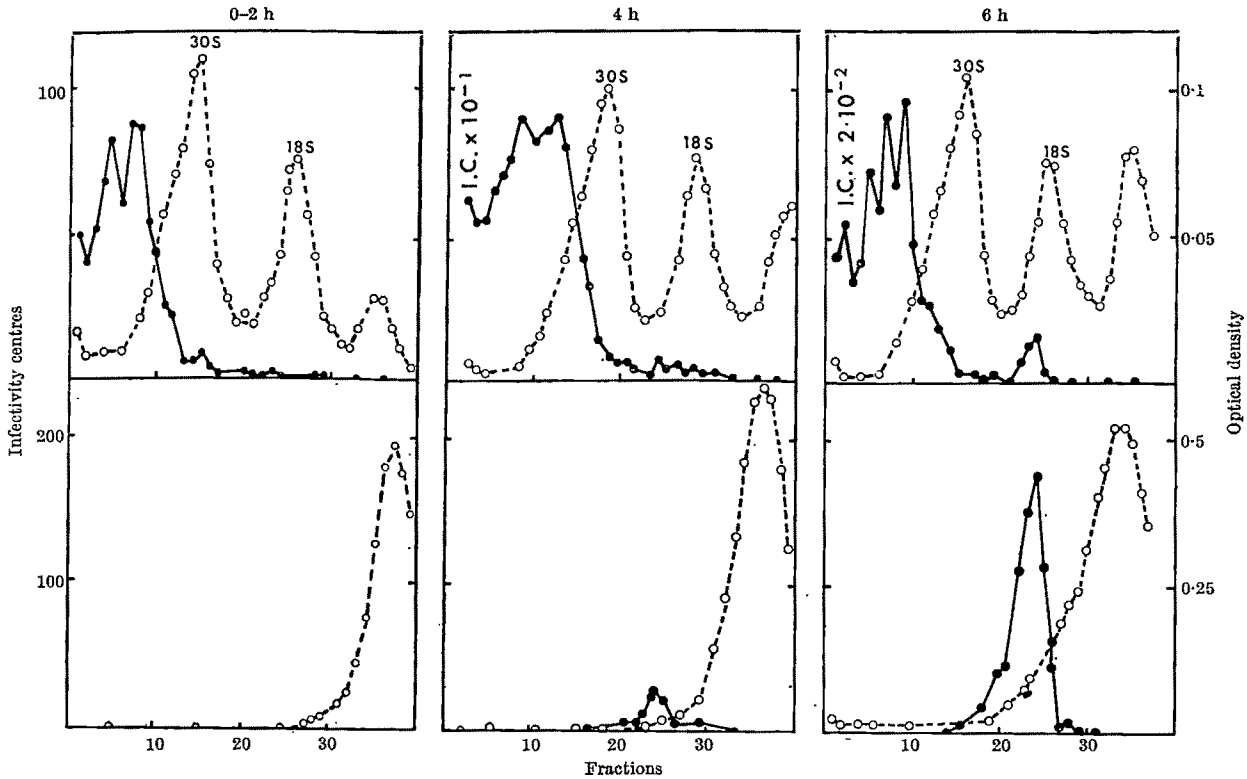


Fig. 1. Sedimentation in sucrose gradient (10–20 per cent) of RNA extracted from Krebs II cells at different intervals after infection by EMC virus (15*H*30, 22,500 r.p.m., in *tris*-hydrochloric acid 0·005 M, EDTA 0·02 M, *p*H 7·2). Top row: (*a*), untreated; bottom row: (*b*), same samples treated by ribonuclease (10^{-1} μg/ml., 30 min 22° C) immediately before centrifugation. Continuous line, infectivity; dotted line, optical density at 260 mμ. The scales of the ordinates in the top and bottom rows are different

on cell membranes, but not yet begun to replicate. It is therefore unlikely that this 'heavy' RNA represents the replicative form.

However, Fig. 1 also shows, and only at times after which viral RNA has begun to replicate, a second peak of RNA infectivity sedimenting in front of the 18 *S* ribosomal RNA component. Its position in the gradient suggests a sedimentation coefficient of about 20 *S*. Though the 20 *S* peak represents only a small proportion of the total infectivity, the amount of this fraction increases with time, especially at the end of the cycle of infection, when cells are dying and releasing virus (Fig. 1 and Table 1).

Table 1. Appearance of Ribonuclease-resistant 20 *S*-Sedimenting Material (Infectivity in Percentage of Control not treated by RNase) in Krebs-II Cells infected with EMC Virus (Multiplicity 10)

Time after infection (h)	Experiment I RNA extracted by variant 1 (%)	Experiment II Extraction variant 2	
		Fraction extractable by phenol (%)	Remaining fraction extractable by sodium dodecylsulphate (%)
0*	0 (< 0·1)	—	—
2	0 (< 0·1)	—	—
4	0·1	0·05	—
5	—	0·05	3
6	1	0·8	65
14 (90% dead cells)	12	—	—

* Immediately after adsorption of virus at +4° C.
RNase treatment: Armour crystallized RNase (10^{-1} μg/ml., 30 min, 22° C in *tris* 0·005 M EDTA 0·02 M *p*H 7·2).

Treatment by pancreatic ribonuclease (0·1 μg/ml., 22° C, 30 min) results in a dramatic disappearance of the 37 *S* infective peak, while the infectivity of the 20 *S* component is unaffected (Fig. 1*b*). Moreover, its slow sedimentation and ribonuclease resistance allow separation of this RNA from the rest of the RNA present.

Similar experiments performed with RNA's extracted by variant 2 of the method described show that only a small part of the total infectivity lies in the fraction not extractable by phenol. Up to 5 h after infection, the infectivity recoverable from this fraction has similar properties of sedimentation and RNase sensitivity to the bulk of the infective RNA extracted by phenol. However, in the later stages of infection, accumulation of the 20 *S* form occurs faster here than in the fraction extractable by phenol (Table 1).

Physical and chemical properties of the 20 S infective form. Sufficient amounts of impure 20 *S* material have been obtained from batches of dying infected cells to enable its physical properties to be measured. Variant 1 of the extraction technique was used, and the product centrifuged on a sucrose gradient. Infective fractions sedimenting in the 20 *S* region were pooled together and concentrated by ethanol precipitation. Studies using biological criteria were performed without further purification.

(i) *Ribonuclease resistance.* In solutions of moderate ionic strength, the infectivity of the 20 *S* form totally resists destruction by the enzyme up to a concentration of 1 μg/ml. in the conditions of time and temperature mentioned here. Under the same conditions, the infectivity of RNA sedimenting in the 37 *S* region, whether extracted from infected cells or from purified virus, is reduced to a value of less than 0·001 per cent of that of control untreated preparations. At lower ionic strength and higher temperature, the 20 *S* form becomes more sensitive to RNase.

(ii) *Effect of formaldehyde.* Preliminary results indicate that the infectivity of the 20 *S* form resists treatment with formaldehyde, in conditions where 37 *S* form has lost 98 per cent of its original infectivity.

(iii) *Solubility in sodium chloride.* The 20 *S* form is soluble in 1·5 M sodium chloride, like DNA and transfer RNA, while the 37 *S* infective form is insoluble, like ribosomal RNA.

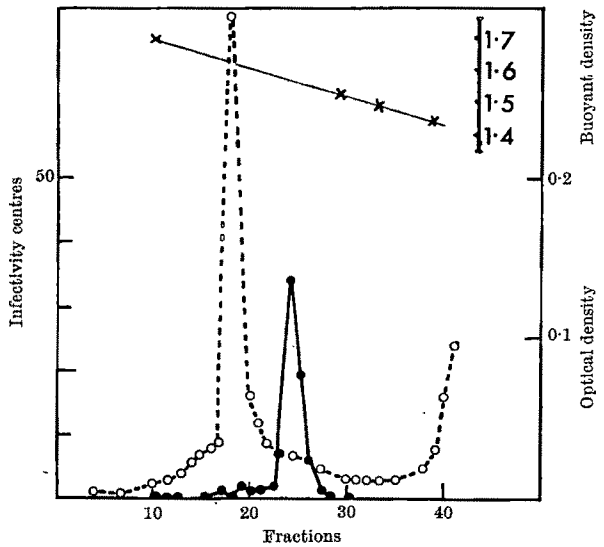


Fig. 2. Equilibrium density centrifugation in cæsium sulphate of 20 *S* infective RNA mixed with an excess of Krebs II ribosomal RNA. (44 h, 33,500 r.p.m. in *SW*39 head.) Continuous line, infectivity; dotted line, optical density at 260 mμ. Density measurements by refractometry

(iv) *Buoyant density in cæsium sulphate.* When centrifuged to equilibrium in concentrated cæsium sulphate, solutions of DNA (double-stranded) have a buoyant density of 1·42 [26], single-stranded ribosomal RNA 1·63 [26]. The latter forms aggregates at high concentration, so that its band-width is narrower than would be expected from its molecular weight. The 20 *S* infective RNA forms a band at a density of 1·57 (Fig. 2) and does not aggregate. Its band-width is comparable with those given by cellular DNA with a molecular weight of 6 millions under the same conditions of centrifugation. The 37 *S* component bands at the place of single-stranded ribosomal RNA.

(v) *Effect of heating on the absorbance of* 260 *mμ.* Only double-stranded DNA and helical polynucleotides exhibit a sharp thermal transition[27]. EMC virus RNA, like ribosomal or tobacco mosaic virus RNA, shows a broad thermal transition owing to the melting out of short intramolecular helical regions (Fig. 3).

To study the effect of heating on the 20 *S* form, we attempted further purification of the material with associated infectivity. The DNase treated product of extraction was precipitated by 1·5 M sodium chloride to remove ribosomal and 37 *S* viral RNA. The supernatant containing oligodeoxyribonucleotides, transfer RNA and the 20 *S* infective form, was precipitated with ethanol, redissolved in 0·15 M sodium chloride, 0·015 M sodium citrate and treated with pancreatic ribonuclease (1 h, 25° C, 0·1 μg/ml.). After removal of ribonuclease by addition of 0·1 per cent of bentonite and centrifugation at high speed, alcohol precipitation and redissolution of the pellet in 0·015 M sodium chloride, 0·0015 M sodium citrate, the final product was fractionated on a column of 'Sephadex *G*200'. The first peak to appear, judged by ultra-violet absorbance at 260 mμ, contained all the infectivity, had ratios $\frac{260}{230}$ and $\frac{260}{280}$ of 2·0, and was orcinol-positive and Dische[28]-negative, indicating the presence of ribose rather than deoxyribose.

The thermal transition of 260 mμ absorbance was examined on the fraction having the highest specific infectivity (5×10^4 plaque-forming units/μg). After a gradual increase up to 70° C, there was a very sharp transition (Fig. 3). The T_m's (melting temperature) were, respectively, 96° C, in 0·15 M sodium chloride, 0·015 M sodium citrate; and 84° C, in 0·015 M sodium chloride, 0·0015 M sodium citrate.

Discussion

All the properties of the 20 *S* infective component are those typical of a molecule having highly organized secondary structure. Before we can be reasonably sure that this is double-stranded RNA, made from a single-stranded chain of viral RNA bound by base pairing to a complementary chain, we must consider whether this molecule could be a DNA-viral RNA hybrid, which would also be resistant to nucleases. However, the buoyant density in cæsium sulphate of such a hybrid would be considerably lower (1·49 [29]); no deoxyribose is detectable; the melting temperature is very high. All these facts, as well as the results of experiments using inhibitors mentioned at the beginning of this article, are against the hybrid hypothesis. Assuming that the material we have found has similar hydrodynamic properties to double-stranded DNA, its molecular weight, calculated from a sedimentation coefficient of 20 *S*, would be $6{\cdot}7 \times 10^6$ (ref. 30). This is about twice the value of the molecular weight found for the single-stranded RNA from purified virus[1,2].

Unfortunately, confirmation of the double-stranded nature of this RNA by determination of its base composition cannot be done with EMC virus, where single-stranded RNA already has ratios adenine/uracil guanine/cytosine very close to one[25]. The necessary confirmation could perhaps be obtained with poliovirus, where adenine/uracil and guanine/cytosine ratios in the single-stranded viral RNA differ from one[31].

If we assume that the double-stranded nucleic acid isolated from our system is really concerned in virus replication, some of its other unusual features have to be accounted for; for example, unlike the double-stranded replicative form of single-stranded φ*X*174 DNA[15], double-stranded EMC RNA only comprises a small proportion of the infective RNA found in infected cells; most of the latter is single-stranded. Experiments in which infected cells have been labelled with radioactive RNA precursors in the presence of actinomycin gave similar results to experiments where RNA was identified by the criterion of infectivity; most of the label becomes incorporated in single-stranded viral RNA, and only a very small amount in the double-stranded material.

So if the mechanism of replication was strictly semi-conservative (all the newly produced chains replicating

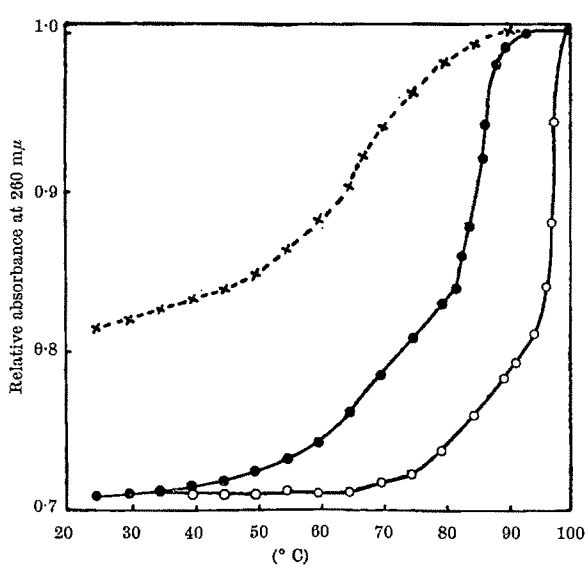


Fig. 3. Absorbance at 260 mμ as a function of temperature. Filled circles, infective 20 *S* material in sodium chloride 0·15 M, sodium citrate 0·015 M; open circles, the same in sodium chloride 0·015 M, sodium citrate 0·0015 M; crosses, single-stranded 37 *S* RNA extracted from purified EMC virus, in sodium chloride 0·15 M, sodium citrate 0·015 M

as well as the infecting chain) it would be necessary to assume that the double-stranded stage has a very transient existence compared with the single-stranded stage. Alternatively, the number of double-stranded molecules may remain very small during active replication; the greater part of the new duplexes formed may be immediately split into their two constituent chains, only one containing the viral information serving to initiate specific protein synthesis on the ribosomes.

In either case, the fate of one of the chains is uncertain. It is unlikely that they both contain the necessary base sequences to direct synthesis of viral protein via the ribosomal system[32]. In HeLa cells infected with poliovirus, the base ratios of RNA, newly synthesized in the presence of actinomycin, resemble those of purified virus RNA, and so do not reflect an equal synthesis of complementary RNA[33,34]. This suggests that the complementary chain may be destroyed soon after replication without attachment to ribosomes.

Bellett and Burness[22] found that infective RNA (presumably single-stranded) extractable from Krebs cells by phenol was at first mostly located in the nuclear fraction, and Zalta *et al.*[23] have reported an increase of infectivity after incubation *in vitro* of nuclei from infected Krebs-II cells. The high proportion of the double-stranded form found in the nuclear fraction of RNA which resists extraction by phenol also suggests that the replication, as well as the opening of the strands, may occur in the cell nucleus. This hypothesis is not incompatible with the results of Franklin[35], which show radioactive precursors located mostly in the cytoplasm of *L* cells infected with Mengo virus; in these rapidly growing cells the nuclear stage of virus replication may be very short compared with the cytoplasmic stage, as has been pointed out by Dulbecco[36]. Indeed, experiments in progress show that in *L* cells also double-stranded material accumulates during the later stages of the cycle.

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DIURNAL RHYTHMS OF ELECTROLYTE EXCRETION IN DEPRESSIVE ILLNESS

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THE term "manic-depressive insanity" was first used by Kraepelin[1] in 1899, although the disease had been previously described by Falret[2] and Baillarger[3] in 1854, who independently reported recurring attacks of mania and depression in the same patient. From their observations, the concept has developed of an endogenous or physiological type of depressive illness which can be distinguished ætiologically and clinically from an exogenous, reactive illness determined primarily by psychological events. In spite of the genetic evidence presented by Slater[4], Kallmann[5] and other workers however, this concept has remained controversial: recently the evidence derived from the exploitation of physical treatments has forced a revaluation of the position and established the need for a careful examination of the physiological status of psychiatric patients.

A classic symptom of endogenous depression, first described by Schneider[6] in 1920, is a diurnal variation of mood in which the depressive process is typically at its maximum in the early hours of the morning and tends to improve during the day. This contrasts with the findings in psychogenic depression where very frequently each new day starts more hopefully than it finishes. It is also claimed that the sleep disturbance in the two types of depression is characteristically different so that in the endogenous depression sleep is most difficult in the early hours of the morning. These diurnal variations in symptomatology and the disruption of the normal pattern of sleep and wakefulness suggest that it might be worth looking for abnormalities in other physiological functions normally showing diurnal rhythms. Such rhythms in the renal excretion of water and electrolytes have intermittently attracted the interest of physiologists since Quincke[7] reported in 1877 that the volume of urine excreted at night is reduced compared with that excreted during the daytime.

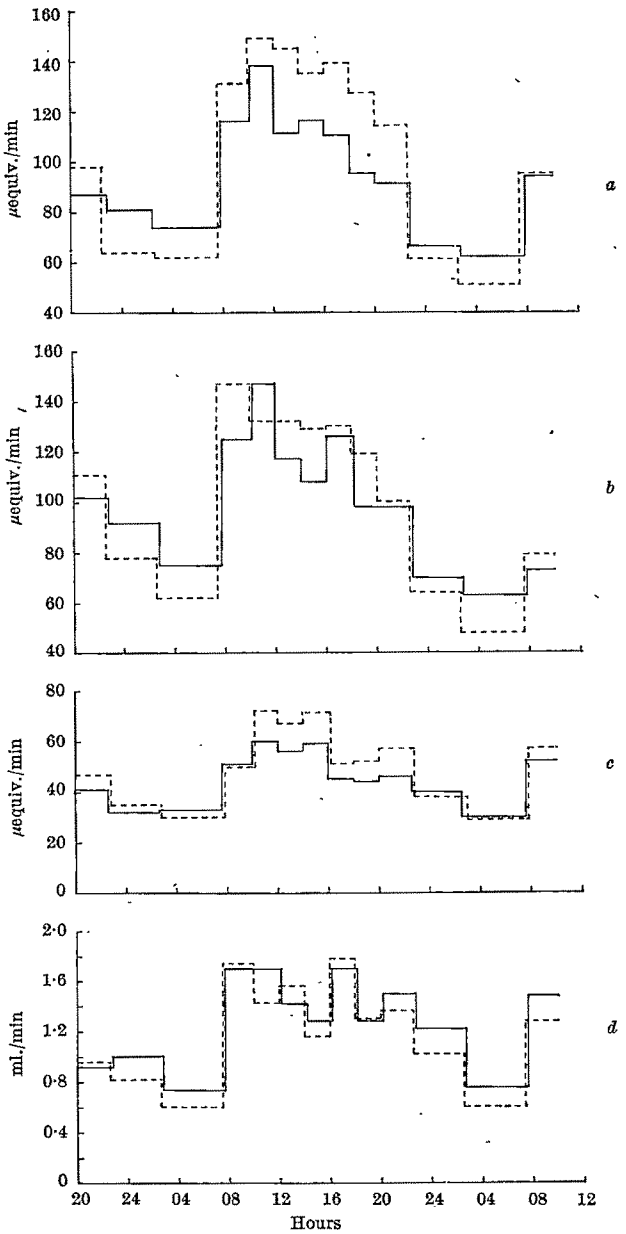


Fig. 1. —, Depression; – – –, neurosis: *a*, chloride; *b*, sodium; *c*, potassium; *d*, urine volume

This article describes briefly the result of an investigation of the renal diurnal rhythms of 20 "endogenously depressed" patients treated in the psychiatric ward at the Royal Free Hospital. These findings are related to similar observations obtained with 25 "neurotic" controls. Observations were made consecutively on all admissions.

The diagnoses were those derived on clinical grounds in the absence of any knowledge of the experimental findings and patients in whom there was any suspicion of schizophrenia or organic disease were excluded from the analysis.

Shortly after admission each patient was placed on a standard régime, which started at 20.00 h when the patient emptied his bladder. The bladder was again emptied at the following times: 22.30; 02.30; 07.30; 10.00; 12.00; 14.00; 16.00; 18.00; 20.00; 22.30; 02.30; 07.30; 10.00. On each occasion the volume and the actual time at which urine was passed were recorded and a specimen kept for analysis. In addition, observations were made on oral temperature, pulse, blood pressure and reaction time. Throughout the test period the patients were kept on a standard hospital metabolic diet.

Fig. 1 shows the mean rates per minute of water and electrolyte excretion for the group of neurotic and for the group of depressive subjects over the different periods of the 38 h of the investigation. In all cases a clear diurnal rhythm is seen with rates higher between 07.30 h and 22.30 h than during the preceding and succeeding night periods. In the case of sodium and chloride, and to a lesser extent potassium, but not water, the amplitude of this diurnal variation is greater in the neurotic group than in the depressives. This difference is not present in the excretion of water and this excludes the possibility that it is brought about by differences in the efficiency of the collection procedures for the two groups. There are no important differences in overall excretion rates, but if the first night is compared with the second, both groups show a tendency to sodium and chloride retention during the second night. This is probably a consequence of the standard diet which had a relatively low sodium content (80 m.equiv./day) and could have influenced excretion rates during the latter half of the test period. Both groups were equally affected and the effect therefore is not relevant to differences between the day/night partitions for the two groups. As would be expected, the results for sodium and chloride excretory patterns were very similar. The depressives when compared with the neurotics excrete less of these electrolytes during the day and more by night. For potassium, the night excretory rates are very similar for the two groups; but by day the excretory rate is greater for the neurotics, giving again for this group a diurnal rhythm of greater amplitude.

In order to assess the significance of these findings and the importance of differences in medication and age, we have expressed the results as day/night ratios for each of the three electrolytes. For each subject the total amount of electrolyte excreted between 08.00 and 20.00 h has been calculated as a percentage of the total excretion

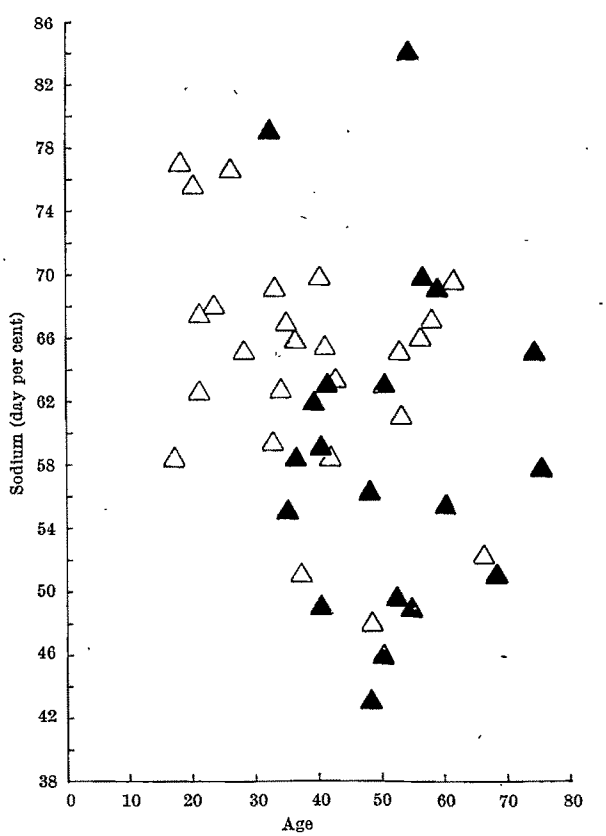


Fig. 2. ▲, Neurotics; △, depressives

over the period of 24 h. The figure for the night is the mean of the two night periods. Since the early morning specimen was collected at 07.30 the 12-h figures have been prorated from the two periods 02.30–07.30 and 07.30–10.00. Ranking these ratios the Mann–Whitney U test shows that the differences between the two groups are statistically significant for sodium ($P = 0{\cdot}012$) and for chloride ($P = 0{\cdot}035$) but not for potassium ($P = 0{\cdot}08$).

Unfortunately, the condition of many patients precluded the possibility of their ceasing treatment for the test and we have therefore assessed the relationship of the day/night ratios to medication. Patients having drug treatment ($n = 15$) were compared with those having no drug treatment of any kind ($n = 30$), those having antidepressant drugs ($n = 11$) with those not having these drugs, and those having phenothiazines ($n = 10$) with those not. In no case was the difference significant nor were any of the differences marked.

Another difference between the two groups of patients which needs to be considered is that of age. The mean age of the depressive group was 50·3 years and that of the neurotic group 37·6 years (Mann–Whitney U test, $P = 0{\cdot}003$). So far as we are aware there is no published data on the relationship of the diurnal excretory rhythm to age. For the present subjects the relationship between the day/night ratios for sodium and age and diagnosis is shown graphically in Fig. 2. Since the distribution of the ratios is reasonably normal, we have calculated the product moment correlations of their ratios with age for the two groups. For the neurotics $r = -0{\cdot}37$; for the depressives $r = -0{\cdot}09$. Neither of these coefficients is significant but that for the combined group ($r = -0{\cdot}308$) just reaches the 0·05 level of confidence. While the regression on age does not account for all the difference between the two groups, there is insufficient data to decide whether the ratios for the two groups are different if age is taken into account. Further observations are being made and will be reported elsewhere. In the meantime it may be observed that the range and the variance of the day/night ratios are greater for the depressives, and that while increasing age might well be associated with a reduction in the amplitude of the diurnal rhythm, there is no reason to suppose that it would produce an actual reversal. Six of our patients showed such a reversal and of these five were diagnosed as depressive.

It seems likely therefore that there are in depressive illness disturbances of the diurnal rhythm of electrolyte excretion which are not secondary to changes in behaviour or diet.

We thank Dr. S. W. Hardwick, Sister Guiver and other members of the staff of the Royal Free Hospital, and Mrs. M. Kerr for advice with statistical analysis. One of us (A. E.) was supported by the Medical Research Council and another (P. B.) by the Mental Health Research Fund.

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USE OF PAPER IMPREGNATED WITH ESTERS OF *O*-PHENYLPHENOL TO REDUCE THE ROTTING OF STORED FRUIT

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In recent years the use of wraps or liners impregnated with diphenyl has become one of the standard methods for reducing the rotting of oranges. Diphenyl wraps cannot, however, be used to reduce the rotting of fruits other than citrus. All deciduous fruits take up diphenyl from diphenyl treated wraps and acquire an unpleasant taste and attempts to find other volatile fungicidal compounds which could be used instead of diphenyl to reduce the rotting of all stored fruits have so far been only partially successful.

The use of diphenyl was first suggested in 1936 [1,2]. I had previously found that wraps impregnated with iodine[3] reduced the rotting of grapes and of tomatoes, and later found that *o*-phenylphenol alone, or in mineral oil[4], or with hexamine[5] reduced the rotting of citrus and some other fruits. Iodized wraps were found to be effective under commercial conditions, but because they stained the wood wool packing they were commercially unacceptable. Wraps containing *o*-phenylphenol when used on a large scale were found to be unsatisfactory because they often caused damage to fruit. More recently Spenser and Wilkinson[6] found that lentical rotting of apples caused by *Gloeosporium* could be reduced by storing them in trays to which liners impregnated with 2.4.5-trichlorophenoxytrichloromethane had been fixed. Direct contact with the treated liners caused damage to the fruit. Eckert and Kolbezen[7] were able to control the spread of *Rhizopus* in packed tomatoes with paper wraps impregnated with active halogen compounds such as dichloro- or dibromodimethylhydantoin or dichloroisocyanuric acid.

It has now been found that it is possible to reduce the rotting of tomatoes, citrus and some other fruits by the use of paper impregnated with certain esters of *o*-phenylphenol, many of which are both volatile and fungicidal, and if introduced in small quantities into the lid of an inverted Petri dish will inhibit or reduce the rate of growth of fungi on the nutrient medium contained in the upper part of the Petri dish.

The extent of damage caused to fruit by contact with paper impregnated with esters of *o*-phenylphenol varies considerably. Some esters caused an extensive browning or pitting of the surface of some fruit within 3–5 days, though with other fruit there was no damage or so little damage as to warrant small scale trials of the extent to which these esters could reduce rotting of these fruits.

In preliminary trials, wraps (10 in. × 10 in.) containing 50 mg of the *o*-phenylphenol esters of caproic, methoxyacetic, benzoic or crotonic acids were not effective in reducing rotting and did not cause damage. But wraps containing 50 mg of the esters of acetic, propionic, butyric or isobutyric acids considerably reduced the rotting of samples of oranges which had been wounded and loaded with spores of *P. digitatum*. The wraps containing isobutyrate caused no damage to the surface of the oranges, those with acetate or butyrate caused a slight pitting and browning of the surface while those with the propionate caused extensive damage.

In other trials, wraps impregnated with the *o*-phenylphenol esters of acetic, butyric or isobutyric acids were found to be as effective as diphenyl wraps in reducing the rotting of citrus; Table 1, for example, shows the relative effects of paper impregnated either with *o*-phenylphenyl acetate or with diphenyl. In this experiment the *o*-phenylphenyl acetate paper caused slight pitting of the

Table 1. No. (per cent) of Oranges rotting (in Samples of 30), wrapped in Plain Wraps or Wraps impregnated with *o*-Phenylphenyl Acetate, or with Diphenyl

(50 mg per 10 in. × 10 in. wrap)

Temperature of storage	Type of wrap	Days in store 6	11	18	27
20° C	Untreated	97	100		
	Diphenyl	40	97		
	o-Phenylphenyl acetate	23	33	80	90
12° C	Untreated	—	100		
	Diphenyl	—	33	84	84
	o-Phenylphenyl acetate	—	26	67	70
5° C	Untreated	—	—	33	100
	Diphenyl	—	—	10	70
	o-Phenylphenyl acetate	—	—	13	50

oranges. The extent of damage caused by *o*-phenylphenol esters depends in part on the amount contained in the wrap and when the amounts per wrap were reduced from 50 to 25 or 12·5 mg the damage was reduced without their effect on rotting being seriously reduced.

The possibility of reducing liability to damage by dissolving the esters in some mineral or vegetable oil, or other carrier before incorporation in the paper has been considered, but a suitable one has not so far been found.

Table 2 shows the extent to which the rotting of English glass-house tomatoes stored under conditions of high humidity was reduced by the use of wraps impregnated with *o*-phenylphenyl acetate. The wraps were effective even at 40° F, a temperature at which tomatoes suffer low temperature injury and are rendered more liable to rotting. The tomatoes suffered neither damage nor taint. Grapes and some soft fruits appear not to be damaged by contact with paper impregnated with *o*-phenylphenyl acetate. Apples and pears are susceptible to injury but

Table 2. No. (per cent) of Tomatoes rotting (in Samples of 25), wrapped in Plain Wraps and in Wraps containing 12·5 mg of *o*-Phenylphenyl Acetate

Temperature of storage	Type of wrap	Days in store 14	17	21	24	28
15° C	Plain	28	64	84	92	92
	Treated	0	0	0	4	12
10° C	Plain	8	20	48	64	68
	Treated	4	4	4	4	4
5° C	Plain	0	0	44	88	96
	Treated	0	0	0	0	0

in some instances rotting has been reduced without damage to the fruit. Hence it appears that *o*-phenylphenyl acetate (12–25 mg per 10 in. × 10 in. wrap) may well prove a substance which could be used in paper to prevent the rotting of tomatoes and some other deciduous fruits, and that it may also prove as effective as diphenyl in reducing the rotting of citrus.

It may be possible that on further investigation some slightly less volatile ester will be found to be generally more suitable than the acetate and further esters are being examined. The isobutyrate, for example, appears to be less damaging to citrus than the acetate. One reason for exploring the use of *o*-phenylphenol compounds is that data already exists on the extent to which *o*-phenylphenol is taken up by fruit[8] and on the toxicity of *o*-phenylphenol[9]. Dipping in a solution of sodium *o*-phenylphenate alone or with the addition of hexamine or excess sodium hydroxide is extensively used to reduce the rotting of fruit and limits have been fixed in the United States and in England to the amounts of *o*-phenylphenol treated fruits may contain.

It is, of course, possible that esters of *o*-phenylphenol could be used in dips, wax coatings, etc., for the preservation of fruits or other materials of biological origin, but it is their use in paper wraps which appears to be of greatest interest.

The *o*-phenylphenol esters were supplied by Borax Consolidated, Ltd., and Theodore St. Just, Ltd. A provisional patent application (*U.K. PA* 25694/63) covering the use of these esters has been filed jointly by the National Research Development Corporation (acting on behalf of the Agricultural Research Council) and Borax Consolidated, Ltd.

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A COMMON LINK BETWEEN PHOTOSYNTHESIS AND RESPIRATION IN A BLUE-GREEN ALGA

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CONTINUING investigations of the Emerson enhancement effect have revealed in the blue-green alga *Anacystis nidulans* a new phenomenon which appears important to the formulation of a two-light-reaction mechanism for photosynthesis. At wave-lengths absorbed predominantly by chlorophyll *a* the light-intensity curve of photosynthesis shows two distinct linear segments as observed in terms of net rate of oxygen evolution. The phenomenon is far more dramatic and more easily measurable than the minor 'non-linearities' previously observed in *Chlorella*[1].

Independent and prior observation of the same phenomenon has been communicated to us by Hoch, Owens and Kok[2]. Their measurements with a mass spectrometer and oxygen isotopes permitted simultaneous measurement of oxygen uptake and oxygen evolution and provide the key to interpretation. In *Anacystis* they could distinguish between two effects of increasing intensity at 700 mμ: (1) a linearly increasing rate of oxygen production; (2) a depression in rate of oxygen uptake which became saturated at about 50 per cent of the rate in darkness.

Our investigations are carried out with a modified Blinks–Haxo polarographic electrode[3], and therefore are less definitive in measuring only changes in net oxygen exchange. At the same time our precision and rapidity of measurement and our use of two symmetrical light beams of 4·3-mμ half band-width[1] have allowed measurements which broaden the interpretation.

Our electrode is a platinum strip, maintained at 0·5 V (versus saturated calomel electrode), which forms the floor of a chamber 250μ deep and separated from flowing nutrient solution above by a 'Cellophane' membrane. Any change in net rate of oxygen exchange by an algal film, as between light and darkness, is reflected by a proportionate change in current flow. In work with *Chlorella* a film of algal cells was deposited directly on the platinum surface; however, blue-green algae deposited in the same way showed a decaying response to a standard illumination. Analysis of concentration profiles in the electrode chamber leads to the belief that algal cells on the cathode surface experience a very low oxygen concentration, a high OH^- ion concentration, and hence a potentially low concentration of carbon dioxide. We were able to obtain

reasonably stable day-to-day response with *Anacystis* by depositing the cells on an agar layer which held them about 100μ above the platinum cathode surface. One cell preparation then provided stable material over a period of a week although within a series of measurements on one day there was a small measurable decay.

Figs. 1 and 2 show the results of a set of experiments which have been repeated several times with variations only in detail. At a wave-length absorbed predominantly by phycocyanin the rate of net oxygen evolution increases linearly with intensity and the curve extrapolates approximately to the origin (curve *D*). At wave-lengths absorbed predominantly by chlorophyll *a* the corresponding curves (*A*, *B*, *C*) always show two linear segments: a high initial slope at low intensity followed by a lower secondary slope at high intensities. From the analysis of Hoch, Owens and Kok[2], we attribute the high initial slopes to the combined effects of a decreasing rate of oxygen uptake and an increasing rate of oxygen production. The lower slopes at higher intensities represent only the actual rate of oxygen production superimposed on a lowered but now constant rate of oxygen uptake.

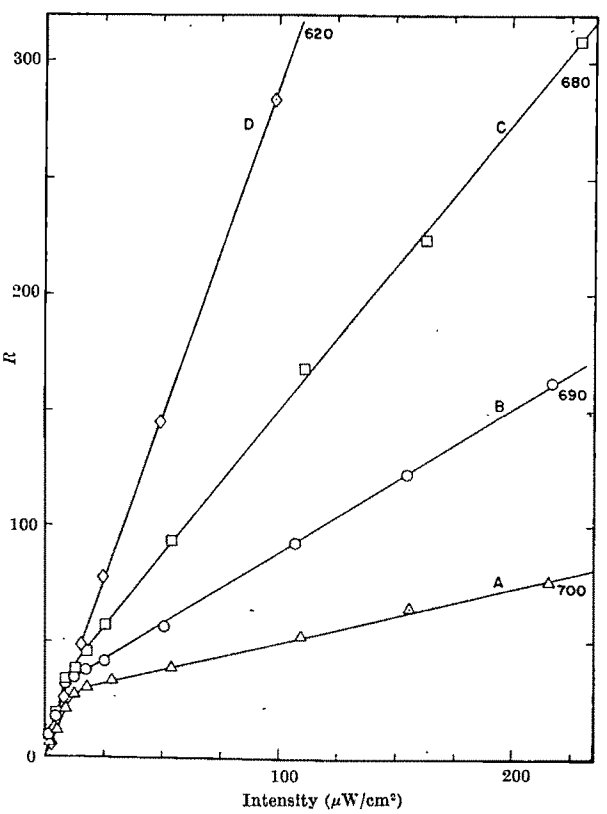


Fig. 1. Net rate of apparent oxygen production, *R*, of *Anacystis nidulans* as a function of light intensity. *R* is measured in relative units of 1×10^{-8} amp change in polarographic current. Each of the curves describes response to the wave-length indicated in mμ. The relatively thick algal film used gave nearly complete light absorption. Experimental temperature was 26° C; the cells had been grown at 37° C under conditions of high cell concentration and high tungsten illumination

Fig. 2 shows the change in rate of net oxygen evolution in response to intensity at 700 mμ when added to a constant background intensity. The reference curve *A* (dark background) is taken from the first set of experiments of Fig. 1. Its slopes are somewhat higher than those of the other curves only because of a measured 10 per cent decay in response which occurred between the two sets of experiments. Curve *E*, obtained with a constant background of 700 mμ, shows only one linear segment extrapolating to zero but otherwise corresponding to the second and lower slope of curve *A*; the low intensity effect has been saturated and obscured by the 700 mμ background.

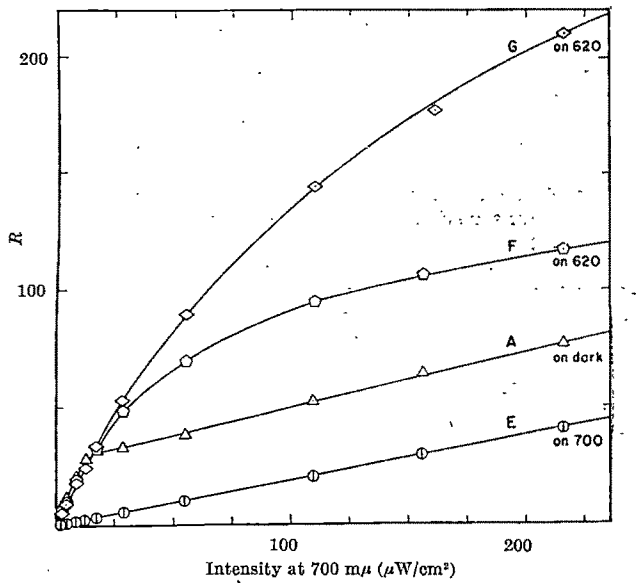


Fig. 2. As Fig. 1, but for response to 700 mμ when superimposed on various background illuminations of constant intensity: *A*, on dark, repeated from Fig. 1 and without correction for small decay in response between the two sets of experiments; *E*, on 180 μW/cm² 700 mμ; *F*, on 35 μW/cm² 620 mμ; *G*, on 145 μW/cm² 620 mμ

Conversely, curves *F* and *G* (620 mμ background) follow the higher initial slope of curve *A* and prolong its duration to an extent dependent on intensity of the 620 mμ background. Curves *F* and *G* demonstrate enhancement. Curve *F*, for the lower level of 620 mμ background, reaches the same final slope common also to curves *A* and *E*; 620 mμ background becomes limiting so that added increments of 700 mμ are not enhanced.

Further treatment of the results is presented in Table 1. The ratio of the slopes of the two linear segments of each of the three wave-lengths is the same as the maximum enhancement attainable at these wave-lengths (columns 2 and 3). The initial high slope at each wave-length (curves *A*, *B*, *C*) is the same as the corresponding slope of its curve when enhanced by 620 mμ background (for example, curve *G*). Columns 4 and 5 demonstrate that for all three of the long wave-lengths chosen there is a common limit to the decrease in oxygen uptake. The actual magnitude of this figure has varied between experiments, apparently depending on thickness of the film and past history of the cells.

Table 1

λ (mμ)	Ratio of slopes initial/ sec	Maximum enhancement*	Magnitude of decrease in oxygen uptake† extrapolated intercept	measured directly‡
680	3·2	3·2	25	28
690	6·2	6·2	27	26
700	10·3	10·6	26	27

* Calculated as $(R_\lambda)_{620}/(R_\lambda)_{700}$.
† In relative units as used in Figs. 1 and 2.
‡ Measured as $(R_\lambda)_{dark} - (R_\lambda)_{700}$ at 25 μW/cm².

Several different lines of investigation[4–7] provide evidence for two light reactions separated by an electron transport system, as originally envisaged by Hill and Bendall[8]. One light reaction, driven by quanta absorbed by accessory pigments, delivers to an electron transport system electrons ultimately derived from the oxidation of water. Electrons pass through the electron transport system in dark reactions and are furnished to a second light reaction. The second light reaction, driven by quanta absorbed by, or transferred through chlorophyll *a*, delivers electrons at a potential capable of phosphopyridine nucleotide reduction.

A low net quantum yield occurs at long wave-length and enhancement becomes possible when the chlorophyll *a* reaction receives quanta at a rate greater than its supply

of electrons. Supplementary light of wave-length absorbed predominantly by accessory pigments increases the rate of the first light reaction and delivers electrons to the second light reaction at a rate potentially in excess. Excess long-wave quanta absorbed by the chlorophyll *a* system then can be used, giving enhancement as measured by oxygen production[1].

In *Anacystis* a higher intensity increment of 700 mμ can be enhanced about 10 times by background light at 620 mμ (Table 1). Hence we estimate that, in the absence of 620 mμ background, approximately 90 per cent of the quanta available to the chlorophyll *a* reaction at 700 mμ is ineffective as measured by oxygen production. However, the effect of a low-intensity increment of 700 mμ light cannot be enhanced as observed in terms of net oxygen exchange. We conclude, therefore, that at long wave-length a large fraction of the quanta available to the chlorophyll *a* reaction can be used effectively in suppressing oxygen uptake. It appears that the electron acceptor for the chlorophyll *a* reaction, the P_{700} of Kok[6], is at least partially a competitor of oxygen as an electron acceptor.

Preliminary experiments with *Anabaena variabilis* show the same phenomenon presented in Fig. 1 except that the initial slopes extend to higher intensities, as might be expected in an alga with a much higher rate of respiration[9]. However, repetition with *Chlorella pyrenoidosa* under present conditions revealed only the minor 'non-linearity' noted previously. Apparently the phenomenon is not general to all photosynthetic systems, but may be common in the blue-green algae. Other observed anomalies[10–12] of effects of light on oxygen exchange do not fit the pattern suggested herein. It seems likely that other interactions between pathways of electron transport for photosynthesis and respiration will be discovered.

For the chlorophyll *a* reaction in algae there now appear to be three possible sources of electrons: (1) the accessory pigment reaction; (2) hydrogen via hydrogenase[13]; (3) respiration.

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ASSYNT DYKES AND THEIR METAMORPHISM

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FOR many years the unravelling of the history of the metamorphic complexes of the Lewisian rocks of the north-west Highlands of Scotland and the Hebrides has been greatly aided by a swarm of dolerite dykes, commonly known as the 'Scourie' dykes. At the beginning of the century, the Geological Survey workers[1] recognized that these dykes, intruded into the Early Gneiss Complex, were later affected by the 'Pre-Torridonian Movements'. Sutton and Watson[2] elaborated on these events and used the terms 'Scourian' and 'Laxfordian' to describe the two metamorphic periods separated by the period of dyke intrusion.

The intrusion of basic dykes with chilled margins into the 'Scourian' complex was thought indicative "that this section of the crust had become rigid enough to fracture and cool enough for basaltic magma to chill rapidly", and that "when the dolerites were intruded all metamorphism had ceased"[2]. Absolute age determinations by Giletti *et al.*[3] showed that at least 800 m.y. separated the two metamorphisms, but the exact position of the dolerite dykes on this extended time scale has until very recently remained unknown.

During the past two years, however, the re-interpretation of these early generalizations by various workers has aroused much controversy. O'Hara[4] has regarded the development of a hornblende-plagioclase ± garnet assemblage in the chilled margins of the basic dykes in the Scourie area as being an autometamorphic effect, best explained by intrusion of the dolerite magma into the gneiss "at a time when the rocks were still deeply buried and relatively hot (a temperature of 300°–500° C would be consistent both with chilling of the magma and with the generation of amphibolite facies assemblages)". Dearnley[5], on the other hand, has suggested that these margins may record the existence of an 'Early Laxfordian' granulite facies metamorphism which he recognizes in the Outer Hebrides.

Evidence that there may be more than one series of basic dykes in the Lewisian has been advanced by Park and Bowes[6] on the basis of the structural relations at Gairloch and they regard the later set as being contemporaneous with the Laxfordian metamorphic event. Wright[7] has put forward similar suggestions. Other workers have, however, expressed disagreement with such interpretations.

O'Hara[8] has described four different types of dykes—an amphibolite, a norite, a typical 'Scourie' dolerite, and thin ultrabasic dykes—in close association near Badcall, Sutherland, and again reiterates his view that these dykes were intruded into hot country rock and that there was no great period of uplift coincident with the intrusion of these dykes.

Thus the main arguments seem to centre on the age or ages of the basic dykes, the conditions under which they were intruded and the variety of dyke types represented. It is the purpose of this article to summarize some of my observations which may be of interest in these respects.

The Assynt district is favoured by perhaps the greatest concentration of intrusive dykes on the whole foreland and has been commonly regarded as an area little modified by the effects of the Laxfordian metamorphism[2,5]. At least eight types of dykes can be recognized in the area:

(1) *The 'Epidiorites' of the Survey.* Quite abundantly represented, thick quartz dolerites with distinctive coarse gabbroic texture. Rather rarely fresh central parts of these dykes are preserved; otherwise the whole suite is completely amphibolized and usually recrystallized in the lower amphibolite facies.

(2) *Dolerite dykes.* Similar to (1), but richer in pyroxene and with less quartz. Two pyroxene types are also represented. As with the epidiorites much of the suite is completely altered.

(3) *Loch Crocach 'Peridotite'*. This dyke and its branches is completely altered throughout its length. Ghost textures in the tremolite–biotite–plagioclase assemblage suggest an original pyroxene-rich dolerite.

(4) *Picritic dykes.* Olivine–orthopyroxene–augite–plagioclase–biotite dykes which are often wide and rather coarse grained. The margins of these dykes are orthopyroxene-rich and not chilled. These dykes are all slightly different from each other, so were probably not intruded simultaneously. They are considerably less altered than the basic dykes in the same areas, though nevertheless altered rock is more abundant than fresh rock.

(5) *Olivine gabbro dykes.* Olivine–augite–orthopyroxene–plagioclase–biotite–kaersutite–ore dykes, probably related to the picrites. These dykes have finer grained augite-rich margins, but are not chilled. Their state of alteration is comparable with that of the picrites.

(6) *Thin finer-grained dolerites found in the vicinity of Loch Assynt.* These dykes have a similar mineralogy to the earlier dolerites—augite–plagioclase–quartz–primary green hornblende–biotite–ore—but the plagioclase displays a characteristic deep brownish clouding. In spite of their narrow width these dykes are considerably less altered than the earlier basic dykes in comparable areas. Occasional chilled veins of these dykes are seen to cut the picrites.

(7) *Two pyroxene dolerites, with minor olivine, which is largely enclosed by the orthopyroxene.* One dyke on the south side of Loch Assynt is completely fresh and has unaltered chilled margins. Some probably related dykes on the north-east side of Loch Assynt show low-grade alteration.

(8) *Thin basic dyke running to the south of and parallel to the picrite ('Teall's Dyke') west of Loch Assynt.* This is an olivine–augite–plagioclase–biotite–ore rock and the absence of quartz and hypersthene, the sodic character of the plagioclase and the high content of biotite suggest that it may be of alkali type. The dyke is unusually fresh, the development of secondary amphibole being limited to an inch or so at the margins.

The order given here is, on the available evidence, the order of intrusion as found in the field, except that there is no evidence as to the relative order of intrusion of the last three dyke types.

What is significant is that the various dyke types have undergone different degrees of alteration and recrystallization, and that the extent of this is roughly related to the order of intrusion, with the earlier dykes being more altered. These features are difficult to reconcile with a period of metamorphism after the intrusion of the various dykes, but the relations become more feasible if it is postulated that the dykes were intruded during and towards the close of a metamorphic episode, with the degree of alteration being dependent on the length of time being spent under metamorphic conditions. It may be mentioned in passing that a high proportion of the gneisses in Assynt have a lower amphibolite or upper greenschist facies mineralogy; pyroxene gneisses have a very limited distribution. Most of the altered basic dykes belong to the lower amphibolite or upper greenschist facies (see also ref. 9).

Confirmatory evidence that the picritic and olivine–gabbro dykes were intruded into hot country rock is provided by the coarse-grained margins of these dykes and also by the complex crystallization history which they have undergone which is difficult to interpret except under conditions of slow cooling at depth. The chilled margins of the basic dykes are probably more a function of the higher viscosity of the magma than of the temperature of the country rock.

Some idea of the relative temperature of the gneiss at the time of intrusion and alteration of the picrites is afforded by the common occurrence of the assemblage olivine–tremolite–chlorite ± talc in the thoroughly altered picrite dykes wherever the original rock contained an excess of olivine. Serpentine is not developed. This stability of olivine in a hydrous environment would indicate temperatures of about 500° C according to the experimental data of Bowen and Tuttle[10] on the MgO–SiO_2–H_2O system.

Another interesting feature of the picrites, particularly with regard to the high proportion of olivine and orthopyroxene they contain, is the complete absence of any indication of the common reaction relationship between these two minerals, as seen in near surface intrusions. Boyd and England[11] have recently shown that the reaction relation is eliminated at high pressures and their experiments suggested a pressure of about 5–6 kbar as the minimum necessary to overcome the relationship. The depths of the Earth's crust corresponding to these pressures would be about 15–20 km, and temperatures at these depths would be about 500° C under typical values for the geothermal gradient of 25°–30°/km. This temperature is not in conflict with that suggested here by the stability of the dyke olivines. Hence these values serve to confirm the view that the Assynt dykes (except perhaps the later three types) were intruded at considerable depth into hot country rock, and that their intrusion clearly does not mark a period of great uplift of the basement rocks.

The effects of the early metamorphism of the basic and ultrabasic dykes are most widespread in the western part of the area, particularly near the belt of west-north-west–east-north-east trending vertical gneisses north of Lochinver. Here the ultrabasic dykes have suffered considerable chemical interchange at their margins with the gneisses or adjacent basic dykes, and the typical assemblage tremolite–chlorite ± anthophyllite of the centre gives way at the margins to a conspicuous green actinolite ± plagioclase rock. However, carbon dioxide has also been an important and widespread influence during the metamorphism such that dolomite becomes a common phase in the assemblages developed, and the anthophyllite and even tremolite are replaced by talc and dolomite. The carbon dioxide has also caused widespread development of scapolite from plagioclase in the green actinolitic margins of the ultrabasic dykes, in some basic dykes, and, in favourable structural situations small masses of gneiss may even be completely replaced by scapolite. Tourmaline is found locally in the dyke margins.

Much of this alteration and metasomatic activity predates the strong shearing characteristic of the Laxfordian. One major shear belt recognized by the Survey workers[1] runs south-eastwards from Clachtoll, following the belt of vertical gneisses, towards Glen Canisp. In this belt the central parts of the ultrabasic dykes have been converted to soft talc–carbonate schists while the green margins remain as more massive actinolite schists. In some extreme cases the central parts of the dykes may be largely sheared out, leaving lenses of green actinolite rock in a matrix of talc schist. All scapolite is destroyed in the sheared rocks.

The three later dyke types listed here have largely escaped the effects of the hydrous metamorphism and the later shearing, though type 6 has suffered marginal shearing in one locality. Their true age must remain for the moment unknown, but it is likely from their fresh and finer grained nature that they are considerably later than the picrites.

The investigations in Assynt therefore confirm O'Hara's contention that the Scourie dykes were intruded at great depth under metamorphic conditions. It is clear also that a variety of dyke types exist in the Lewisian, including possible alkali types, and that, judging from their states of alteration, not all are of the same age. The early dykes, and especially the picrites, record an extensive period of metamorphism and local metasomatism which is demonstrably earlier than the strong shearing and reconstitution of the Laxfordian event, and which is

probably pene-contemporaneous with the dykes themselves.

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SIGNIFICANCE OF DIFFERENCES IN MAGNETIZATION ALONG CERTAIN DOLERITE DYKES

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SYSTEMATIC changes in magnetization have been found by measurements at localities spaced along certain dolerite dykes. The iron-oxide minerals in these dolerites have been investigated and related to a magnetic parameter. The nature of the mineralogical control on the magnetization and the distribution of iron-oxide minerals in the dykes are considered especially in relation to sills fed by the dykes.

Magnetic measurements. The natural magnetization of a dyke is generally the resultant of an induced component, KT (susceptibility × Earth's magnetic field strength) and a remanent component, J_n. Their ratio ($Q_n = J_n/KT$) is used here as a parameter of magnetization. At sixteen localities these components have been calculated from measurements on cylindrical cores cut from specimens collected at points across exposed dykes. An a.c. bridge apparatus was used to measure K and an astatic magnetometer to measure J_n. Two specimens with contrasted Q_n ratios were also a.c. demagnetized and heated to compare their J_n stabilities and the Curie points of their magnetic minerals respectively.

At the same localities and some others vertical force magnetometer profiles were surveyed across the dykes. These anomalies generally had a shape comparable with that due to a uniformly magnetized dyke. This shape is principally determined by the inclination of the magnetization, with respect to the dyke walls, in the plane normal to the dyke and to a lesser extent by the ratio of dyke width (W) to the depth of its top surface below the plane of measurement (D). Since field observations show that the dykes trend east–west, dip nearly vertically and, at exposures, have W/D ratios of 10–20, the geometric parameters are known. Since KT and the stable J_n direction (see below) lie at known inclinations in the plane normal to the dykes, their ratio, Q_n, can be expressed in terms of profile shape, in particular maximum peak/minimum peak amplitudes (R) (Fig. 1).

The results from both methods at three localities, chosen to illustrate the range of Q_n, are shown in Fig. 1. A consistent J_n direction, namely, north polarity directed horizontally towards the south, resistant to a.c. demagnetization, that is, stable, is shown by specimens with a high Q_n value. Other contemporaneous, that is, Permo-Carboniferous igneous, rocks in Britain are similarly magnetized[1–3]. Scattered J_n directions and easy a.c. demagnetization characterize specimens with low Q_n values. Specimens from the intermediate locality show varying degrees of stability[4].

Although specimen values of K and J_n vary in magnitude across these dykes they do so in the same sense tending to minimize variations in Q_n. Since the maximum Q_n range encountered between specimens from one locality is 0·3–0·7 whereas the maximum range of average values between localities is 0·1–2·0 the latter are more important. They are shown in Table 1 with profile estimates.

The tendency for profile values of Q_n to exceed specimen values could be a result of outcrop sampling since the former apply to volumes of rock extending to a depth below outcrop greater than the dyke widths where J_n may be effectively larger due to more consistent orientation[3].

The profile Q_n values along dykes in the vicinity of sills are shown in Fig. 2. Localities where profiles depart from simple dyke anomalies are indicated by a question mark or a specimen value in brackets. These localities are generally where the magnetization changes abruptly and are probably lateral discontinuities, such as en echelon offsets, in the dykes. For individual dykes Q_n varies mainly with K (Fig. 2, section along dyke C).

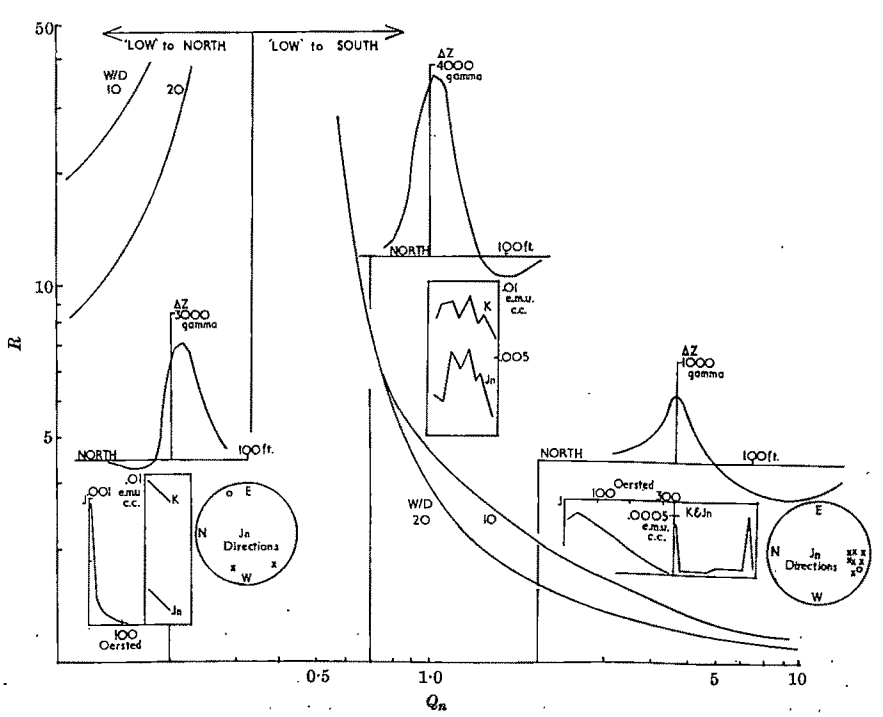


Fig. 1. Magnetometer profiles related to Q_n values and specimen measurements

Table 1

Locality	*A2*	*A4*	*A6*	*A7*	*A11*	*B2*	*B4*	*B5*	*B6*	*C3*	*C6*	*C9*	*C11*	14
Profile	2	1	0·7	0·9	2	0·1	0·8	0·8	0·4	2	0·7	0·2	2	0·7
Q_n Specimen (average)	1·2	1·0	0·6	0·4	0·6	0·1	0·5	0·5	0·1	2·0	0·4	0·1	0·7	0·7

Table 2

Q_n value of specimen	2·0	1·2	1·0	0·5	0·2	0·03
Magnetic fraction (% wt.)	0·5	5·4	7·9	9·2	10·3	16·8
Magnetite/ilmenite in above	3	5	5	4	10	> 20
$TiFe_2O_4$ in magnetite	0·48	0·21	0·18	0·17	0·19	0·24
FeO (% wt.)	10·6	8·6	9·8	8·7	9·3	8·2
Fe_2O_3 (% wt.)	2·5	3·9	3·6	4·8	4·0	5·0
TiO_2 (% wt.)	2·3	2·0	2·2	2·2	2·2	2·2

Mineralogical measurements. Observations, in reflected light, on cores from fifteen specimens show two variables in the character of their magnetite grains. Most apparent are differences in the frequency of ilmenite lamellæ within the magnetites; less obvious is a patchy alteration affecting those magnetites containing most ilmenite.

The specimens range from those with up to 80 per cent of their opaques as fresh, ilmenite-poor, magnetites with low Q_n values to those with rather altered magnetites containing many ilmenite lamellæ and up to 30 per cent of their opaques as separate ilmenite grains and having high Q_n values. Sulphides, mainly pyrite, may be more abundant in the latter specimens.

The alteration is to a mineral of lower reflectivity than the magnetite, that is, not typical maghemite. Comparable magnetites with ilmenite in a 400-ft. sill associated with the dykes are partially altered to maghemite and also have a stable remanence[3]. Petrographically similar Tertiary dolerite sheets in Japan have an unstable remanence where their magnetites are altered to maghemite but show increasing stability in proportion to the volume of ilmenite lamellæ in their magnetites[5]. The reversed magnetization of Mull Tertiary basalts is associated with ilmenite exsolution lamellæ in their magnetites[6].

For quantitative comparison with magnetic properties the strongly magnetic grains were separated from the powder (< 100 mesh) of six specimens covering a range of Q_n values (Table 2). All these contained 10–15 per cent weight opaque minerals (estimated from micrometric counts), but their magnetic fractions decreased with increasing Q_n. The proportion of ilmenite, as lamellæ, to magnetite increased with Q_n (X-ray determinations). The amounts of $TiFe_2O_4$ in solid solution in the magnetite, inferred from cell dimensions, and the amounts of FeO, Fe_2O_3 and TiO_2 in the rocks as a whole showed no systematic changes.

Mineralogical control of magnetization. It is apparent that the fresh magnetite grains, generally 0·25–0·5 mm diameter and containing only a few traces of ilmenite lamellæ, give rise to an unstable remanence. On heating, a specimen of this type showed a single Curie point at 510° C consistent with a magnetite of low $TiFe_2O_4$ content[7].

Some feature of the magnetite in the composite altered magnetite/ilmenite grains must give rise to the stable remanence. The cell dimensions and Curie point (590° C) of this magnetite show it to be similar in composition to that in the dolerites with unstable remanence.

The differences in shape and size of the magnetite in the two types of grains suggest a possible cause for their magnetic differences. Coercivity increases with decreasing grain size; the effect being significant for grains less than 0·1 mm diameter and large for grains less than 0·02 mm diameter[8]. The sub-division of the 0·5 mm composite grains by their ilmenite lamellæ may reduce the magnetite to about 0·1 mm. However, some specimens showing ilmenite lamellæ but only minor magnetite alteration are relatively unstable suggesting that the alteration is the more important factor. Although the alteration product is not typical maghemite, the stable magnetization may be comparable with the thermochemical remanence consequent on oxidation of titanomagnetite at elevated temperatures[9].

Q_n values related to dykes which fed sills. The dykes are part of an east–west trending Permo-Carboniferous swarm in the Midland Valley of Scotland[10]. Individual dykes are about 100 ft. wide, have near vertical margins and some can be traced for more than 20 miles. Petrologically similar quartz-dolerites and tholeiites occur as sills[10], up to 400 ft. thick, which have two main outcrops, more than 10 miles apart, on the flanks of the Central Coalfield Syncline. A borehole at Rashiehill[11], near the synclinal axis, proved sill *X–X′* at depth. West of the sharp Riggin Anticlinal axis (*X*-outcrop), sill *X–X′* pinches out near dyke *E* and also thins out south-westwards[12].

Since sills *X–X′* and *Y–Y′* lie at different levels and terminate against dyke *C*, it probably fed them[12]. Similarly, dyke *G* fed sill *Z* and dyke *A* may have fed the lowest sill at *X′*. Dyke *A* may have fed the sills at *X*, which it meets at their thickest point. The dyke east

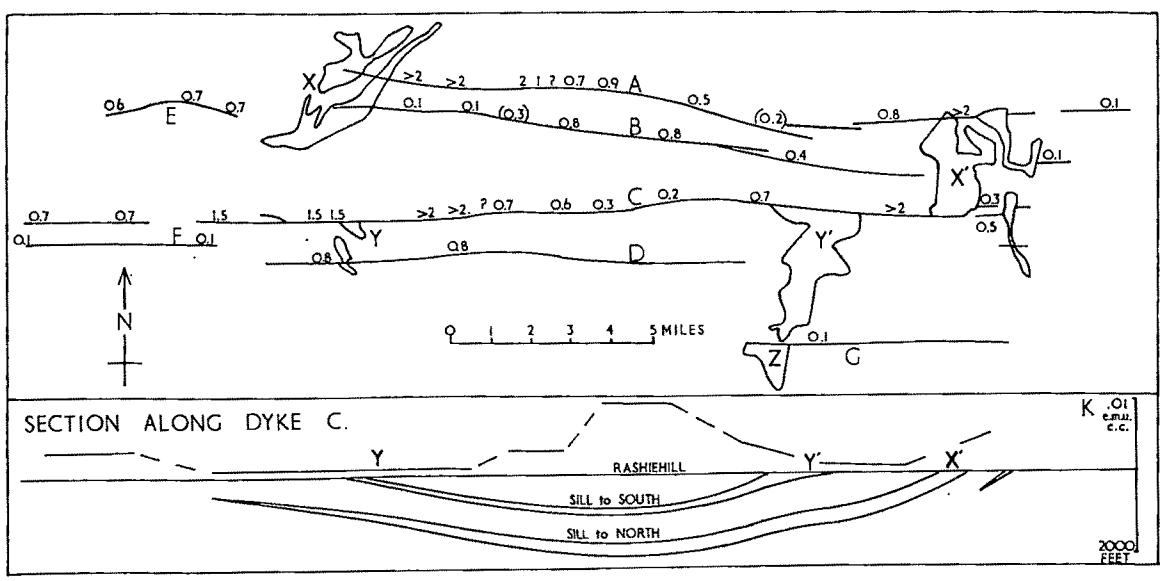


Fig. 2. Distribution of Q_n values along dykes related to sills

of X' and in line with dyke B could be a feeder from its mapped relationships. Dyke B probably intrudes sills at X (ref. 12.)

The distribution of Q_n values along the dykes which fed sills (Fig. 2) suggests that the iron-oxide minerals in these dykes vary with distances above and below the sills. Thus both dykes A and C show an increase in susceptibility, that is, low Q_n values, where they rise farthest above the sills near the centre of the Syncline. They both show high Q_n values where they are immediately above the sills. Dykes east of the sill outcrops X' and Z, that is, immediately below them, show low Q_n values. It is possible that the low Q_n values on dykes A, C, B and F indicate the former presence of now completely eroded higher sills in the areas of the Riggin and south-west of it and also in the axial region of the Syncline.

The dyke rocks immediately above the sill connexions are probably similar to the normal sill dolerite since the Rashiehill borehole core material also has a stable remanence and contains composite magnetite/ilmenite grains[3]. Comparably magnetized dolerites do, however, occur at some localities along dykes of the same swarm near Auchterader, 25 miles to the north, which intrude Lower Old Red Sandstone strata and are unlikely to be closely connected with sills. Low Q_n values along dykes are, so far, restricted to those shown in Fig. 2.

It appears then that the different modes of iron-oxide crystallization and alteration in the dykes have at least been influenced by conditions occurring during the crystallization of the magma in the sills fed by them. The magnetite/ilmenite intergrowths could be derived from a homogeneous phase by exsolution and oxidation. Such a primary phase would have crystallized at higher temperature under more reduced conditions than the magnetites carrying only traces of ilmenite[13]. The later alteration of magnetite associated with ilmenite suggests the effect of hydrothermal solutions, end-products of sill crystallization[14], affecting both the rocks in sills and overlying parts of connected dykes.

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A MATHEMATICAL MODEL OF A PERCEPTUAL MAZE TEST

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IN the majority of intelligence tests no attempt is made to explain the difference in difficulty between test items, and few workers have tried to find any objective measure of increasing complexity to correlate with empirical tests of intelligence[1]. The essence of this problem is the development of families of test items which can be ordered theoretically in a systematic way so that this order can be related to subjective difficulty.

If the individual items in a test can be ordered logically then the relationship between these items can be given a mathematical form. This can be achieved more elegantly and almost certainly more fruitfully if the nature of the test material is such that its physical or perceptual characteristics can themselves be expressed simply in mathematical terms. Many non-verbal intelligence tests require the subject to discriminate spatial or geometric relationships. There is no inherent reason why this type of test should not be constructed systematically, but in practice this is almost never attempted. We describe here a test the perceptual characteristics of which can be rigorously defined and for which a mathematical notation can be developed to describe any parameters which might be related to the subjective difficulty of different items. This test is derived from a perceptual maze test which experimental studies have already shown to be sensitive to organic brain damage[2]. Other investigations have shown that it has a high reliability, and that scores obtained on the test correlate with those obtained on other tests of intelligence[3].

Fig. 1 shows a sample item from the test discussed in the present article. The subject is required to find a pathway from the bottom vertex to the top row which satisfies the following conditions: it must lie only along the small-dotted lines; it must be unidirectional in a general upward sense; it must pass through the greatest number of the larger black dots. Apart from the physical dimensions of the lattice and the target dots there are three variables: (*a*) the number of horizontal rows in the lattice (n) (the vertex is excluded); (*b*) the saturation (s), that is, the ratio of number of target dots to the total number of lattice points, the vertex again being omitted; (*c*) the arrangement of the dots on the lattice, a 'pattern' factor (p).

The first two parameters are clearly amenable to exact treatment and have already been made the subject of an experimental study[4]. The analysis of the 'pattern' factor (p), however, presents a formidable problem. For a lattice with 16 rows, and a variable number of dots, there are 2^{152} (more than $5{\cdot}7 \times 10^{45}$) different patterns; even with s specified, for example, at 50 per cent, there are $\binom{152}{76}$, more than $3{\cdot}6 \times 10^{44}$ patterns. Clearly the value of making an analysis of the pattern factor will depend on the practicability of developing a method of classification which can be related to psychological concepts which may explain the problem solving behaviour involved.

The psychological interest of this test material lies not in the ways in which the problem can be solved—there is no difficulty in devising a computer programme to produce a solution, and we are indebted to Mr. C. D. Alway for a particularly elegant strategy which once learnt enables most human subjects to solve fairly rapidly any pattern of any reasonable size—but in the ways in which the majority of human subjects actually solve the problems and the reasons why these fail, when the complexity of the items increases. It has been suggested that the commonest strategy is a perceptual scanning in which links are sought between areas which appear to contain

relatively high concentrations of target dots. One source of difficulty is that relationships which are irrelevant to the task, for example, horizontal groupings, may remain perceptually attractive. The topological relationships between the target dots can thus be considered from two aspects. The first of these takes account only of the rules of the problem and is 'task-orientated'. It is concerned with such questions as whether one dot is accessible from another, and how many dots may be obtained on pathways between two dots. The second aspect considers perceptual relationships between dots which are irrelevant to the task but which may, nevertheless, influence subjects in their choice of solution pathways.

What is needed, therefore, is a notation which not only permits a comprehensive description of every possible path but which also facilitates an analysis in terms of the remaining relationships between the target dots. We present here such a system and illustrate briefly how it might be developed for analysing some of the task-orientated pattern complexities which it seems possible may be related to the subjective difficulty of test items.

A system of co-ordinates (p,q) is defined, where $p = 0,1,2 \ldots n$ = the row number, numbering from the bottom vertex to the top; and $q = 0,1,2 \ldots p$ = the position of the point in the p^{th} row, numbering from left to right. Necessary and sufficient conditions for the accessibility of a point (p_j, q_j) from a point (p_i, q_i) are then:

$$p_j > p_i$$
$$p_j - p_i \geqslant q_j - q_i$$
$$q_j \geqslant q_i$$

and it may easily be shown that:

n_{ij} = No. of pathways from the point (p_i, q_i) to the point (p_j, q_j)

$$= \binom{p_j - p_i}{q_j - q_i}$$

Suppose that the maze dots are labelled 1, 2, 3 . . . r by starting at the bottom vertex and working along consecutive rows from left to right, and that the dot labelled i is situated at the point (p_i, q_i) for $i = 1, 2, 3 \ldots r$, then the number of pathways between dot i and dot j is:

$$n_{ij} = \binom{p_j - p_i}{q_j - q_i}$$

In addition we define, for $i = 1, 2 \ldots r$,

n_{oi} = number of pathways from the bottom vertex, $(p,q) = (o, o)$, up to the dot i

$$= \binom{p_i}{q_i}$$

and n_{if} = number of pathways from dot i up to the top row of the maze $= 2^{n-p_i}$.

Of course, n_{of} equals 2^n.

We may now define the matrix $N = (n_{ij})$ for $i, j = 0, 1, 2 \ldots r, f$. The matrix is square and of order $(r + 2)$. It is also clear that $n_{ij} = o$ if $i > j$, or if $i = f \neq j$: and if we further define $n_{ii} = o$ for $i = 0, 1, 2 \ldots r, f$, then N is an upper triangular matrix with zeros down the main diagonal. In this notation o and f are equivalent to dots in the sense that they are points at which paths start and end, and so they will be referred to as such in the following. But it must be remembered that o and f are never targets and hence never contribute to the score.

If m = maximum score obtainable on the maze there exists a sequence of matrices:

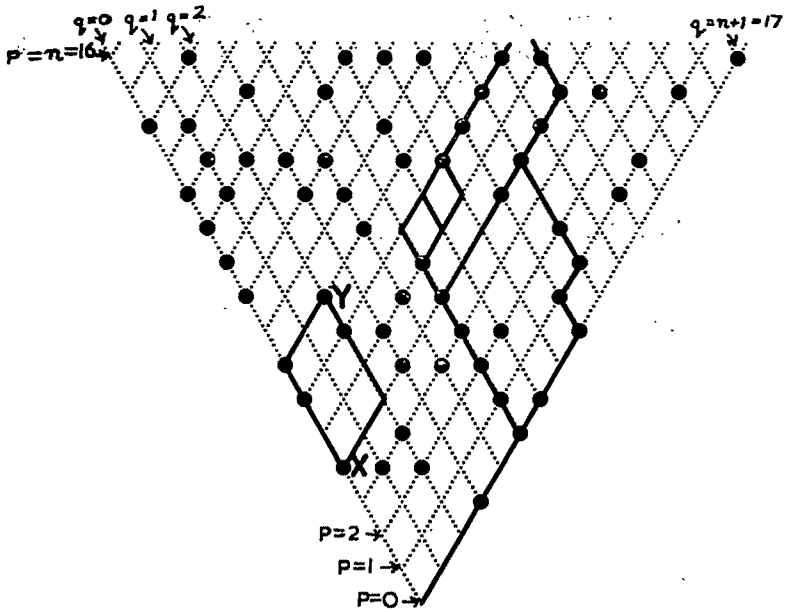


Fig. 1. An example of a test item incorporating information to illustrate some of the mathematical concepts derived in the text. Here the maximum score, m, is 11, and there are 5 solution pathways (that is, $v_{of}^{(12)} = (M^{12})_{of} = 5$), which are drawn in. However, the number of solution sets of dots = 3 (that is, $v_{of}^{(12)'} = (M'^{12})_{of} = 3$). The small parallelogram section on the left-hand side of the figure illustrates the co-ordinate system. The dot at lattice point X is designated by $i = 2$, and the lattice co-ordinates of dot j (pt. X) are $(p_i, q_i) = (4,0)$: for the dot at lattice point Y we have $j = 20$, and $(p_j, q_j) = (9, 2)$. Then the total number of pathways between dot i and dot j (that is, between X and Y) is given by:

$$n_{ij} = \binom{p_j - p_i}{q_j - q_i} = \binom{9 - 4}{2 - 0} = 10$$

The number of these pathways which pass through 0, 1 and 2 dots between dot i and dot j are given respectively by:

$$V_{ij}^{(0)} = M_{ij} = 2; \quad V_{ij}^{(1} = (M^2)_{ij} = 6; \quad V_{ij}^{(2)} = (M^3)_{ij} = 2$$

$$V^{(\alpha)} = (v_{ij}{}^{(\alpha)}), \; i, j = 0, 1, 2 \ldots r, f; \; \alpha = 0, 1, 2 \ldots m$$

where $v_{ij}{}^{(\alpha)}$ = number of pathways between dots i and j passing through α dots (excluding dots i and j) for $i, j = 1, 2 \ldots r$. $v_{oi}{}^{(\alpha)}$. $v_{if}{}^{(\alpha)}$ are similarly defined for $i = 1, 2 \ldots r$. Each of the $V^{(\alpha)}$ matrices is of the same form as N and it may be shown that $V^{(\alpha)} = M^{\alpha+1}$ where M is a matrix such that $M = N(I + N)^{-1}$ and I is the $(r + 2) \times (r + 2)$ unit matrix.

The set $v_{of}{}^{(\alpha)}$, $\alpha = 0, 1, 2 \ldots m$, is the frequency-of-scores set for the whole maze: and the sets with different suffixes give the corresponding frequency-of-scores set for different portions of the maze.

Possibly useful statistics, which may be easily derived from the foregoing are:

(1) The total score matrix $T^{(1)} = N^2$, giving the total score between two dot points (excluding these dots in totalling the score), and likewise between the vertex and a dot, the vertex and the top and a dot and the top.

(2) The sum of squares of scores matrix $T^{(2)}$
$$= N^2(I + 2N)$$

(3) The variance of scores matrix $T^{(3)}$, where:

$$t_{ij}{}^{(3)} = \frac{1}{n_{ij}} \left[t_{ij}{}^{(2)} - \frac{(t_{ij}{}^{(1)})^2}{n_{ij}} \right]$$

If the whole-maze frequency-of-scores set (that is, $v_{of}{}^{(\alpha)}$) for each member of the set of mazes of given parameters (n, r) is considered, there are $\binom{x}{r}$ members of the set, where $x = \dfrac{n(n + 3)}{2}$ = number of lattice points; then totalling these frequencies over all mazes of the set, it may be shown that the total frequency-of-scores set is given by:

$$v_T{}^{(\alpha)}(n,r) = \sum_{\substack{\text{all mazes} \\ \text{of } (n,r) \text{ set}}} v_{of}{}^{(\alpha)} = 2^n \binom{n}{\alpha} \binom{x - n}{r - \alpha}, \; \alpha = 0, 1, 2 \ldots n.$$

The expected frequency-of-scores set for a (n,r) maze is therefore:

$$\left[\nu_T^{(\alpha)}(r,n) \Big/ \binom{x}{r} \right], \ \alpha = 0, 1, 2 \ldots n.$$

These and other statistics concerning the distribution of scores may be used to define parameters for the maze which may be related to subjective difficulty.

In this notation m_{ij} equals the number of pathways between dot i and dot j with zero score ($i, j = 0, 1, 2 \ldots r, f$): and if $m_{ij} > 0$, then we may say that dot j is directly accessible from dot i. Developing this concept we may use a binary notation in the form of matrix $M' = (m'_{ij})$ $(i, j = 0, 1, 2 \ldots r, f)$ where $m'_{ij} = 1$ if $m_{ij} > 0$ (that is, if j is directly accessible from i) and $= 0$ otherwise. Matrices $V^{(\alpha)\prime}$, $T^{(1)\prime}$, $T^{(2)\prime}$, $T^{(3)\prime}$ may also be derived corresponding to the unprimed matrices given here. The basic difference between the two sets of matrices is that in the first set the number of different pathways with score α between dots i and j is considered, whereas in the second series the number of different sets of α dots lying on pathways between dots i and j is the underlying concept. Thus, for example, $\nu_{of}{}^{(m)\prime}$ gives the number of different solution sets of dots.

Further, the dots (not including o and f) may be divided into n groups corresponding to the n horizontal maze rows, and the vector (d_p) may be defined, where $d_p =$ number of dots on the p^{th} horizontal maze row $(p = 1, 2 \ldots n)$.

The matrix M' may now be partitioned into sub-matrices $R_{\alpha\beta}$ where α, β refer to maze rows; here 'maze rows' are taken to include a zero row and an f row and so $\alpha, \beta = 0, 1, 2 \ldots n, f$. $R_{\alpha\beta}$ is a $d_\alpha \times d_\beta$ matrix which gives direct accessibility relationships between dots on the α^{th} row and the β^{th} row. Again, $R_{\alpha\beta} = O$ if $\alpha \geqslant \beta$.

Since one theory describing maze problem-solving activity postulates that individuals differ in the size of the perceptual unit which they use, it seems relevant to illustrate one way in which the present approach could be used to analyse this aspect of the problem. To do this we have defined as a Δ any maze-linked set of dots. A Δ is designated by $\delta(\nu,\gamma)$ where ν is the number of dots in the Δ and γ is the number of gaps, or vacant sites, interspersed between the dots. Now considering M' in the partitioned form $R_{\alpha\beta}$ $(\alpha, \beta = 0, 1, 2 \ldots n, f)$ which is an upper triangular matrix with zero sub-matrices $R_{\alpha\alpha}$ in the main diagonal, then it will be seen that the $\delta(\nu,\gamma)$'s are given by the $(\nu - 1 + \gamma)$th parallel of sub-matrices of $M'^{\nu-1}$. If the partitioned matrix $M'_{(\rho)}$ is defined so that $M'_{(\rho)}$ has the same first ρ parallels of sub-matrices as M' but all other parallels consist of zero sub-matrices, then it will be seen that the $\delta(\nu,\gamma)$'s are given by the $(\nu - 1 + \gamma)$th parallel of sub-matrices of $M'^{\nu-1}_{(\gamma+1)}$. In the particular case of the complete solution Δ's, that is, the $\delta(m + 2, n-m)$'s these are given by the $(n + 1)$th parallel of sub-matrices of $M'^{m+1}_{(n-m+1)}$. In other words, $M'_{(n-m+1)}$ and not M' need be considered for the solution. Thus for a maze with $n = 16$, $m = 12$ (that is, $n - m + 1 = 5$), the subject need only consider direct accessibility relationships between dots which are separated by not more than five maze rows.

The foregoing mathematical analysis is one of several which might be equally or more valuable. It is presented in this form because it deals primarily with the target dot relationships and so lends itself to a study of the problem-solving activity involved. It has, we hope, been developed sufficiently to show that a systematic analysis of this test material is both practical and potentially fruitful.

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DISTORTION OF VISUAL SPACE AS INAPPROPRIATE CONSTANCY SCALING

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DISTORTIONS of visual space associated with certain simple patterns have been investigated since the beginning of experimental psychology[1], and many theories have been proposed[2], but so far none, in my opinion, has been satisfactory in explaining these so-called 'geometrical' illusions. Figs. 1, 2 and 3 show representative illusions of the kind we are considering.

The traditional theories fall into three classes: (1) That certain shapes produce, or tend to produce, abnormal eye movements. (2) That some kind of central 'confusion' is produced by certain shapes, particularly non-parallel lines and corners. (3) That the figures suggest depth by perspective, and that this 'suggestion' in some way distorts visual space.

The eye movement theories are difficult to support because the illusions occur undiminished when the retinal image is optically stabilized on the retina[3], or when the figures are viewed as after-images following illumination by a bright flash of light. Further, since distortions can occur in opposed directions at the same time (as with the Müller–Lyer figure[4] (Fig. 1*a*) it is difficult to see how either overt or incipient eye movements could be involved. The various 'confusion' theories all suffer from vagueness, and they give us no idea as to why the distortions should occur in the observed directions, or only in certain kinds of figures. The perspective theory[2] is inadequate because it does not suggest why or how perspective should produce distortions in flat figures, but it does imply a generalization which seems to hold true of all the known illusion figures, and this gives a clue vital to understanding the origin of the illusions.

The illusion figures may be thought of as flat projections of typical views of objects lying in three-dimensional space. For example, the outward-going Müller–Lyer arrow figure is a typical projection of, say, the corner of a room—the fins representing the intersections of the walls with the ceiling and floor—while the in-going arrow is a typical projection of an outside corner of a house or a box, the converging lines receding into the distance. The following generalization seems to hold for all the illusion figures thought of in this way: The parts of the figures corresponding to distant objects are expanded and the parts corresponding to nearer objects are reduced. Thus in the Müller–Lyer figure the vertical line would be further away in the diverging case, and is expanded in the illusion, and vice versa, while in the Ponzo figure

the upper horizontal line would be farther away and it also is expanded in the flat illusion figure.

Given that this generalization holds for all the illusions, why should these distortions occur ?

Do we know of any other perceptual phenomena involving systematic perceptual modification of the retinal image ? There is a well-known set of phenomena which certainly does involve perceptual modification of retinal images—size constancy[5,6]. This is the tendency for objects to appear much the same size over a wide range of distance

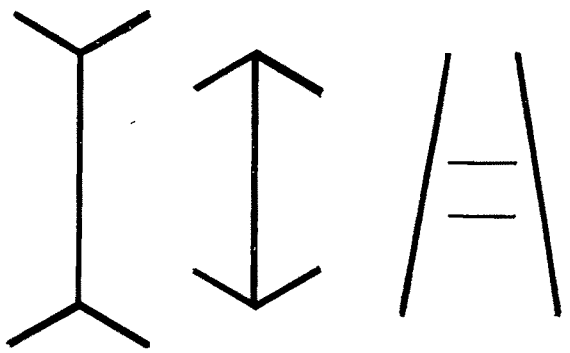


Fig. 1. (*a*) The Müller-Lyer; (*b*) the Ponzo illusion

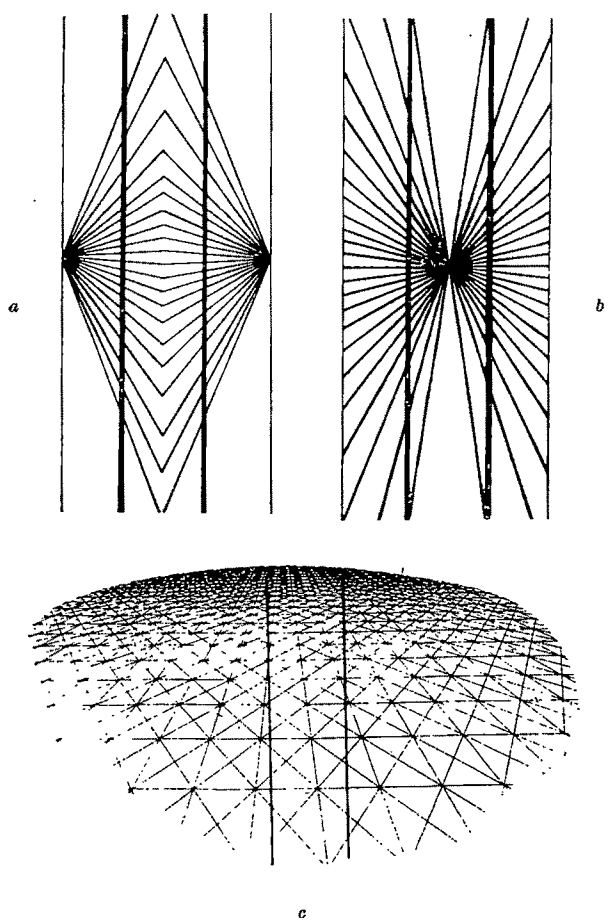


Fig. 2. (*a*) and (*b*) Alternative forms of the Hering illusion. The vertical lines are bowed inwards and outwards, respectively. (*c*) An illusion showing how parallel lines indicating distance seem to diverge when presented on a texture gradient. (The texture taken from Gibson, *The Perception of the Visual World*. 1951)

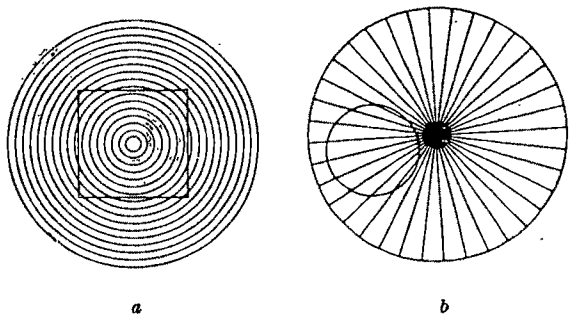


Fig. 3. Further distortions to be expected on the distance hypothesis; the concentric circles and spokes set the constancy scaling by indicating depth. (Figures, though not interpretation, from Orbison, *Amer. J. Psychol.*, 52, 39; 1939)

in spite of the changes of the retinal images associated with distance of the object. We may refer to the processes involved as constancy scaling. Now in constancy scaling we find known processes which not only could but also must produce distortion of visual space if the scaling were set inappropriately to the distance of an observed object. It is strange that apparently only one writer, Tausch, has considered constancy in connexion with the geometrical illusions[7].

We can see our own scaling system at work in the following demonstration of Emmert's law[8]. The after-image of a bright light is 'projected' on to a series of screens lying at various distances, or a single screen moved away or towards the observer. Although the effective retinal image is constant, the after-image perceived as lying on a screen looks larger the farther the screen is from the observer. Complete constancy would give a doubling in size for each doubling of distance, and the amount of scaling can be quantified under various conditions for stationary or moving screens[9,10].

Clearly inappropriate constancy scaling would produce distortion of visual space, but why should this occur with the illusion figures which are in fact flat and are generally seen to be flat ? It is generally assumed that constancy scaling depends simply on apparent distance (as Emmert's law might suggest); but if we are to suppose that constancy scaling can operate for figures clearly lying on a flat surface we must challenge this assumption, and suggest that visual features associated with distance can modify constancy scaling even when no depth is seen. If we are to suppose that the illusions are due to misplaced constancy scaling, we must suppose that the scaling can be set directly by depth features of flat figures, and that the scaling is not set simply as a function of apparent distance as is generally thought to be the case.

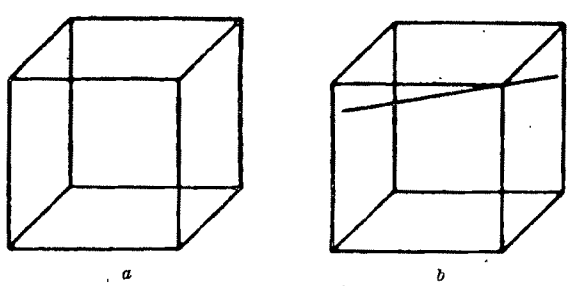
Perspective drawings and photographs are seen to depict objects as if they lay in three dimensions, and yet at the same time they appear flat, lying on the plane of the paper, and so they are perceptually paradoxical. The surface texture of the paper evidently prevents the perspective from making the objects appear truly three dimensional, for if we remove all texture and view with one eye, then perspective drawings can look as impressively in depth as the real world viewed with one eye.

We have presented the well-known illusion figures with no background texture—by making wire models coated in luminous paint so that they glow in the dark, or using back illuminated transparencies—and we find that, viewed with one eye, they look three dimensional, provided the angles are not marked exaggerations of perspective. The Müller–Lyer arrows, for example, look like corners and not like flat projections when presented as luminous figures in the dark, and those parts which appear most distant are the parts which are expanded in the illusions as normally presented on textured paper. What happens to the distortions when we remove the background texture

is complex, and will be discussed more fully elsewhere; but, in general, distortions are reduced or disappear.

Emmert's law may suggest that constancy scaling arises directly from apparent distance; but there is retinal information indicating the distance of each position of the screen, and possibly this might serve directly to set the scaling. However, the following demonstration shows conclusively that scaling can occur simply as a function of apparent depth and independently of retinal or other sensory information.

Fig. 4*a* shows the well-known Necker cube figure—a skeleton cube which reverses spontaneously in depth so that sometimes one face, sometimes another, appears the nearer. As shown on textured paper, it is paradoxical in the manner described here—it looks as if it were in depth and yet it is seen to be flat on the paper. By making a luminous model of this figure, and viewing it in the dark, we find that it still reverses but now it looks like a true three-dimensional figure, and it undergoes size changes—the apparently farther face looking somewhat larger than the nearer, showing that constancy scaling is now operating. Since the retinal image remains unchanged it follows that the scaling is set under these conditions as a simple function of apparent distance. This is shown most dramatically with a three-dimensional luminous cube. This looks like a true cube when seen correctly, but when perceptually reversed in depth it looks like a truncated pyramid, the apparently front face being the smaller[11].



a *b*

Fig. 4. (*a*) The Necker cube. This reverses in depth. When viewed as a self-luminous figure, the apparent front looks smaller, the back larger. (*b*) Humphrey's figure. The oblique line is seen as slightly bent; the direction of bending being determined by the angle against which it is placed, and not by the way the cube appears to lie in depth

It thus appears that there are two ways in which constancy scaling can be set. We may name these:

(1) *Primary constancy scaling.* This is set by perspective or other features normally associated with distance. These features can be at variance with apparent distance in special cases, such as the illusion figures. (We call it 'primary' because it seems to be primitive, and to be mediated by neural systems situated early in the perceptual system.)

(2) *Secondary constancy scaling* is set simply by apparent distance, and this may be a function of previous knowledge and is not necessarily tied directly to visual information. Its existence is suggested but not proved by Emmert's law; but it is conclusively demonstrated with the ambiguous self-luminous objects which change their shape systematically according to which faces appear nearer or farther though there is no change in the retinal image. Errors in apparent distance should produce distortion of visual space via this secondary scaling system, and the well-known moon illusion may be an example.

Although the self-luminous figures do clearly demonstrate what we have called the secondary constancy scaling system, what clear evidence have we for the primary system, supposed to be set by typical depth cues even in the absence of depth perception? For our present purpose it is much more important to demonstrate the existence of primary than secondary scaling. To get evidence for primary scaling entirely independent of the illusions is very difficult, but the following is at least suggestive.

(1) It has been noticed by Humphrey[12] that a straight line drawn across a corner of a Necker cube (Fig. 4*b*) appears bent. Now this is particularly interesting because the direction of bending is the same which ever way the cube appears to lie in depth. It is bent in the direction to be expected if constancy scaling is operating from the typical perspective interpretation of the angle against which the line lies.

(2) In primitive races living in houses without corners the geometrical illusions are reduced[13,14]. If learning is important, this would be expected.

(3) In a case of a man blind from the first few months of life, but gaining his sight after operation fifty years later, we have found that the illusions were largely absent, and his constancy appeared abnormal or absent although he could at that time, some weeks after the corneal graft operation, recognize common objects[15]. This has been noted in other cases. (In fact, it was this observation which suggested to me this kind of theory of the illusions.)

We should expect the different scaling systems to have somewhat different time-constants, and we are attempting to measure these to establish their separate existence quite apart from considerations of distortions of visual space.

It further may be suggested that figural after-effects —distortions similar to the geometrical illusions, but produced as a result of prolonged viewing of a suitable stimulus pattern and transferring to a second test pattern—may be due to the primary scaling being set by depth features present in the stimulus pattern, this scaling taking some time after lengthy fixation to become appropriate to the second test pattern, so the second pattern is distorted by scaling carried over from the earlier pattern. Preliminary experiments are providing strong evidence that figural after-effects can be thought of in this way, and such a theory would have advantages over present theories of the figural after-effects which are *ad hoc*, involve dubious physiological speculation and fail to make useful predictions[16,17].

In attempting to give a general account of all illusions involving systematic distortions of visual space, either while viewing a figure or following on prolonged viewing, and relating the distortions to a known perceptual phenomenon—size constancy—we have not attempted to specify the neural processes involved, and we believe this to be impossible at this time. Recent work on recording from the visual regions of the cat's brain while presenting the eyes with moving or fixed patterns[18] gives promise that the underlying neural mechanisms may soon be revealed.

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LETTERS TO THE EDITOR

ASTRONOMY

A Supernova Remnant in the Large Magellanic Cloud

THE proximity of the Large Magellanic Cloud offers an unparalleled opportunity for the detailed comparison of an external galaxy at optical and radio wave-lengths. Recently the 210-ft. steerable reflector at the Australian National Radio Astronomy Observatory has been used to survey the Large Cloud at a number of wave-lengths between 11 cm and 220 cm. The results of this work have been presented at the recent International Astronomical Union/International Scientific Radio Union Symposium No. 20 (ref. 1).



Fig. 1. $H\alpha$ photograph of *N49* taken with the Uppsala Schmidt telescope

The high resolving power and sensitivity of the 210-ft. reflector at short wave-lengths enabled the radio emissivities of about 40 emission nebulæ in the main body of the Large Cloud to be determined. Observations of these *H*II regions were carried out at 21 cm and 11 cm where the aerial beam-widths at half-intensity points are 14′ of arc and 7·5′ of arc respectively and the receiving systems allow temperature differences of 0·1° K to be measured. For each *H*II region, the expected flux density at 11 cm was calculated from the $H\beta$ flux densities of Dickel, Aller and Faulkner[2] and the $H\alpha$ flux densities of Doherty, Henize and Aller[3] and compared with the observed flux density. In most cases reasonable agreement was obtained between the radio and optical observations which was consistent with a thermal origin of the radio emission.

However, in the case of one small emission nebula listed as N49 in the catalogue of Henize[4], the flux density at 11 cm ($1{\cdot}3 \times 10^{-26}$ W m^{-2}(c/s)$^{-1}$) is some 15 times greater than that calculated from the $H\alpha$ intensity. This suggests that a considerable proportion of the radio emission could be of non-thermal origin. Our measured position at 11 cm of (1950·0) R.A. $05^h\ 26^m{\cdot}0$, Dec. $-66°\ 08'$, is within 0·5′ of arc (our experimental accuracy) of the optical position. Combining our intensity measurements of *N49* at 11·21 and 75 cm and that of the Mills, Slee and Hill[5] source (05–64) at 85·5 Mc/s, the spectrum has an index of -1 which clearly indicates a non-thermal origin of the radiation. Mills, Slee and Hill give an estimate of the east–west diameter of 0·5′ of arc which is in fair agreement with the overall optical size of 1′ of arc.

Fig. 1 shows the region of *N49*. The photograph is from a 7-min exposure in $H\alpha$ light with the 20/26-in. Schmidt telescope of the Uppsala Southern Station. It may be noted that no exciting star can be seen in the nebula either in Fig. 1 or on longer exposures. On a 1-h exposure in ultra-violet light with the Uppsala Schmidt telescope the central part of *N49* is filled by weak emission but no stellar object appears. The nebula shows a looped filamentary structure characteristic of remnants of galactic supernovæ. Henize[4] also reported strong OI emission from *N49* which is another characteristic feature of these objects[6].

It therefore appears reasonable to assume on the basis of these radio and optical observations that *N49* is a supernova remnant in the Large Magellanic Cloud and is the first such remnant to be discovered in an external galaxy. The outer diameter of the looped structure of *N49* is 16 parsecs and the inner diameter is 6 parsecs, using 55 kiloparsecs as the distance to the Large Cloud. If we assume an expansion rate of 5,000 km/sec for the supernova shell with no deceleration due to collision with interstellar matter then the minimum age is 1,500 years. The intrinsic radio luminosity of *N49* is only 5 times less than Cassiopeia *A*, the most intense of the known galactic supernovæ. It is of interest to note that the spectral indices of the radio emission from Cassiopeia *A* and *N49* are very similar and much 'steeper' than that of other galactic supernovæ.

If Shklovsky's[7] formula for the variation of flux density with radius of supernova remnants is applied, it is found that *N49* would be intrinsically 1,000 times more intense than Cassiopeia *A* at comparable ages. Such supernovæ in the first few years of their existence would be readily observable with existing radio telescopes in galaxies many megaparsecs distant.

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RADIO ASTRONOMY

Angular Size of Radio Stars

The existence of a number of radio sources with an angular extension less than 1″ is well known[1]. Some of them were identified with star-like objects and therefore they are referred to as radio stars. Among these are 3*C*-48, 3*C*-119, 3*C*-147, 3*C*-273, 3*C*-286. Their non-thermal spectra and the evidence of a rather strong linear polarization suggest the synchrotron mechanism of radio emission. Twiss[2], Razin[3], and Le Roux[4] have shown that a self-absorption of synchrotron radiation by relativistic electrons must occur at frequencies $f < f_1$, f_1 depending on relativistic electrons concentration, normal component of magnetic field $H_\perp$, spectral index of radio emission α (the spectrum being of the form $S(f) \propto f^{-\alpha}$), and on linear dimension of radio-emitting region. At frequencies below f_1 the spectrum of radio emission is $S(f) \propto f^{2.5}$ whatever α is, thus f_1 is a frequency where maximum flux density of a radio source occurs.

Using well-known formulæ for synchrotron radiation, it is possible to derive f_1 as a function of the observational values of flux density $S(f)$, apparent angular diameter θ and spectral index α:

$$f_1 = K(\alpha)\,[S(f_2)\,.\,\theta^{-2}\,.\,f_2^{\alpha}\,.\,10^{33}]^{\frac{1}{2.5+\alpha}}\,H_\perp^{\frac{1}{5+2\alpha}} \quad (1)$$

where $K(\alpha)$ is the slowly varying function of spectral index α of order of unity, $S(f_2)$—flux density in M.K.S. units at a frequency $f_2 > f_1$, not affected by self-absorption.

$H_\perp$ is the only term in equation (1) which cannot be obtained through direct radio astronomy measurements, but there is a good chance for a reasonable use of equation (1), since f_1 appears to be quite insensitive of $H_\perp$ ($f_1 \propto H_\perp^{1/7}$). In this communication $H_\perp$ is equal to 10^{-4} gauss.

Considerations of possible red shift effects show that f_1 in equation (1) must be increased by a factor $(1 + Z)^{\frac{1}{5+2\alpha}}$, where $Z = \frac{\Delta\lambda}{\lambda_0}$; for $Z < 10$ the red shift factor is less than 1·4. Therefore, equation (1) is also insensitive to red shift.

Inspection of equation (1) reveals dependence of self-absorption on the surface brightness temperature of a radio source. The sources with very high brightness temperature due to self-absorption must show a sharp 'fall-off' of the flux density at metre wave-lengths, while in sources with relatively low-brightness temperature the 'fall-off' occurs at decametre wave-lengths and is due mainly to absorption by ionized hydrogen. For example, the critical frequency f_1 for self-absorption in *M*31 (Andromeda nebula) is 0·1 Mc/s, in Cygnus-*A*, 10 Mc/s, in 'radio-stars' more than 20 Mc/s, depending on their angular size.

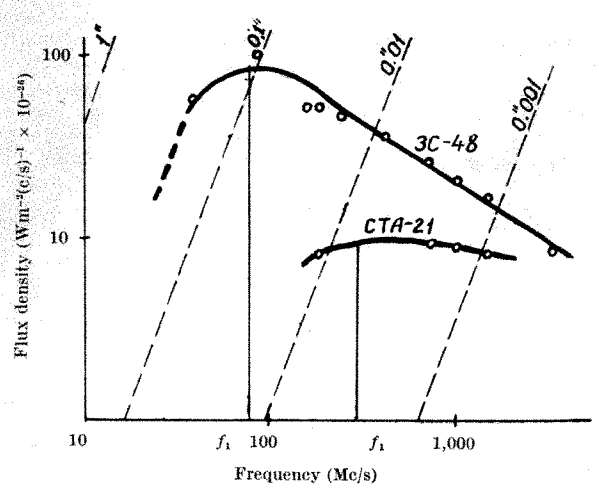


Fig. 1. Spectra of radio sources 3*C*-48 and *CTA*-21. Dashed lines represent equation $\log S = 2{\cdot}5 \log f + \log 0{\cdot}14\,\theta^2$ (S in 10^{-26} W per m² per hertz, f in Mc/s, θ in sec of arc)

The spectrum of 3*C*-48 is shown in Fig. 1, all data except for 81·5 Mc/s are taken from ref. 5 and flux density at 81·5 Mc/s is taken from 2*C* catalogue. The spectrum shows a distinct maximum near 70 Mc/s, which may be due to self-absorption. Apparently the self-absorption of synchrotron radiation may account for observed correlation of spectrum curvature and high brightness temperature[6].

It is possible from equation (1) to derive an expression for the apparent angular diameter in terms of maximum flux density and critical frequency f_1 (including the red shift correction):

$$\theta = 4{\cdot}3 \times 10^{16}\,[S(f_1)]^{0.5}\,f_1^{-1.25}\,H_\perp^{0.25}\,(1 + Z)^{0.25} \quad (2)$$

The radio source 3*C*-48 then turns out to be 0·14″ in diameter. Fig. 1 also shows a spectrum of radio source *CTA*-21 with a maximum near 300 Mc/s, which corresponds to an angular diameter 0·01″. Dashed lines in Fig. 1 represent possible positions of maxima of spectra for radio sources with angular diameters of 1″; 0·1″, 0·01″ and 0·001″.

Comparison of Fig. 1 with spectra of all 160 discrete sources given in ref. 5 shows that there are no sources with apparent angular diameters less than 0·02″, except for *CTA*-21 and *CTA*-102 which may be of the order of 0·01″. The same conclusion holds for all sources of revised 3*C* catalogue. This means that angular diameters of all non-thermal sources known greatly exceed angular diameter of stars and therefore so-called radio stars identified with star-like objects in fact are not stars and there are no stars among the radio sources known at the present time.

This conclusion may be of interest to cosmology regarding the minimum angular size of radio sources predicted by cosmological models.

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A Radio Source with a Very Unusual Spectrum

Conway, Kellerman and Long[1] have recently determined the spectra of 160 radio sources in the wave-length range of 10 cm–26 m. Their results were obtained by combining measurements at nine wave-lengths made at the California Institute of Technology, Mullard and Nuffield Radio Observatories. At wave-lengths longer than 20 cm they found that most of the spectra could be represented by power laws and that the median spectral slope was 0·7. Two notable exceptions are the sources *CTA* 21 and *CTA* 102 (ref. 2) which have curved spectra with maxima in the region of 30–50 cm. These two objects were originally found from observations at 30 cm whereas all the others were selected from the 3*C* (ref. 3) and the Mills, Slee and Hill (MSH) (ref. 4) catalogues. As these surveys were made at wave-lengths of 2 and 3·5 m respectively, selection from them would tend to discriminate against objects of the type of *CTA* 21 and *CTA* 102. Kellerman (private communication) has pointed out that it is important to know the relative population of such sources because of their possible influence on source counts at different wave-lengths.

We have recently made a survey of the sky south of −20° declination at a wave-length of 75 cm with the

210-ft. telescope. Sources of flux density greater than 4×10^{-26} W m^{-2} (c/s)$^{-1}$ were afterwards observed at 22 and 11 cm. The catalogue comprises 300 objects with galactic latitude greater than $\pm 10°$ and of these 230 occur in the MSH catalogue. On linear extrapolation of the spectra defined by the 22 and 75 cm measurements, some 50 would be below 15×10^{-26} W m^{-2} (c/s)$^{-1}$ at 3·5 m and thus might not be expected in the MSH catalogue. On the same basis, the other 20 would be well above the lower limit of the MSH survey. The absence of a few of these could be due to their proximity to side-lobe regions of the cross-aerial associated with strong sources. However, the remainder could be explained in terms of strong curvature in their spectra between 75 cm and 3·5 m. For 3 out of the 230 sources common to both surveys there is reasonably direct evidence of maxima in the spectra in the decimetre wave-length range. It seems, therefore, that the frequency of occurrence of objects such as *CTA* 21 and *CTA* 102 lies somewhere between 1 and 6 per cent and thus they should not have a significant effect on source counts.

The most unusual case we have of a curved spectrum (Fig. 1) is that of the source which we have designated 1934–63. Its co-ordinates are:

R.A. 19h 34m 46s ± 4s (1950)
δ −63° 49·4′ ± 0·5′

Between 10 and 50 cm the flux densities were measured relative to Hercules-*A* as the two were comparable in this range; above 70 cm they were measured relative to a nearby source, MSH 19–56, which has about the same flux density at 75 cm and has a well-defined power-law spectrum. The angular size of the source is not known, but as it does not noticeably broaden the aerial beam at 11 cm it must be less than 2′ of arc. Linear polarization is less than 2 per cent at 11 cm and less than 1 per cent at 22 cm. A 74-in. plate taken by Dr. Bengt Westerlund at Mount Stromlo Observatory shows no prominent visible counterpart for the source although there are several galaxies of about eighteenth magnitude in or near the error area.

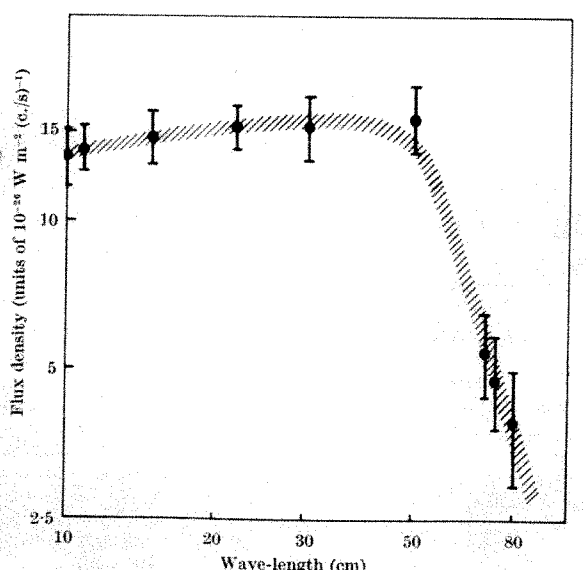


Fig. 1. Radio spectrum of the source 1934–63. Both scales are logarithmic

The marked difference between the spectra of *CTA* 21 and *CTA* 102 and this new object lies in its extremely sharp long-wave-length cut-off. The gradual slopes of *CTA* 21 and *CTA* 102 are just consistent with the assumption of radiation from an almost mono-energetic electron spectrum. With 1934–63 the sharp cut-off appears to rule out an origin solely in an emission process and suggests the presence of an absorbing or reflecting medium in front of a normal non-thermal source. Even in this case extremely high values of optical depth or plasma critical frequency are required. A knowledge of the angular size and thus surface brightness temperature is required before possible models can be profitably investigated.

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PHYSICS

Stimulated Compton Scattering of Electrons by Standing Waves of Light

The production of very intense visible radiation by lasers has facilitated investigations of the interactions between photons and electrons. A recent communication shows that for the case of a beam of multi-GeV electrons incident head-on upon a ruby-laser beam, Compton scattering produces an appreciable quantity of high-energy scattered photons, which largely preserve their initial polarization[1]. Thomson scattering of a laser beam by a beam of 2-KeV electrons has been observed experimentally[2].

It is interesting that, thirty years ago, Kapitza and Dirac calculated the probability of reflexion of very-low-energy electrons from standing waves of light[3]. Because the standing wave-train gives rise to stimulated Compton transitions, Bragg's law is obeyed, one-half the light wave-length being equivalent to the lattice spacing. This, of course, is a non-linear effect distinct from that cited in the previous paragraph. Using Thomson's formula, the probability of an electron being deflected is shown to be:

$$\frac{e^4 L}{2m^2c^2h^2\nu^4 v}\int I_\nu I'_\nu d\nu$$

L is the length of the electron path through the light beam and v is the electron velocity. I'_ν is the energy of the stimulating beam per unit area per unit time per unit frequency-range. I_ν is the corresponding quantity for an element of the incident beam, the total energy per unit area per unit time of which is $I_0 = \int I_\nu d\nu$.

It was concluded by the authors that the experiment could not be made with the apparatus then available. For example, the probability of deflexion of 25-eV electrons from standing waves produced by a mercury arc delivering 1 W at 5461 Å with a band-width of 0·1 Å is about 10^{-14}.

The probability depends on the square of the intensities, however, and the advent of lasers would seem to bring the experiment within range of possibility. Using values characteristic of ruby lasers produced commercially—power output 10 kW and line-width of 0·04 Å—the probability of electron deflexion is increased to the order of 10^{-7}. Experimental problems, while far from trivial, appear to be surmountable. The primary and deflected beams lie very close together, and separating them may be somewhat difficult.

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Basic Concepts of Measurement and the Michelson–Morley Experiment

One of the reasons for the success of the scientific method is the rigorous control of the conditions of measurement. The effect of varying one parameter is examined while the other conditions are maintained constant. It must not be overlooked, however, that some of the conditions cannot be varied, and their effects cannot therefore be examined in this way. All measurements which have been made so far have been carried out on the Earth's surface where there is a strong gravitational field due to the Earth, a rotational velocity, and a centrifugal force balancing the Sun's gravitational field.

The effect of this permanent environment is usually ignored. It is ignored, for example, in the definition of the units of measurement. Although the units of mass length and time are regarded as independent this is not quite true since their experimental realization must be effected in the gravitational field having dimensions involving both length and time. Moreover, since the Earth's gravitational field cannot be varied and indirect effects produced by the field cannot be examined, no allowance can be made for them. Possible effects of the field are ignored in experiments concerned with electricity and magnetism and in the formulation of the laws of electromagnetism including the velocity of propagation of electromagnetic waves.

In view of these unavoidable difficulties care must be taken in the interpretation of experimental results and theoretical predictions. The laws of physics developed in this particular environment might be expected to hold within this environment but not necessarily outside it. Consider the Earth revolving around the Sun. Its gravitational field moves with it, the gravitational field of the Sun is always balanced by the centrifugal force and the rotation of the Earth on its axis remains the same. The environment is unaltered and the laws might be expected to remain valid. In particular there is no reason to expect a change in the speed of light in consequence of the Earth's movement around the Sun and a null result would be expected for the Michelson–Morley experiment.

If, however, we assume that the particular environment can be ignored and that the speed given by Maxwell's theory is the 'free-space' speed, as is usually done, then a positive result would be expected. The simplest interpretation of the null result is that this extrapolation to 'free-space' propagation cannot be made. There is nothing to indicate whether the environment affects the propagation of light or the units of length and time; but if, as in the special theory of relativity, the velocity of light is made constant by definition, then it is the units of length and time which must be affected.

The contraction of length and time. The null result of the Michelson–Morley experiment can be brought into accord with the conception of free space velocity if the unit of length is changed so that the arm of the instrument in the direction of the movement around the Sun is reduced in length in the ratio $(1-v^2/c^2)^{1/2}$. Then, in order to preserve the constancy of the velocity of light, the unit of time must be modified in the same way. This purely empirical adjustment of the units is to be regarded as a first attempt to free the units of measurement from the restriction placed by the Earth's environment.

If one seeks a physical cause for the necessity of these adjustments it seems reasonable to look for it in the Earth's environment which is known to differ from the ideal 'free-space' condition.

Einstein's special theory of relativity. It seems to me that the derivation of the time and space contraction in Einstein's special theory is equally empirical. It is true that Einstein's paper[1] contains an important section on the synchronization of clocks in which it is pointed out that distant clocks cannot be synchronized unless some assumptions are made about the velocity of light. This section is particularly relevant to experiments that may be made in the future with atomic clocks in artificial satellites, but it is not relevant to any measurements that have been made in the past. Nor do these ideas play an important part in the derivation of the Lorentz transformations in Einstein's paper. He uses them to define time in such a way as to eliminate the ordinary pre-relativity Doppler effect, but this is a convenience rather than a necessity. It has been shown that the well-known clock paradox is predicted by the theory only by changing the meaning of the symbols during the course of the argument[2–4]; but there seems to be a more serious contradiction which has not yet been explained or indeed aroused any comment. This is, that although the theory is in accord with experimental results obtained in the Earth's environment, and was indeed inspired by the difficulty in explaining the Michelson–Morley result, the theory itself is deliberately restricted to another environment in which there is no gravitational field.

An experimental approach to gravitation. The main purpose of this communication is to direct attention to the fact that the restrictions in the environment placed by the gravitational field have at last been removed. Satellites have so far been used mainly to overcome the effects of the Earth's atmosphere on the absorption of electromagnetic and other radiations and to investigate the magnetic field and ionization at greater distances from the Earth than is possible by older methods. But the balancing of the Earth's gravitational pull by a centrifugal force may be a more important advantage. It may be possible to investigate gravitational and inertial effects by controlled experiment. Even simple experiments like Newton's rotating bucket might yield interesting results; and techniques now exist for measuring the precise frequency of atomic oscillators travelling in satellites both by means of recording instruments in the satellite and radio transmissions to Earth.

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Thermal Resistivity of Irradiated Graphite

Mason and Knibbs[1] have recently measured the thermal resistivity of polycrystalline graphite of various crystallite sizes irradiated for various doses at 250°–275° C. They explain the extra phonon scattering arising from the irradiation by attributing it to interstitial clusters, one cluster per crystallite-plane, and they reckon that vacancies make only a small contribution. This explanation is not entirely satisfying. For, at all crystallite sizes studied[1], the increase of thermal resistivity on irradiation was found experimentally to be comparable with the thermal resistivity before irradiation, implying that the mean free path of scattering from irradiation-induced defects, L_i, is comparable with the mean free path for crystallite-boundary scattering, L_a, which is taken as equal to the mean crystallite diameter. This means that if there is only one scattering defect per crystallite-plane, then the diameter of this defect is a substantial fraction of the crystallite diameter, and therefore the concentration of surviving interstitials is large, say at least 10 per cent. With a dose of only 177 MWD/Ate, however, the number of displaced atoms calculated by the Kinchin–Pease method[2] is only about 2 per cent and at 250° C a large proportion of these are lost by recombination with vacancies; this is confirmed by electron microscopy of single crystals[3] and by stored energy results[4]. There cannot,

therefore, be enough interstitials to construct the defects postulated.

As an alternative approach, we regard the grain boundary as a sink or gutter for interstitials, and we assume that free clusters do not occur in fine-grained material of the type studied. If the vacancies are dispersed throughout the lattice as immobile singles and their concentration is n_0 per lattice atom, and if we assume that the rates of reaction between the mobile interstitials and (*a*) the vacancies and (*b*) the crystallite boundary are proportional to the perimeter of the vacancies and of the boundary respectively[5], we deduce that:

$$dn_0/dt = cL_a/n_0L_a^2$$

where c is a constant of order unity, L_a is the crystallite size measured in units of 1·62 Å (the mean diameter of a graphite atom), and where t represents dose in units of 10^4 MWD/Ate, this being the dose in which all lattice atoms are displaced on the average once by the irradiation. Integrating, we obtain $n_0 = (2ct/L_a)^{1/2}$. The phonon-scattering cross-section of a vacancy is of the order of its geometrical cross-section[6], so the corresponding mean free path, L_v, is of the order of $1/n_0$ atoms. The thermal resistivity is therefore represented theoretically by:

$$\frac{1}{K} = \text{const.}\left(\frac{1}{L_a} + \frac{1}{L_v}\right) = \text{const.}\left(\frac{1}{L_a} + (2c)^{1/2}\frac{t^{1/2}}{L_a^{1/2}}\right)$$

The experimental results of Mason and Knibbs[1] can be represented well enough by:

$$\frac{1}{K} = 1{,}040\left(\frac{1}{L_a} + \frac{t^{1/2}}{L_a^{1/2}}\right) \quad (K \text{ in c.g.s.u.})$$

which is of the required form. By comparison, $c = 0{\cdot}5$. This value is satisfactorily near unity; I shall not discuss it further here, as it includes various factors the theoretical value of which it is difficult to estimate accurately.

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GEOLOGY

New Isotopic Age Measurements from the McMurdo Sound Area, Antarctica

Subsequent to the publication[1,2] of potassium/argon dates from basement rocks of the McMurdo Sound area, a few additional dates have been obtained. As shown in Table 1, the potassium/argon dates now available represent a variety of igneous and metamorphic rock types. Each reported date is based on an average of two or three determinations of radiogenic argon content.

Table 1. Potassium/Argon Ages from the McMurdo Sound Area*

Sample No.	Rock type	Mineral dated	Age ($\times 10^6$)	Reference	Location
ATZ-1	Monzonite gneiss	Biotite	425 ± 15	1	77° 40′ S., 162° 45′ E.
ATZ-2	Lamprophyric dyke	Biotite	458 ± 20	1	77° 40′ S., 162° 45′ E.
ATZ-3	Marble	Biotite	500 ± 20	1	77° 26′ S., 163° 48′ E.
GD-1	Paragneiss	Biotite	500 ± ?	2	77° 24′ S., 163° 40′ E.
P-1	Diorite	Biotite	496 ± 15		77° 25′ S., 163° 50′ E.
P-2	Basic dyke	Biotite	524 ± 15		77° 25′ S., 163° 50′ E.
AS-1	Lamprophyric dyke	Biotite	520 ± 30		77° 43′ S., 162° 40′ E.

* Except for *GD*-1, determinations were carried out by Geochron Laboratories, Inc., Cambridge, Mass., and constants used were: $\lambda\beta = 4{\cdot}72 \times 10^{-10}$/year and $\lambda_e = 0{\cdot}585 \times 10^{-10}$/year and $^{40}K/K = 1{\cdot}22 \times 10^{-4}$ g/g.

A comparison of samples *ATZ*-3 and *P*-2 emphasizes the importance of realizing that the potassium/argon ages are interval estimates. The central figure for *ATZ*-3, a marble sample, is 500 m.y. The corresponding figure for *P*-2 is 524 m.y. In this case, field observation reveals that the basic dyke cuts the marble, shows no sign of post-intrusion metamorphism, consequently is younger than the marble. Consideration of the reported intervals reveals that the dyke may be as much as 11 m.y. younger than the marble without contradicting the potassium/argon determinations.

The reported ages suggest that there may actually have been two distinct periods of activity: (1) Late Cambrian–Early Ordovician (510 ± 25 m.y.) metamorphism of previously existing sediments, accompanied by the intrusion of certain igneous systems; (2) Late Ordovician–Early Silurian (440 ± 25 m.y.) intrusion of later systems. Observations by McKelvey and Webb[3] and Allen and Gibson[4] in Wright Valley and Victoria Valley respectively also suggest that the basement intrusives of the McMurdo Sound area may be divided into two groups of slightly different ages (Table 2). Present results tend to strengthen this hypothesis.

Table 2. Tentative Correlation of Marble Point with Wright Valley Intrusives

	Pearn (1963)	McKelvey and Webb (1962)
		Wright intrusives
Late Cambrian	Brewer Hill granite	Dais granite
	Bill Hill granodiorite	Theseus granodiorite
(*P*-2)	Bill Hill 'trap'	Loke microdiorite
(*P*-1)	Surko Creek diorite	(No apparent equivalent)
		Victoria intrusives
Ordovician	Surko Creek granite	Vida granite
	Surko Creek and Air Strip 'trap' dykes	Vanda lamprophyres

The Marble Point locality has recently been examined in some detail[5], and the intrusive units recognized by McKelvey and Webb in the Wright Valley area have been tentatively identified. It is likely that some of the intrusives of Taylor Valley also are referable to the two groups of McKelvey and Webb.

From Antarctica in general there have been more than 65 isotopic dates reported in the 450–550 m.y. range[6]. An extensive period of metamorphism–plutonism occurred during early Palæozoic time with a peak of activity indicated in lower Ordovician. The geographical distribution of 400–550 m.y. dates extends over the entire peripheral area of eastern Antarctica from the Conrad Mountains (72° 00′ S., 9° 30′ E.) to Cape North (70° 40′ S., 165° E.) and south to McMurdo Sound Region, Victoria Land. Such distributions suggest that a widespread period of severe orogenic activity affected the whole eastern Antarctic region about 450–550 m.y. ago.

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Norwegian Minerals and Rocks of Possible Caledonian Origin

In a recent communication[1] an outline is given of the sequence of metamorphic and structural events which affected the Lewisian rocks of the Glenelg inlier. In this outline a period of folding, about 740 million years old, and certainly older than 560 million years, is considered to form part of the Caledonian orogeny. Further can be mentioned the intrusion of the Ben Vuroch granite into Dalradian sediments between 700 and 500 million years ago[2].

These results are of importance for the discussion of the Norwegian part of the Caledonian chain. Age determinations[3] of minerals and rocks from three districts are referred to here. They may all be of Caledonian origin:

(1) The Rødsand mine is situated in the Molde–Kristiansund district, central Norway. A molybdenite from the mine gave the rhenium/osmium age 540 (± 10%) million years.

(2) Two micas from pegmatites in the county of Sogn and Fjordane, Southern Norway, gave the potassium/argon ages 582 and 590 million years.

(3) Potassium/argon determinations of micas in the carbonatite in the Fen area, 119 km south-west of Oslo, gave the ages 565, 590 and 603 million years.

According to my investigations in the Molde–Kristiansund district, we here have a Precambrian–post-Precambrian Caledonian stratigraphic sequence. The Precambrian part of this sequence is quite as important as the post-Precambrian part. A Precambrian basement is not found.

The two pegmatites in the county of Sogn and Fjordane are both situated in gneisses. These gneisses may belong to a Caledonian stratigraphic sequence, but are probably parts of the Precambrian basement. In both cases the rocks may have undergone an early Caledonian metamorphism.

According to Prof. J. Tuzo Wilson[4], the graben in the South Norwegian sparagmite region can be considered to belong to the Caledonian orogeny. The Fen area may have originated in connexion with these faults.

The age determinations given here indicate that the Precambrian–Cambrian part of the Norwegian Caledonian history is more important than is generally assumed.

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A Beacon Sandstone: its Petrology and Hydrocarbon Content

After the ill-fated Scott expedition of 1910–13 a valuable suite of Antarctic rocks, collected by Sir Raymond Priestley, was donated to the University of Nottingham. In it was a specimen from the Beacon Sandstone series collected from Cape Royds. This we have investigated to see whether such a series might conceivably possess the properties of reservoir or source rocks for oil.

The Beacon Sandstones extend throughout eastern Antarctica in the region of the Great Fault Scarp. The succession, consisting of nearly horizontal strata interleaved with dolerite sills, characterize the tabular mountains for more than a thousand miles[1]. In some places, such as north of the Mackay Glacier, the series is more than 3,500 ft. thick and has been subdivided into three main lithological divisions[2]. Unfortunately, we do not know to which of these subdivisions the Priestley specimen belongs.

In hand specimen it is a light-coloured, very coarse-grained, hard quartzite with numerous small pebbles. The sorting is rather poor and the grain size varies from 10 mm down to silt grade interstitial sediment. The majority of the pebbles are of unstrained quartz, the rest are of granite, gneiss, schist and quartzite. Very little cement has been introduced, and while a small proportion of the grains have a fringe of secondary quartz most of them are welded along common margins. Sporadically distributed throughout are small chalky patches and also small holes often lined with chalky material. On X-ray analysis this chalky substance has proved to be albite-rich alkali felspar. It is not known why the felspar is in this condition, and further investigation is required. Of interest and significance to the present study are euhedra and granular aggregates of sphene. While no metamorphic significance is normally attached to this mineral, in some instances elsewhere, it is clear that it has begun to crystallize at higher temperatures than associated epidote. The minimum temperature of formation of epidote is 160° C, so that of sphene must be higher. If the recrystallization of the sphene and the quartz were at the same time, then the formation of the quartzite must have occurred above 160° C.

In essence, therefore, this is a quartzite of low porosity of which the induration and association with dolerite intrusions would, at first sight, appear to rule out the possibility of the survival of hydrocarbons. Moreover, the rock was digested with hydrofluoric acid in order to

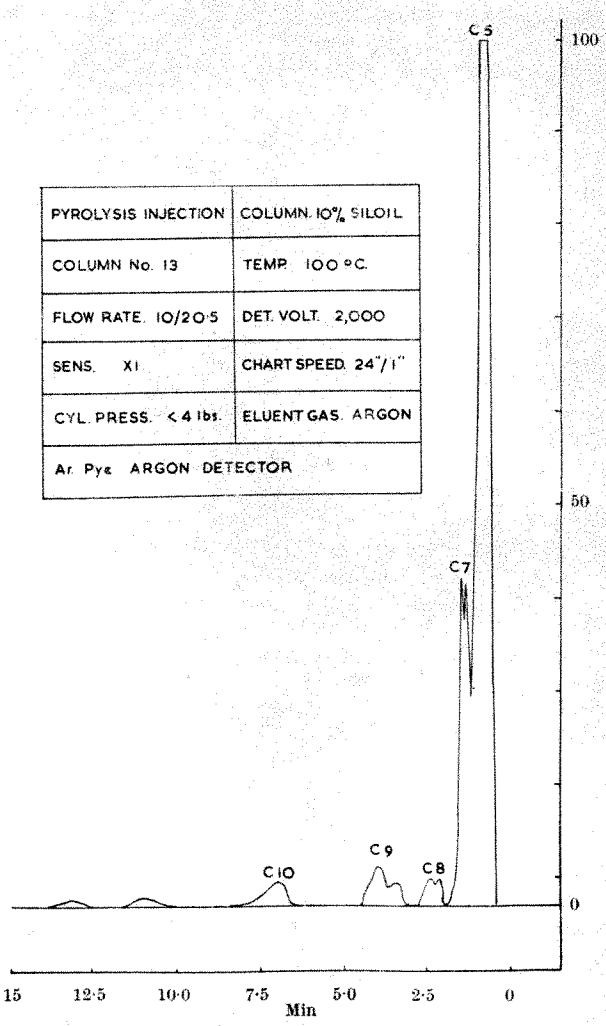


Fig. 1

scan the sediment for micro-organisms such as dinoflagellates. No trace of these or other organic matter was found. Nevertheless, seven powdered specimens were subjected to the version of gas chromatography known as 'pyrochromatography'[3].

All seven samples yielded identical chromatograms and Fig. 1 is a typical example. Of the ten compounds extracted from the specimen five consist of paraffins ranging from C_5 to C_{10}; the remainder have not yet been identified. These oils differ from the indigenous oils of normal sediments not only in containing no light fraction but also in the narrow range of their boiling points, and from those of metamorphosed oil-bearing strata in containing no naphthenic or phenolic compounds. In other words, they are neither original nor residual but are probably migrants which have been fixed by condensation.

It would be imprudent to speculate too far from one sample but the following conditions might be envisaged. The presence of sphene suggests that during metamorphism, presumably by the dolerites, the Beacon Sandstone was subjected to a temperature in excess of 160° C. Adjacent to the intrusions higher temperatures would develop accompanied by hydrothermal activity derived from the inherent water content of the invaded sediments. Consequently, by a process akin to steam distillation, the volatile constituents of the sediments would migrate laterally and upwards throughout the thermal aureole and would condense as soon as they found themselves in a rock with a temperature lower than their own boiling point. Such a thermo-dynamic process would account for the narrow range of saturated hydrocarbons found in this specimen. The fact that the boiling point of the highest recorded C_{10} paraffin is 174° C, which is very little above the minimum temperature indicated by the sphene, would support such a hypothesis. Moreover, such an explanation seems to indicate that this considerable column of upper Palæozoic and Mesozoic sediments might well contain source rocks capable of producing oil. Unfortunately, so far as we know, there are no structures capable of concentrating such oil into an oilfield, but from the nature of these deposits there might conceivably be stratigraphic traps.

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CHEMISTRY

Decontamination of Synthetic Effluent by Flotation

Interested by the communication of Sebba[1], some scanning tests were undertaken in order to ascertain whether the flotation of ions could be used for the decontamination of radioactive effluent. The results obtained may be considered as being indicative and of limited value only, owing to the fact that no special efforts have been made to achieve optimal conditions, to use foam stabilizers, etc.

The glass flotation cell used is illustrated in Fig. 1. Encouraging results have been obtained by use of a foam column of 60 cm and by introducing the surfactant, that is, sodium lauryl sulphate, 25 cm underneath the liquid inlet. The foam column proved to be very stable at $pH = 2$ if distilled water was used for effluent make-up. When tap water, containing about 40 p.p.m. Ca^{2+}, was used, less-stable columns were obtained. In this case, a foam stabilizer should be used. Each run lasted at least 2 h; equilibrium was attained after 1 h.

Table 1

No.	Tracer	Carrier (p.p.m.)	Chemicals added		Decontamination factor*	
			Cu^{2+} (p.p.m.)	$Fe(CN)_6^{4-}$ (p.p.m.)	Flotation	Flocculation
1	^{134}Cs	—	—	—	1·4 ± 0·2	—
2	^{134}Cs	1	—	—	1·1 ± 0·0	—
3	^{134}Cs	—	15·1	11·5	375 ± 125	150 ± 50
4	^{134}Cs	1	15·1	11·5	90 ± 30	45 ± 20
5	^{134}Cs	—	15·1	11·5	25 ± 6	32 ± 6
6	^{89}Sr	—	—	—	2·5 ± 0·5	—
7	^{89}Sr	—	—	—	2·4 ± 0·1	—
8	^{89}Sr	—	15·1	11·5	1·7 ± 0·3	1·3 ± 0·1
9	^{89}Sr	1	15·1	11·5	1·3 ± 0·2	1·1 ± 0·1
10	^{152}Eu	—	—	—	330 ± 150	—
11	^{152}Eu	1	—	—	400 ± 90	—
12	^{152}Eu	—	15·1	11·5	200 ± 60	1·6 ± 0·2
13	^{152}Eu	1	15·1	11·5	750 ± 250	1·1 ± 0·0

Flotation runs, $pH = 2$; 25 p.p.m. sodium lauryl sulphate; distilled water, except No. 5, where tap water was used; liquid flow-rate, 0·7 m/h; air flow-rate, 1 m/h, except No. 5, 1·55 m/h. Flocculation runs, in beakers under slow stirring during 0·5 h; distilled water; phase separation by centrifugation directly after stirring; $pH = 2$.
All decontamination factors are average values from two or more runs; during each run three or more samples were taken after 1 h of operation when equilibrium was attained.
* Concentration column influent over effluent.

Some results are given in Table 1. It appears that decontamination by straight ion flotation is high for Eu^{3+} and relatively low for Sr^{2+} and Cs^+. In order to improve the Cs^+ decontamination, a copper ferrocyanide floccule was formed in the solution. It appeared that the ion flotation of Eu^{3+} is not affected but that the Cs^+ decontamination improved markedly. Sr^{2+} decontamination remains almost unchanged, which becomes understandable after comparing the flotation results with the reference flocculation runs that were executed simultaneously under comparable conditions. In every case the effluent from the cell was completely clear and contained no floccules. On the whole, flotation gives equal or better results than does flocculation. It is noted that the flocculation, especially of Sr^{2+} and Eu^{3+} for which other chemical treatments give better results, was done under relatively unfavourable conditions, since at $pH = 2$ only copper ferrocyanide could be formed as precipitate, and this is a specific scavenger for Cs^+ only. It is, however, very likely that if favourable conditions can be found, the same conclusion will be arrived at, although the influence

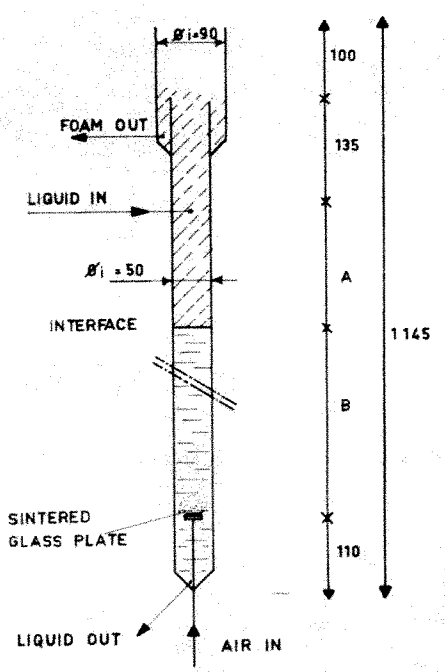


Fig. 1. Flotation cell (× 0·1). *A*, Distance from liquid inlet; *B*, distance from air inlet

of other contaminants present in waste effluent, such as organic materials, is unknown.

An interesting result of the flotation runs was that the separated foam volume represented a very small part of the volume of effluent treated. The average value obtained for more than 30 runs was 0·01 vol. per cent. This is of great importance for the treatment of radioactive effluent since the concentrated radioactive phase, foam + floccule, can possibly be stored insolubilized or contained without any further volume reduction.

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Analysis of Reversibly Interacting Systems by Differential Boundaries in Electrophoretic and Sedimentation Experiments

Differential boundaries, which can be formed in Tiselius electrophoresis cells or in special ultracentrifuge cells[1,2] between solutions differing slightly in composition, may prove to have distinct advantages over ordinary boundaries for the study of rapidly reversible interacting systems. This possibility is being explored theoretically by considering model systems in which it is assumed that the transport of species occurs in rectangular cells under a uniform force field, that the mobilities or sedimentation coefficients of reacting species are independent of concentration[3] and that reaction is governed by the simple form of the law of mass action.

To illustrate this approach we consider a model in which reactants A_1 and A_2 form complexes C_1 and C_2 reversibly in solution, according to the equations $A_1+A_2=C_1$ and $A_1+C_1=C_2$. Suppose an experiment is performed in which a solution is made up with A_1 and A_2 at constituent molar concentrations $\overline{m}_1$ and $\overline{m}_2$, and that a differential boundary is formed in a suitable cell between this solution and another with A_1 and A_2 at the slightly different constituent molar concentrations $\overline{m}_1+\Delta\overline{m}_1$ and $\overline{m}_2+\Delta\overline{m}_2$: then it can be shown[4] that the initial differential boundary must split into as many boundaries as there are basic constituents present. These boundaries should possess little structure, and will appear in the schlieren pattern simply as 'blips' drawn out by diffusion. The information required from the experiment is the velocities and areas of these blips.

In the present case two boundaries will form. Let their velocities be V_1 and V_2 and the changes in total concentration (wt./vol.) across each boundary, as deduced from the areas of the blips, be ΔW_1 and ΔW_2. Then since ΔW_1 and ΔW_2 are linearly related to $\Delta\overline{m}_1$ and $\Delta\overline{m}_2$, provided the latter are sufficiently small[4], we may write:

$$\begin{aligned}\Delta\overline{m}_1&=\sigma_{11}\,\Delta W_1+\sigma_{12}\,\Delta W_2\\ \Delta\overline{m}_2&=\sigma_{21}\,\Delta W_1+\sigma_{22}\,\Delta W_2\end{aligned}\qquad(1)$$

where σ_{11}, σ_{12}, σ_{21} and σ_{22} are appropriately valued coefficients. By repeating the experiment for different values of $\Delta\overline{m}_1$ and $\Delta\overline{m}_2$, the values of these four unknown coefficients can be determined.

Let v_1 and v_2 be the sedimentation velocities of A_1 and A_2 and assume that these are known since they are properties of the pure constituents. Also let the sedimentation velocities of the complexes C_1 and C_2 be u_1 and u_2 and consider these as unknown. Further, let the unknown free molar concentrations of A_1, A_2, C_1 and C_2 in the solution be a_1, a_2, c_1 and c_2 respectively. Then having determined V_1, V_2 and σ_{11}, σ_{12}, σ_{21}, σ_{22} experimentally, these six unknown quantities, together with two, necessarily positive, unknown multipliers λ_1 and λ_2, can be found[4] from the eight equations:

$$a_1+c_1+2c_2=\overline{m}_1 \qquad(2)$$

$$a_2+c_1+c_2=\overline{m}_2 \qquad(3)$$

$$a_1+c_1+4c_2=\sigma_{11}^2\lambda_1+\sigma_{12}^2\lambda_2 \qquad(4)$$

$$a_2+c_1+c_2=\sigma_{21}^2\lambda_1+\sigma_{22}^2\lambda_2 \qquad(5)$$

$$c_1+2c_2=\sigma_{11}\sigma_{21}\lambda_1+\sigma_{12}\sigma_{22}\lambda_2 \qquad(6)$$

$$v_1a_1+u_1c_1+4u_2c_2=\sigma_{11}^2V_1\lambda_1+\sigma_{12}^2V_2\lambda_2 \qquad(7)$$

$$v_2a_2+u_1c_1+u_2c_2=\sigma_{21}^2V_1\lambda_1+\sigma_{22}^2V_2\lambda_2 \qquad(8)$$

$$u_1c_1+2u_2c_2=\sigma_{11}\sigma_{21}V_1\lambda_1+\sigma_{12}\sigma_{22}V_2\lambda_2 \qquad(9)$$

These equations are linear in a_1, a_2, c_1, c_2, u_1c_1, u_2c_2 and λ_1, λ_2, and their solution is therefore unique. After these equations have been solved, the equilibrium constants k_1 and k_2 for the two reactions $A_1+A_2\rightleftharpoons C_1$ and $A_1+C_1\rightleftharpoons C_2$ are immediately obtained from the mass action equations $k_1=(a_1a_2)/c_1$ and $k_2=(a_1c_1)/c_2$.

Equations (2) and (3) merely define the constituent molar concentrations of A_1 and A_2. The remaining six equations are obtained by considering the flux of A_1 and A_2 in their free and combined state through the two differential boundaries, assuming that A_1 and A_2 are in chemical equilibrium with the complexes C_1 and C_2 at all times.

This method of analysing a differential boundary experiment can be generalized to deal with more complicated systems. The advantage of differential boundaries over ordinary boundaries lies in the fact that the parameters of an interacting system can be found by solving linear equations instead of non-linear differential equations. It remains to be seen what limitations are implied by the simplifying assumptions mentioned here, or are inherent in the experimental measurement of differential boundaries.

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Fibres of Guanine Nucleosides and Nucleotides

It has been shown by Gellert, Lipsett and Davies[1] that guanylic acid will form a gel under certain *p*H conditions and they were able to draw fibres from gels of the disodium salt of 3′-guanylic acid and of the free acid, 5′-guanosine monophosphate. From X-ray diffraction studies of these fibres the foregoing workers concluded that they consisted of a helical arrangement of the ribose nucleotides.

We have been studying corresponding deoxyribose compounds and we have observed gel formation in deoxyguanosine, deoxyguanosine-5′-monophosphate and the ammonium salt of deoxyguanylic acid. In addition we studied gels of the mixed 2′-, 3′-guanosine monophosphate disodium salts. (Gellert *et al.* separated these isomers before forming fibres.)

Deoxyguanosine. Solutions were prepared in the *p*H range 4–7 by dissolving 25 mg/ml. in a warm buffer solution (disodium hydrogen phosphate and citric acid), and on cooling a gel formed in the solution. From this gel it was possible to draw fibres suitable for X-ray diffraction studies which showed that the material was oriented.



Fig. 1. X-ray diffraction patterns. *a*, Fibres of deoxyguanosine; *b*, oriented crystals of deoxyguanosine; *c*, fibres of mixed disodium salts of 2′- and 3′-guanosine monophosphates

Occasionally, however, the substance crystallized and no gel was observed. If the gel is stirred it crystallizes and then it is not possible to draw fibres. Gel formation was observed at *p*H 4, 5 and 6, but not at *p*H 7. Attempts to form gels with guanosine were unsuccessful.

Fig. 1*a* is the X-ray diffraction pattern from deoxyguanosine fibres. There is a strong meridional reflexion at 3·4 Å on the second layer line and a strong equatorial reflexion at 20·5 Å. Six additional equatorial reflexions were observed, and they can all be indexed satisfactorily on the basis of a hexagonal lattice with $a = 23{\cdot}9$ Å. The 3·4 Å spacing along the fibre axis suggests that the molecules are packed so that the guanine bases are perpendicular to the axis with two units, possibly related by a 2-fold screw axis, forming the repeat distance along the axis. The number of molecules forming a co-planar set may be three as in some synthetic polynucleotides[2] or it might be four as suggested for guanylic acid gels[1]. The deoxyguanosine as supplied is in the form of extremely fine needles which tend to form a 'mat' with the needles lying parallel to one another and from this material it is possible to choose an oriented powder specimen. The X-ray diffraction pattern of such a specimen, kept stationary, is shown in Fig. 1*b*. Again there is a strong reflexion on the meridian on the second layer line at approximately the same spacing (3·58 Å) as for the gel fibres but the equatorial reflexions are different. X-ray Weissenberg photographs of a single needle recrystallized from water indicated that the lattice was orthorhombic with $a = 11{\cdot}0$ Å, $b = 59{\cdot}7$ Å, $c = 7{\cdot}16$ Å. The density determined by flotation in a calibrated gradient column of carbon tetrachloride and *n*-hexane was 1·535 g/cm³, which indicated 16 molecules per unit cell. From these measurements it would seem that there is no water of crystallization in the cell. If larger crystals can be grown it is hoped to examine the structure of deoxyguanosine in the same detail as that of guanine[3], but so far it has not been possible to get sufficiently large crystals.

Deoxyguanosine-5′-monophosphate and deoxyguanylate, ammonium salt. Both these substances formed gels at *p*H 5 and *p*H 6 but it was difficult to draw satisfactory fibres for X-ray diffraction examination. In each case the photographs which were obtained showed a strong meridional reflexion at 3·35 Å. This indicates, as for deoxyguanosine, that the bases are stacked in planes perpendicular to the fibre axis.

Mixed isomers 2′-, 3′-guanosine monophosphate, disodium salts. When a solution (*p*H 6) of these mixed isomers was allowed to stand for over a week at room temperature a whitish gel formed which could be drawn quite easily into fibres several inches long. The X-ray diffraction diagram (Fig. 1*c*) is different from the above deoxyguanosine diagrams and from the diagrams obtained by Gellert *et al.*[1] with the separated isomers. The layer line spacing is approximately 16·0 Å with a strong reflexion on the meridian at 3·25 Å (5th layer). This could indicate that the molecular units are arranged on a helix with five units per turn of the helix. There are six reflexions on the equatorial layer line and these can be indexed on the basis of a hexagonal lattice with $a = 27{\cdot}1$ Å. A molecular unit could be a tetramer as suggested by Gellert *et al.* but we do not find in any of our patterns the strong meridional reflexion at 6·75 Å which is a feature of their 3′-isomer patterns.

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Formation of Electrons and of Atomic Hydrogen in γ-irradiated Ice

In ice at 77° K containing acids such as sulphuric, perchloric and orthophosphoric, irradiated with γ-rays, hydrogen atoms were first detected by electron spin resonance by Livingston, Zeldes and Taylor[1]. In neutral ice, however, no hydrogen atoms were observed at 77° K although some were detected at 4° K[2]. It has been suggested[3] that radiation-produced electrons, $(H_2O)^-$, which have been shown[4] to occur in the radiolysis of aqueous solutions, may react with a hydrogen ion in the acidic ice to produce a (stable) hydrogen atom:

$$(H_2O)^- + H^+ \rightarrow H + H_2O \qquad (1)$$

In an analogous manner we may expect that a Brønsted acid such as $H_2PO_4^-$ may react to form a hydrogen atom, by a proton transfer, according to the equation:

$$(H_2O)^- + H_2PO_4^- \rightarrow H + HPO_4^{2-} \qquad (2)$$

Such reactions have been suggested to occur in aqueous solution[5]. In the case of bisulphate, one would have the analogous reaction:

$$(H_2O)^- + HSO_4^- \rightarrow H + SO_4^{2-} \quad (3)$$

although there are reasons to suggest that here, as in the other cases, the reaction goes to a greater or lesser extent through an unstable intermediate, namely $(HSO_4)^{2-}$, which may break up according to:

$$(HSO_4)^{2-} \rightarrow H + SO_4^{2-} \quad (4)$$

and possibly also to some extent:

$$(HSO_4)^{2-} \rightarrow OH^- + SO_3^- \quad (5)$$

To ascertain whether or not hydrogen atoms are formed under these conditions, experiments were carried out with ice containing different solutes. Cylinders of crystalline ice weighing about 0·03 g were formed by rapidly freezing the appropriate solution to 77° K in 0·15-cm diameter tubes. These cylinders were then removed from the glass tubes and irradiated directly in contact with liquid nitrogen with cobalt-60 γ-rays at a dose rate of 0·17 megarads/h. Tests with degassed and non-degassed solutions showed no difference and the results reported are for non-degassed solutions. After a total dose of 2·5–8 megarads, the ice cylinders were transferred to a standard quartz-tipped electron spin resonance Dewar, thus avoiding any possible electron spin resonance signals from the irradiated sample tube, and were examined with a 'Microspin' electron spin resonance spectrometer operating at 9,200 Mc/s.

The appearance of hydrogen atoms was found to be of quite general occurrence in the systems containing certain oxyanions. Hydrogen atoms were recognized by their characteristic hyperfine doublet split by 505 gauss. The yields of hydrogen atoms per 100 eV, $G(H)$, for the various solutes are shown in Table 1. The yield, for example, in solutions containing $H_2PO_4^-$, was determined by direct comparison, under identical experimental conditions, with the hydrogen atom yield in glassy 0·20 mole fraction sulphuric acid, for which $G(H) = 1·66$ has been reported[6]. This comparison was made in high-purity quartz tubes in which the background hydrogen atom signal from the quartz itself was negligible. The other yields were obtained by comparison with a standard sample containing $H_2PO_4^-$ as solute. The relative values were reproducible to within less than 15 per cent. These represent initial yields since the hydrogen atom yields were found to be linear with dose up to 10 megarads.

Fig. 1 shows that the hydrogen atom yields are linear with concentration of the solute. It is significant that hydrogen atoms are formed even in irradiated ices containing neutral salts as solutes. The linearity shown in Fig. 1 as well as the consistent reproducibility of the results indicate that homogeneity of the solute was retained on rapid freezing.

It was also found that the observed hydrogen atom electron spin resonance signals in ices containing $H_2PO_4^-$ or HSO_4^- could be decreased by the addition of various second solutes which are known to be good electron scavengers in aqueous solutions. This is as predicted if the hydrogen atoms do originate by equations (2)–(4). Such

Table 1. Hydrogen Atom Yields per 100 eV ($G(H)$) in Irradiated Ice containing Various Solutes at 77° K

Solute	pH	$G(H)$ (for 1·0 M solute)
NaH_2PO_4 (1·0 M)	4·3	0·27
KH_2PO_4 (1·0 M)	4·3	0·27
Na_2HPO_4 (0·2 M)	9·2	0·07*
Na_3PO_4 (0·6 M)	12·6	0·03*
$NaHSO_4$ (1·0 M)	1·2	0·14
Na_2SO_4 (1·0 M)	6·0	0·04
$NaHCO_3$ (1·0 M)	8·2	0·01
K_2CO_3 (1·0 M)	11·3	0·006

* Extrapolated linearly to 1·0 M concentration.

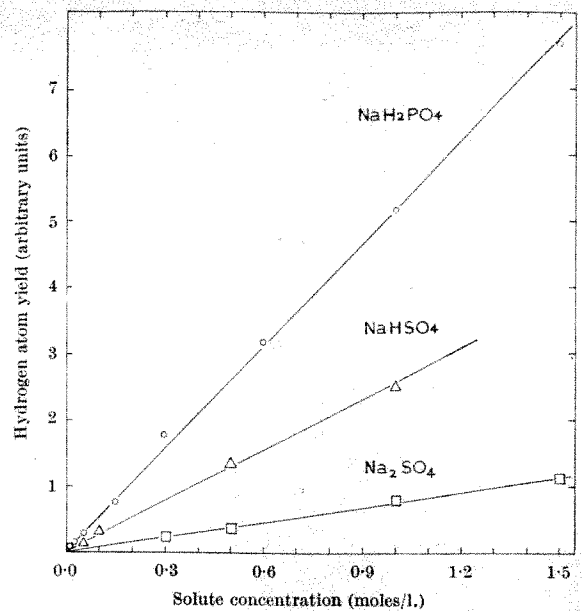


Fig. 1. Dependence of hydrogen atom yields on concentration of solutes in irradiated ice at 77° K. ○, NaH_2PO_4; △, $NaHSO_4$; □, Na_2SO_4

solutes include nitrous oxide, nitrate, nitrite and acetone, which latter would react according to:

$$(H_2O)^- + CH_3.CO.CH_3 \rightarrow [CH_3.CO.CH_3]^- + H_2O \quad (6)$$

In this case a seven-line electron spin resonance spectrum is observed which one has reason to attribute to the acetone negative ion-radical. The kinetics of such competition reactions in the solid and the paramagnetic species produced are at present being studied. In the case of SO_4^{2-} as solute anion these scavengers were found to have no effect on the hydrogen atom electron spin resonance signal.

While a mechanism represented by equations (2)–(4) may account for all or part of the hydrogen atoms observed in the cases of the Brønsted acid solutes: $H_2PO_4^-$, HPO_4^{2-}, HSO_4^- and HCO_3^-, the hydrogen atoms found in the SO_4^{2-}, PO_4^{3-} and CO_3^{2-} systems should arise from the water molecules by a different mechanism. It would appear that the solute anion is necessary for the 'stabilization' of the hydrogen atoms at 77° K (since at this temperature hydrogen atoms are not observed in irradiated pure ice) and that the hydrogen atoms observed in ices containing these latter solutes, which do not contain hydrogen, are directly produced only from water molecules which are in the solvation shell of these solute anions. This may explain the fact that these hydrogen atoms are not affected by the presence of scavenging solutes.

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Stimulated Singlet–Triplet Transitions in Organic Phosphors

Morantz *et al.*[1] have reported that stimulated emission may be observed from the triplet states of organic phosphors. The emission was characterized by a regular series of spikes of intensity implying an oscillatory condition due to the amplification of the stimulated emission as in similar experiments on ruby. Workers in other laboratories, including our own, have failed to detect this effect. We have, however, succeeded in producing the triplet state of such a compound by the reverse process of direct singlet–triplet absorption using a typical flash photolysis apparatus. This is interesting in its own right as it paves the way for studies of triplet states in the absence of excited singlet states, something which hitherto had not been thought possible. Furthermore, the small fraction of molecules excited in this way is in agreement with calculations which suggest that a prohibitively high inversion population density is required to obtain oscillation in a resonant cavity with such compounds using flashes of energy less than 1,000 joules. Experience suggests that even with a 100-fold increase in flash intensity, such concentrations would be difficult to realize.

The phosphors used were naphthalene and 1-bromonaphthalene which have similar singlet–singlet absorption spectra both in position and intensity whereas the singlet–triplet absorption, although in the same spectral region, is much more intense for 1-bromonaphthalene. In the apparatus used the quantum output of a 640-joule flash was found, by potassium ferrioxalate actinometry[2], to be $1{\cdot}8 \times 10^{19}$ quanta. These were detected in a cylindrical reaction vessel (20 cm long, 2 cm internal diameter) which was placed between two photolysis lamps within a highly reflecting enclosure. To prevent the production of triplet phosphor by singlet–singlet excitation followed by intersystem crossing, the mechanism usually responsible for triplet states observed in flash experiments[3], filter solutions were placed in an outer jacket surrounding the reaction vessel. The spectra of the filter solutions, together with the spectra of solutions of naphthalene and 1-bromonaphthalene in liquid paraffin, the solvent chosen for this work, are illustrated in Fig. 1.

Any transient spectra formed in the degassed solutions were recorded on photographic plates ~50 μsec after the photolysis flash using a second spectroscopic flash. The results, summarized in Table 1, show that absorption in the region of the singlet–triplet transitions gives rise to the triplet state of 1-bromonaphthalene but not that of naphthalene. The negative result obtained in this wavelength region for naphthalene confirms the adequacy of

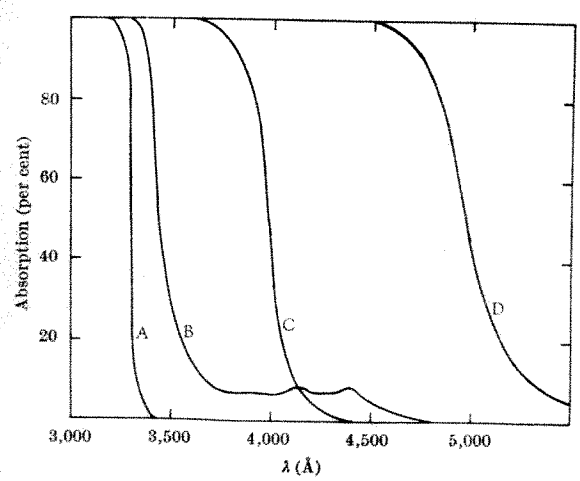


Fig. 1. Absorption spectra of phosphor and filter solutions (path-length, 1 cm). *A*, Naphthalene in liquid paraffin (0·43 mole/l.); *B*, 1 bromonaphthalene in liquid paraffin (1·1 mole/l.); *C*, filter 1 (400 g/l. $NaNO_2$, 10 g/l. $NaNO_3$ in water); *D*, filter 2 (40 g/l. K_2CrO_4, 120 g/l. $NaNO_2$ in water)

Table 1. Transients detected in flash photolysis experiments using degassed liquid paraffin solution within a 'Pyrex' reaction vessel

Filter	Transients: Naphthalene (0·43 mole/l.)	Transients: 1-Bromonaphthalene (1·1 mole/l.)
None	Triplet naphthalene	Triplet 1-bromonaphthalene
Filter 1 Transmission above 3700 Å	None	Triplet 1-bromonaphthalene ($\sim 10^{-5}$ mole/l.)*
Filter 2 Transmission above 4500 Å	None	None

* Assuming the triplet–triplet extinction coefficient to be 10^4 (cf. ref. 4).

filter 1 in preventing singlet–singlet absorption. In fact filter 1 also cuts out part of the singlet–triplet absorption region.

Since the energy output of gas-filled flash lamps is almost independent of wave-length[5], the fraction of molecules raised to the triplet state by direct absorption may be calculated from the relation:

$$\frac{\Delta n}{n} = B\rho(\nu) = \frac{Qh\nu}{\Delta\nu c}\,\frac{c^3}{8\pi h\nu^3}\,A$$

where A and B are Einstein coefficients for spontaneous and stimulated emission, n is the number of molecules in the ground state, $\rho(\nu)$ is the radiation density at any frequency ν, Q is the quantum output of the photolysis flash in the frequency range $\Delta\nu$. For organic phosphors A may be put equal to $\frac{\gamma_p}{(1-\gamma_F)}\tau_p$, where γ_p, γ_F are the quantum yield of phosphorescence and fluorescence and τ_p is the measured phosphorescence lifetime. The calculated values of $\Delta n/n$ are given in Table 2. These values are in good agreement with the amount of triplet 1-bromonaphthalene formed by direct absorption detected by experiments (Table 1) provided allowance is made for absorption by filter 1 in the singlet–triplet absorption region. The negative result in the case of naphthalene is also to be expected.

Table 2. Calculated values for the fraction of molecules expected to be excited to the triplet state by direct absorption $\Delta n/n$ and the critical inversion concentration (Mc) necessary for oscillation in a resonance cavity under the conditions specified in the text

Compound	First triplet level (cm^{-1})	τ_p (sec)	γ_p	γ_F	$\Delta n/n$	Mc
Acetophenone	26,000 (ref. 6)	$8{\cdot}0 \times 10^{-3}$ (ref. 8)	0·63 (ref. 8)	—	9×10^{-5}	3×10^{-1}
Benzophenone	24,400 (ref. 6)	$6{\cdot}0 \times 10^{-3}$ (ref. 8)	0·84 (ref. 8)	—	2×10^{-4}	2×10^{-1}
Pyrazine	26,000 (ref. 7)	$2{\cdot}0 \times 10^{-2}$ (ref. 9)	1·00 (ref. 7)	—	6×10^{-5}	2×10^{-1}
Naphthalene	21,300 (ref. 6)	2·6 (ref. 9)	0·09 (ref. 8)	0·37 (ref. 8)	9×10^{-8}	3×10^{3}
1-Bromonaphthalene	20,700 (ref. 6)	$1{\cdot}8 \times 10^{-2}$ (ref. 9)	0·55 (ref. 10)	—	5×10^{-5}	5×10^{-1}

Since stimulated emission and absorption are equally probable processes the experimental evidence indicates that even if we had an inversion concentration of 1 mole/l. of triplet 1-bromonaphthalene a high-energy flash would only induce a relatively small proportion back to the ground-state. On the other hand, the use of a resonant cavity could provide amplification of the stimulated emission if the increase in mode intensity per traverse due to stimulated emission were greater than the losses due to absorption and scattering in the medium and the mirrors. The threshold inverted population density necessary for oscillation may be expressed[11]:

$$Mc = \frac{8\pi\nu^2\alpha}{c^2 g(\nu_c) L A}\,\frac{1{,}000}{N}\ \text{moles/l.}$$

where L is the distance between the mirrors, and α is the fractional loss per pass when the amplifying medium is neutral, $g(\nu_c)$ is a shape function representing the emission spectrum which we have assumed to be Gaussian in form. Mc is calculated for a series of phosphors taking $L = 1$ cm, as used by Morantz[1], and assuming $\alpha = 0{\cdot}01$, which is probably a lower limit. As can be seen from Table 2, the required triplet concentrations are all greater than 10^{-1} mole/l., 100 times the total concentration of phosphors

used by Morantz. Stockman *et al.*[12] have suggested that the fluorescence of organic compounds might provide a more probable method of obtaining an oscillatory condition.

We thank Dr. K. R. Lea for interesting discussions on this topic.

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Equilibria in Water–Alcohol Mixtures

In order to describe accurately the kinetics of nucleophilic substitutions carried out under alkaline conditions in aqueous alcoholic solutions, in which both hydroxy and alkoxy groups are introduced, it is necessary to know the equilibrium constant (K) of the reaction:

$$R\,OH + OH^- \rightleftharpoons RO^- + H_2O$$

$$K = \frac{[OH^-]\,[ROH]}{[OR^-]\,[H_2O]}$$

in order to calculate the respective concentrations of the two ions.

Caldin and Long[1] have reported investigations on ethanol–water mixtures containing sodium hydroxide in which TNT was used as an indicator. The purple colour obtained is supposedly due to the TNT anion produced by the equilibria:

$$BH + OH^- \rightleftharpoons B^- + H_2O \;(BH^- = \text{TNT})$$

$$BH + OEt^- \rightleftharpoons B^- + EtOH$$

the concentration of B^- varying with the ratio $[OH^-]$ $[OEt^-]$. The procedure is similar to that of Hine and Hine[2], who used 4-nitrodiphenylamine as indicator. We have been unable to reproduce the results of Caldin and Long and have some evidence that TNT does not ionize in the simple manner shown here.

However, where the two reactions:

$$R' - X + OH^- \xrightarrow{k'_2} R'OH + X^-$$

$$R' - X + OR^- \xrightarrow{k''_2} R'OR + X^-$$

occur simultaneously it is possible to obtain values of k'_2 and k''_2 and also of K directly from kinetic measurements providing the concentrations of $R'OH$ and $R'OR$ can be measured.

If the two reactions proceed independently of each other the ratio of rates will equal the ratio of products and will be constant throughout the reaction:

$$\text{that is, } \frac{k''_2[OR^-]}{k'_2[OH^-]} = \frac{[R'\,OR]}{[R'\,OH]} = Y \qquad (1)$$

Y may be determined experimentally, but k'_2 and k''_2 are not directly measurable since $[OH^-]$ and $[OR^-]$ are not known. However, an overall rate constant k_2 for the conversion of $R' - X$ can be measured, such that:

$$\frac{d[R'X]}{dt} = k_2[R'X]\,([OH^-] + [OR^-])$$

$$\text{and } k_2 = k'_2\,\frac{[OH^-]}{[OH^-] + [OR^-]} + k''_2\,\frac{[OR^-]}{[OH^-] + [OR^-]}$$

substituting from equation 1

$$k_2 = k'_2[OH^-]\left(\frac{1+Y}{[OH^-] + [OR^-]}\right) = k'_2[OH_0^-]\left(\frac{1+Y}{[OH_0^-] + [OR_0^-]}\right) \qquad (2)$$

where $[OH_0^-]$ and $[OR_0^-]$ are initial concentrations.

On the assumption that k'_2 is unaffected by the addition of alcohol, the value of k'_2 found from a separate experiment in water may be substituted into equation (2), and since k_2 and Y are known experimentally for any particular water–alcohol mixture and $([OH_0^-] + [OR_0^-])$ is equal to the known concentration of alkali taken, the values of $[OH_0^-]$ and hence $[OR_0^-]$ are readily determined and enable equilibrium constant K and also the rate constant k''_2 to be calculated.

The chlorotriazinyl derivative (I) (0·05 M) was allowed to react in mixtures of water and ethanol containing 0·048 N sodium hydroxide at 60° C to give the results shown in Table 1. Total conversion was measured by electrometric titration of liberated chloride, the formation of II(a) by

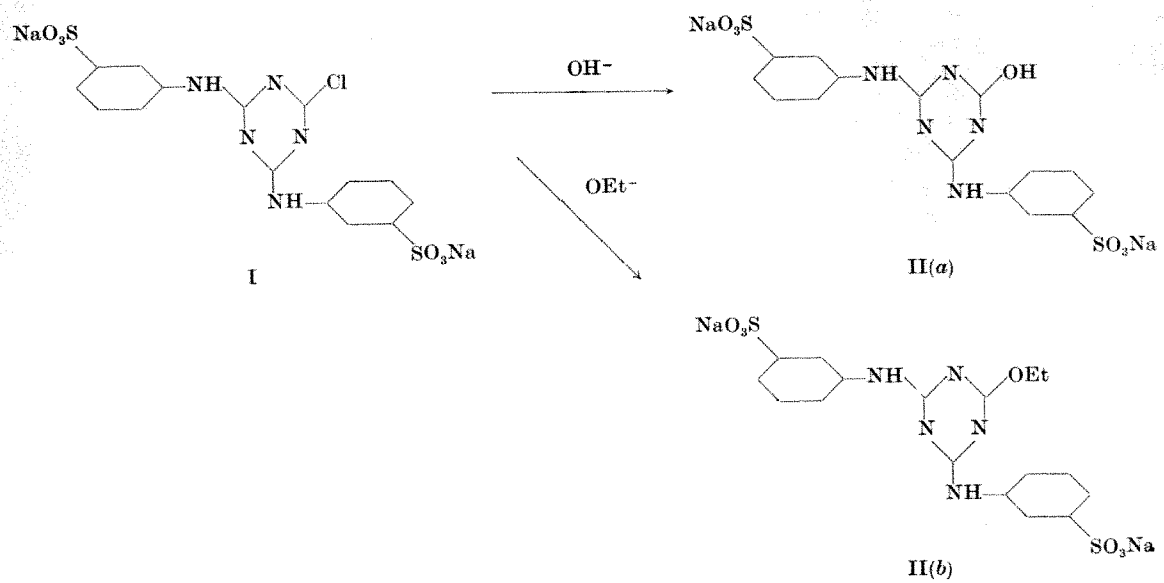


Table 1

Vol. % EtOH	10	15	20	30
k_2 (min^{-1} mol^{-1} l.)	0·39	0·42	0·53	0·62
Y	1·2	1·75	2·6	3·3
K	0·17	0·13	0·17	0·27
k''_2 (min^{-1} mol^{-1} l.)	1·3	0·95	1·3	1·5

k'_2 = 0·21 min^{-1} mol^{-1} l.

The values of Y were constant throughout any one experiment.

potentiometric titration of the $-OH$ group and II(b) by difference.

Pocker[3] reports a value of c. 4 (equivalent to Y) for the final ratio of the products of reaction of isopropyl bromide in 60 per cent ethanol at 70° C.

The assumption that k'_2 is independent of ethanol is equivalent to assuming that the activity coefficients of the reactants are also independent of ethanol, or alternatively that they all vary to the same extent. The constancy of the values of K and k''_2 appears to justify the assumption up to 20 per cent ethanol, but some allowance for variation of activities may be necessary at higher alcohol concentrations and we are examining this possibility.

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Characterization of the Products of the Action of Bee Venom Hyaluronidase

Previous workers[1] have reported that bee venom contains a hyaluronidase, but the products of its action on hyaluronic acid have not been characterized. In the work recorded here bee venom was obtained from several thousand bees using a novel method of collection. Trays of bees, preferably >15 days old, were induced to sting into thin sheets (<0·005 in.) of silicone rubber by subjecting them to a mild electric shock. Under these conditions the bee can withdraw its stinging apparatus and the venom is deposited as a globule on the underside of the sheet where it quickly dries to a crystalline form. The bees themselves appear to become ravenous and will devour large quantities of honey, after which they appear to suffer no adverse effects. In fact, marked bees have been noted working normally on the combs 12 days after venom extraction. The venom crystals (about 0·1 mg/bee) retain their hyaluronidase activity for at least a year if stored at 2°.

Sodium hyaluronate (205·9 mg; $[\alpha]_D^{20} - 68·5°$, c, 0·4 in 0·05 M phosphate citrate buffer pH 4·75) which had been isolated from umbilical cords was dissolved in 0·05 M phosphate citrate buffer (6 ml.; pH 4·75) and incubated for 24 h at 37° with bee venom (47·5 mg) in physiological saline (9 ml.). After neutralization with dipotassium hydrogen phosphate, the digest was boiled, centrifuged and the supernatant freeze-dried. This product, in water (5 ml.), was applied to a 'Dowex 21K' (H.COO$^-$) column[2] (l. 265 mm, diam. 11 mm). Stepwise elution with 270 ml. portions of 0·015 M, 0·05 M, 0·15 M, 0·3 M, 0·5 M, 0·8 M and 1 M formic acid as shown in Fig. 1 gave fractions I (4·3 mg), II (63·3 mg), III (60·5 mg) and IV (0·4 mg) located with the carbazole reagent[3]. Fractions I, II and III were homogeneous on a paper chromatogram irrigated with butan-1-ol/acetic acid/water (44 : 16 : 40) and had mobilities corresponding to those of a disaccharide (Rglucurone 0·5), a tetrasaccharide (Rglucurone 0·27), and a hexasaccharide (Rglucurone 0·18), respectively. The oligosaccharides gave positive reactions with the Morgan–Elson spray[4], indicating that an N-acetylglucosamine residue carries the reducing group in each oligosaccharide, and suggesting that bee venom hyaluronidase is a β-hexosaminidase.

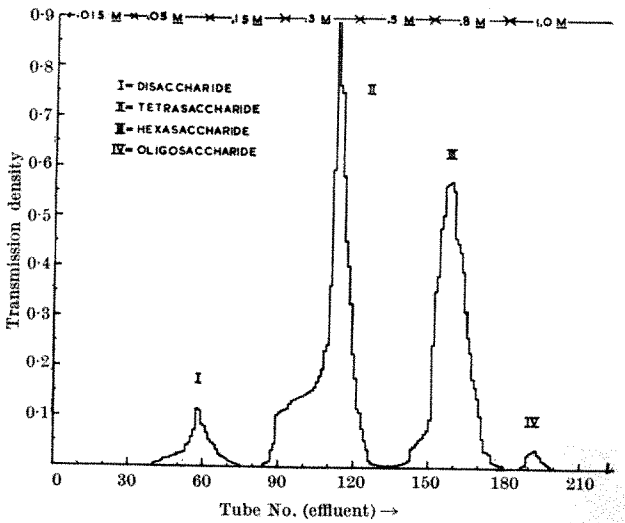


Fig. 1. Ion exchange separation of oligosaccharides on 'Dowex 21K' (H.COO$^-$)

Molecular weight determination using alkaline hypoiodite[5] confirmed the designations of tetrasaccharide (found, 742 : $C_{28}H_{44}N_2O_{23}$ requires 777) and hexasaccharide (found, 1111 : $C_{42}H_{65}N_3O_{34}$ requires 1155) to components II and III respectively. The tetrasaccharide did not consume bromine[6] and no ultra-violet absorption[6] was detectable at 232 mμ, indicating the absence of α,β-unsaturation. Infra-red spectral data[7] confirmed that both the tetrasaccharide and the hexasaccharide were of the saturated type and that in this they resembled similar oligosaccharides obtained by the action of testicular hyaluronidase[2] on hyaluronic acid. Assay of the uronic acid content with carbazole[3] revealed 44·9 per cent in the tetrasaccharide and 45·9 per cent in the hexasaccharide. After hydrolysis with 3 N hydrochloric acid at 105° for 4 h the hexosamine[8] contents were 43·6 and 41·7 per cent (calculated as glucosamine), respectively, and indicated equimolar ratios of uronic acid and hexosamine in both oligosaccharides. The tetrasaccharide had $[\alpha]_D^{25} - 52·2°$ (c, 0·46 in water) while the hexasaccharide showed $[\alpha]_D^{25} - 66·1°$ (c, 0·56 in water).

Partial hydrolysis and de-N-acetylation of the tetrasaccharide (2·2 mg.) was effected with N sulphuric acid (0·25 ml.) for 50 min at 90°. After neutralization (barium carbonate), and centrifugation, part of the diluted supernatant was reacted with the Elson–Morgan reagents[8]. The absorption maxima of the chromogen produced with the deacetylated tetrasaccharide appeared at 510 mμ compared with 530 mμ for reference glucosamine. These results are indicative of 1→3 glycosidic linkages[9] in the oligosaccharide, which is also supported by its pronounced alkali lability. The foregoing evidence and the fact that the bee venom tetrasaccharide showed identical properties, and had an infra-red spectrum indistinguishable from that of a tetrasaccharide produced by testicular hyaluronidase action, indicates that the major products of exhaustive treatment of hyaluronic acid with bee venom hyaluronidase are the tetrasaccharide GpAl$\xrightarrow{\beta}$3 GNAcl$\xrightarrow{\beta}$4 GpAl$\xrightarrow{\beta}$3 GNAc and the hexasaccharide GpAl$\xrightarrow{\beta}$3 GNAcl$\xrightarrow{\beta}$4 GpAl$\xrightarrow{\beta}$3 GNAcl$\xrightarrow{\beta}$4 GpAl$\xrightarrow{\beta}$3GNAc. Bee venom hyaluronidase should prove useful in future structural investigations of hyaluronic acid since with the isolated hexasaccharide it gave only the tetrasaccharide and a disaccharide and showed no evidence of a transglycosylation reaction. Bee venom also appears to degrade chondroitin sulphate A and C, heparin and blood group A and B substances. The nature of this action and whether it is due to the same β-glucosaminidase have yet to be determined.

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BIOCHEMISTRY

Effect of Ultrasonics on the Adenosine Triphosphatase Activity and Actin-binding Ability of L-Myosin and Heavy Meromyosin

Asakura[1] recently discovered that *F*-actin retains its native properties under ultrasonic vibration. In a more recent investigation Ohnishi and Ohnishi[2] reported that the ATPase activity of glycerinated muscle fibres increases under the same conditions. It would appear, therefore, that both structural muscle proteins are resistant to ultrasonic treatment. Physicochemical studies on the effect of ultrasonics on L-myosin and the meromyosins have shown in this laboratory that L-myosin and heavy meromyosin were readily fragmented, whereas light meromyosin was relatively resistant to vibration[3]. The effect of ultrasonics on the biochemical properties of L-myosin and heavy meromyosin will be described in this paper.

L-myosin[4] and heavy meromyosin[5], 6·0 mg/ml., in 0·6 M potassium chloride, *p*H 7·0, were vibrated in the model *U*–20 biosonic apparatus (Will Corporation) at 20 kc/s in the cold room. The temperature of the myosin solutions was kept below 10° C during vibration.

ATPase activity was measured in a water bath of 25° C with magnetic stirring in the test tubes. For the determination of the ATPase activity of L-myosin or heavy meromyosin in the presence of calcium, incubations were carried out in systems containing: 0·1–0·2 mg protein/ml. 35 mM potassium chloride, 10 mM histidine buffer, *p*H 7·0, or 10 mM *tris*(hydroxymethyl)aminomethane buffer, *p*H 9·0, 10 mM calcium chloride, and 5 mM ATP. For the determination of the magnesium-activated actomyosin ATPase activity, the system contained: 0·25 mg L-myosin/ml., 0·025 mg *F*-actin/ml., 36 mM potassium chloride, 10 mM histidine buffer, *p*H 7·0, 1 mM magnesium sulphate, and 1 mM ATP. All the determinations were performed in duplicate for a period of 2–5 min. Care was taken that not more than 20 per cent of the added ATP was hydrolysed. The inorganic phosphate liberated was determined by the method of Rockstein and Herron[6]. The results are expressed as μmoles of inorganic phosphorus liberated per mg of L-myosin, heavy meromyosin, or actomyosin per min.

Actomyosin formation at high ionic strength was determined in a system containing: 1·5 mg L-myosin/ml., and 0·3 mg *F*-actin/ml. or 3·0 mg heavy meromyosin/ml. and 0·6 mg *F*-actin/ml., 0·5 M potassium chloride, 20 mM histidine buffer, *p*H 7·0, and 0·5 mM magnesium sulphate at 25° C. The actomyosin formation is expressed according to Weber and Portzehl[7] as ATP sensitivity.

Actomyosin formation at low ionic strength was determined by measuring the amount of *F*-actin bound to L-myosin as described previously[8]. In order to increase the sensitivity of the determination, the *F*-actin was labelled with isotopic calcium[9], and the amount of bound *F*-actin was determined by radioactivity measurements. It was also shown previously that no exchange of the labelled *F*-actin-bound calcium occurred in the presence of L-myosin and magnesium at low ionic strength[9]. The system contained 0·5 mg L-myosin/ml., 0·05 or 0·1 mg ^{45}Ca-*F*-actin/ml. (4,980 counts/min/mg *F*-actin), 40 mM potassium chloride, 10 mM histidine buffer, *p*H 7·0, and 1 mM magnesium sulphate at 25° C. The precipitated actomyosin was separated immediately by centrifugation and any remaining actin in the supernatant was determined by drying 3-ml. samples of the supernatant in stainless steel planchets under infra-red lamps and counting in a windowless gas-flow counter (Tracerlab). All countings were performed in duplicate. The actomyosin formation at low ionic strength is expressed as g *F*-actin-bound per g of L-myosin at the two different *F*-actin concentrations used. No sedimentation of *F*-actin was found in the absence of L-myosin.

Actin was purified according to the procedure of Mommaerts[10].

Fig. 1 shows the effect of ultrasonics on the ATPase activity and actomyosin formation of L-myosin. In the course of vibration, L-myosin simultaneously loses its calcium-activated ATPase activity, both at *p*H 7·0 and 9·0, and its magnesium-activated ATPase activity in the presence of *F*-actin. These ATPase activities of L-myosin are lost to a greater extent than its ability to combine with *F*-actin both at high and low ionic strength. For example, the actomyosin formation is almost unchanged after 20 min of vibration, whereas the different ATPase activities are decreased by about 50 per cent. Fig. 2 shows that heavy meromyosin is affected by ultrasonics in the same way as L-myosin in regard to its ability to combine with *F*-actin at high ionic strength and its calcium-activated ATPase activity at *p*H 7·0.

These results show, therefore, that the ATPase activity of L-myosin does not withstand ultrasonic vibration, contrary to its resistancy when present in the myofibrils[3]. They show, furthermore, that vibration affects

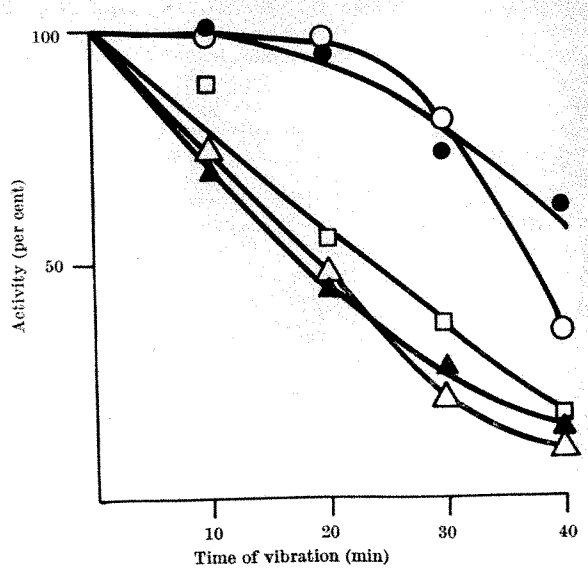


Fig. 1. Effect of ultrasonic vibration on the characteristic properties of L-myosin. ○, actomyosin formation at high ionic strength (100 per cent = 112); ●, actomyosin formation at low ionic strength (100 per cent = 0·088 and 0·173 for the two different actin concentrations); □, magnesium-activated actomyosin ATPase activity (100 per cent = 0·26 μmoles inorganic phosphorus/mg protein × min); △, calcium-activated L-myosin ATPase activity at *p*H 7·0 (100 per cent = 1·03 μmoles inorganic phosphorus/mg protein × min); ▲, calcium-activated L-myosin ATPase activity at *p*H 9·0 (100 per cent = 1·26 μmoles inorganic phosphorus/mg protein × min)

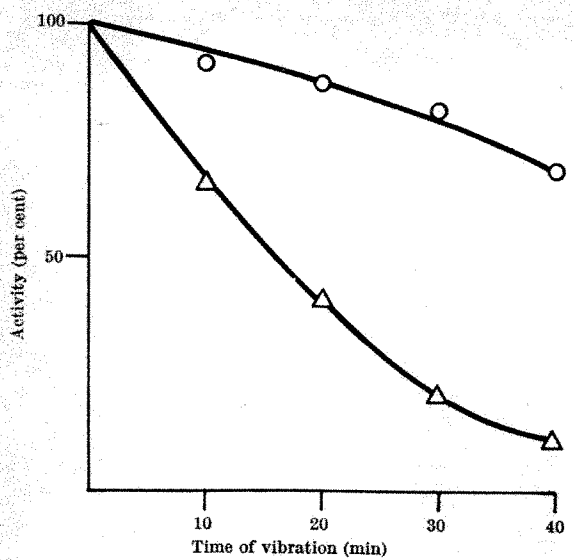


Fig. 2. The effect of ultrasonic vibration on the characteristic properties of heavy meromyosin, ○, actomyosin formation at high ionic strength (100 per cent = 120); △, calcium-activated ATPase activity at *p*H 7·0 (100 per cent = 1·29 μmoles inorganic phosphorus/mg protein × min)

differently the ATPase activity and actin-binding ability of both L-myosin and heavy meromyosin. We have reported earlier that L-myosin isolated from iodacetamide-treated actomyosin reacted with actin but did not show ATPase activity[11]. It appears, therefore, that the site(s) of L-myosin or heavy meromyosin which interact with ATP and actin are different.

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Effect of Orotic Acid on the Synthesis of Citrovorum Factor and of Methionine in Liver of the Chick

IN a dietary condition in which only B_{12} of the vitamins and methionine of the amino-acids are active for growth in the chick, it has been demonstrated that orotic acid is a growth factor[1]. One biochemical hypothesis on the effect of orotic acid in B_{12} and methionine deficiency is that orotic acid acts in the metabolism of the 1–C unit.

In the work recorded here the part played by orotic acid on the metabolism of 1–C unit has been investigated, considering its effect in comparison with B_{12} and with methionine on the utilization of β-carbon of serine for the conversion of folic acid to citrovorum factor (CF) and for the biosynthesis of the methyl group of methionine. The experiments were carried out *in vitro* on liver homogenates of B_{12}-deficient chicks or dietary treated with vitamin B_{12}, or with methionine, or with orotic acid.

New Hampshire × White Leghorn chicks from a commercial hatchery were used. The animals were put into four groups of 25 chicks each, and, beginning from one day of age, one group received the vitamin B_{12}-deficient diet[2] and the other three groups were kept on the same diet supplemented for each 100 g with 10 μg vitamin B_{12}, 150 mg DL-methionine, and 10 mg orotic acid respectively. The chicks were maintained in standard electrically heated batteries with screen wire floors. Food and water were supplied *ad libitum*.

At the end of the test period (5 weeks), 6 chicks of each group were killed, the livers were removed and immediately tested for folic acid to CF conversion and methionine synthesis[3]. The quantity of CF and methionine formed, considered as the measure of enzyme systems, was valued microbiologically.

CF was assayed with *Leuconostoc citrovorum* A.T.C.C. 8081. Bacto 'CF assay Medium' (Difco Laboratories Inc.) was used as basal medium and 'Leucovorin' (Lederle) as the standard. The cultures were incubated for 72 h at 37° C and growth was measured by titrimetric procedure.

Methionine was assayed with *Leuconostoc mesenteroides P*60, A.T.C.C. 8042. Bacto 'Methionine' assay 'Medium' (Difco Laboratories Inc.) was used as basal medium and DL-methionine was used as standard. The growth was measured titrimetrically after an incubation period of 72 h at 37° C (Table 1). Nitrogen was determined on each homogenate by the usual micro-Kjeldahl technique.

As shown in Table 1, analysed for statistical significance, the livers of chicks treated with dietary vitamin B_{12} ($P < 0{\cdot}01$), with methionine ($P < 0{\cdot}05$), with orotic acid ($P < 0{\cdot}001$), converted folic acid to CF more effectively than those of chicks fed the unsupplemented deficient diet. These results indicate that the enzyme system which leads to the synthesis of CF from folic acid and β-carbon of serine is influenced by methionine and by orotic acid, as well as by B_{12}.

Table 1 shows that the livers of chicks treated with vitamin B_{12} ($P < 0{\cdot}01$), with methionine ($P < 0{\cdot}05$), and with orotic acid ($P < 0{\cdot}05$) synthesize methionine from homocysteine and serine more effectively than those of deficient chicks. These results indicate that all three substances are involved in this synthesis.

The effect of dietary methionine on the CF and methionine formation could be due to a better biosynthesis of the specific enzymes and demonstrates the sensitivity of these processes to the amino-acid deficiency.

The action of B_{12} could be attributed not only to an increased biosynthesis of enzymes[4] and to a metabolic regulation of cofactors concerned with 1–C metabolism[5], but also to a direct coenzymatic mechanism[6,7].

Table 1. SYNTHESIS OF CITROVORUM FACTOR AND METHIONINE BY LIVER HOMOGENATES FROM CHICKS FED B_{12}-DEFICIENT DIET AND SUPPLEMENTED WITH B_{12}, WITH METHIONINE, WITH OROTIC ACID

Supplements to 100 g deficient diet	Body-weight after 5 weeks	Nitrogen content mg/g of liver	μg CF formed per g of liver	μg methionine formed per g of liver
None	257 ± 8·06* (25)†	28·6 ± 1·48* (6)†	19·74 ± 1·87* (6)†	437·7 ± 27·8* (6)†
10 μg vitamin B_{12}	328 ± 8·18 (25)	30·3 ± 1·87 (6)	31·77 ± 3·32 (6)	653·9 ± 67·2 (6)
150 mg DL-methionine	339 ± 7·74 (25)	29·7 ± 2·87 (6)	28·84 ± 3·63 (6)	535·5 ± 27·3 (6)
10 mg orotic acid	315 ± 7·21 (25)	31·4 ± 1·19 (6)	41·18 ± 1·58 (6)	658·6 ± 94·1 (6)

* Standard error of the mean. † No. of animals.

Reaction mixtures contained: 5 ml. 20 per cent liver homogenate in 0·08 M sodium potassium phosphate buffer *p*H 6·3 along with 5 ml. same buffer and aliquots of solutions containing 10 mg L-serine, 10 mg L-homocysteine, 100 μg folic acid; total volume to 11 ml. Incubation was at 37° C under N_2 for 2 h.

With regard to orotic acid, it is not possible with the preliminary results at our disposal to define the action mechanism of this substance.

Orotic acid could in fact act indirectly in the biosynthesis of specific enzymes through an increase of soluble RNA involved in the transport of amino-acids[8], or, more directly, as a precursor of the pyrimidine coenzymes in the metabolic processes connected with the utilization of β-carbon of serine. This is in part justified by the observation that CMP plays a part in a reaction in which the serine is involved[9].

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Nature of Humic Acids

The idea that humic acids are heteropolycondensates[1] of phenolic substances with or without the participation of amino-acids is now widely accepted. Three possible sources of the phenols are postulated: (1) phenolic materials such as flavonoids leached from the plant debris; (2) phenolic units formed during the decomposition of lignin; (3) phenolic substances synthesized by soil micro-organisms which may have been utilizing carbohydrates. However, attempts to identify the units in the condensate have been hampered by the absence of degradative procedures, which yield monomers with unchanged substitution patterns and intact carbon side-chains. Previous chemical investigations have, therefore, been primarily concerned with elemental analysis and the determination of active groups[1,2] which are mainly carboxyl and phenolic hydroxyls and possibly smaller quantities of quinonoid and primary alcohol groupings.

The indication that the first step in the biological degradation of humic acid[3,4] was a reductive step led us to re-investigate chemical reductive methods. Sodium amalgam which had been used previously in the reduction of humic acid[5] was found most successful. The humic acid was dissolved in sodium hydroxide and reduced with sodium amalgam in an atmosphere of oxygen-free nitrogen at 100°–110° C. The reaction mixture was acidified and the phenols extracted with ether. Yields of 10–14 per cent of phenolic material were recovered after the first reduction. Repeated reductions gave a total yield of phenol material corresponding to 30–35 per cent of the original material and the residue gave a further yield of phenols on fusion with potassium hydroxide at 280° C.

After successful reduction of humic acid from a podzol[6] under *Pinus sylvestris*, humic acids extracted from a cultivated chernozem near Prague, Czechoslovakia, a rendzina at Swyncombe, Oxfordshire, collected by Mr. J. Elston, a humic soil developed under the moss *Bryum argenteum*, collected from Signy Island, South Orkneys, by Dr. M. W. Holdgate, and a sample of humic acid extracted by Mr. P. Howard from an oak–ash mull at Humphrey Head, Lancs., were also reduced. The phenols were separated by two-way chromatography on thin-layer plates of silica gel using acetic acid (10 per cent v/v)/chloroform and ethyl acetate (45 per cent v/v)/benzene. In Table 1 the phenolic materials which have been identified from the different samples are recorded. It must be emphasized that at this stage these identifications are provisional only, and depend on chromatographic evidence and colour reactions.

The chromatographic patterns differ in humic acids of different origins and provide a 'finger-print' technique, which may remove some of the present confusion which arises from a lack of criteria for the characterization of this class of compounds.

The contribution of lignin from the overlying vegetation to humic acid formation was shown by the presence of relatively unchanged lignin residues. Vanillic, syringic, *p*-hydroxybenzoic, guaiacyl- and syringyl-propionic acids have been identified. Syringyl residues occur in humic acids formed under deciduous hardwood vegetation (oak) but are absent under coniferous softwoods (pine), where vanillic acid predominates. The assumption that these are lignin-derived components was supported by the complete absence of such residues in humic acid developing in a lignin-free environment below Antarctic clumps of the moss *Bryum argenteum*.

Other degradation products which may be used to distinguish between different humic acids include phloroglucinol, methyl-phloroglucinol, protocatechuic acid, resorcinol, 3 : 5-dihydroxybenzoic acid, pyrogallol and 2 : 4-dihydroxytoluene. Several of these can be obtained by degradation of model substances of the C_6—C_3—C_6 type. They may represent a flavonoid contribution of the parent vegetation or, alternatively, could be derived from soil microbial syntheses where 1–3–5 substitution patterns are quite common.

Reductive cleavage of fully methylated podzol humic acid suggests that the isolated monomers are linked by ether bridges. The phenolic hydroxyl groups of vanillic, protocatechuic and *p*-hydroxybenzoic acid are protected during methylation and these units are released unchanged. Units of the 'flavonoid' group are replaced by partially methylated derivatives (mono-methoxy-phloroglucinol, *m*-methoxyphenol, etc.), indicating that these possess some free unbonded phenolic hydroxyl groups as well as those involved in linkage.

Table 1

		Source of humic acid					
		Podzol	Humic acid (L. Light and Co., Ltd.)	Rendzina	Chernozem	Mull humus (oak)	Antarctic moss humus
Possible flavonoid units	Phloroglucinol	+ + +	+ + +	Trace	+ +	−	+ +
	Resorcinol	+ + +	+ + +	+	+ + +	+ +	+ +
	Methyl-phloroglucinol	+ +	+ +	Trace	+ +	−	+
	2 : 4-Dihydroxytoluene	+ +	+ +	Trace	+	−	+
Possible lignin units	*p*-Hydroxybenzoic acid	+	+	+ +	+	+ +	−
	Vanillic acid	+ +	+ +	+ + +	+ +	+ +	−
	Syringic acid	−	−	+ +	+	+ +	−
	Protocatechuic acid	+	+	+	+	+	−
	Guaiacyl-propionic acid	+	−	+ +	+	−	−
	Syringyl-propionic acid	−	−	+	+	−	−
	Pyrogallol	+	+	+	+	−	−
	3 : 5-Dihydroxybenzoic acid	+ +	+	Trace	+	−	+

The reductive cleavage of saccharo-humic acid, which is often discussed in humic acid studies, did not reveal any phenolic units. This confirms the widely accepted view that saccharo-humic acid is quite unrelated to soil humic acids. A native spruce lignin supplied by Dr. Handley, of Oxford, was readily reduced by the same technique to give a mixture of more than 30 phenolic compounds, including *p*-hydroxybenzoic acid, which indicates the probable existence of non-methylated phenol residues in soft-wood lignin, and a number of substituted phenyl residues with C_1, C_2 and C_3 side-chains.

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Presence of Ribonucleic Acid in the Initial Yolk Globules of Oocytes of *Aplysia depilans* L.

Previous work carried out with the electron microscope has indicated that the formation of yolk globules of some species of Mollusca and Echinodermata occurs by aggregation of protein particles[1,2]. Experiments have also indicated the possibility of obtaining in neoplastic cells an artificial aggregation of the particles[1–3]. Work on oocytes has shown the ribosomal nature of the particles, and it is thus possible that RNA is present in the product of protein synthesis.

Investigations have been carried out in order to solve this problem. The vitellogenesis of oocytes of *Aplysia depilans* L. (Mollusca, Opistobranchia), in which the final yolk globules (Fig. 2, *g″*) are formed by products coming from the transformation (Fig. 1, *g′*) of initial large yolk globules (Fig. 1, *g*), which are found in relatively young oocytes[4], has been examined.

The following stains were used for light microscopy: Unna-Pappenheim, Dominici, toluidin blue; for fluorescent microscopy, acridine orange. Control with ribonuclease ('Crystalline' of Light and Co.) for all methods was carried out. In this way observations of the presence of RNA in the aforementioned initial yolk globules was made possible. Einarson's method was also used; but it was not found to be suitable. With Unna-Pappenheim's method, there have been indications that a diminution of the pyroninophilia of the globules is due to digestion with ribonuclease. Clear-cut results have been obtained with Dominici's method and with treatment with acridine orange. In the final yolk globules the tests always gave negative results.

Further tests made on other material—whether germinal or somatic—could perhaps render it possible to generalize the idea that ribonucleoproteins, after the incorporation of amino-acids, are traceable, at least in an initial phase of formation, in the products of protein synthesis. More information on the function and destiny of cytoplasmic ribosomes ('cytoribosomes')[2] could therefore be obtained.

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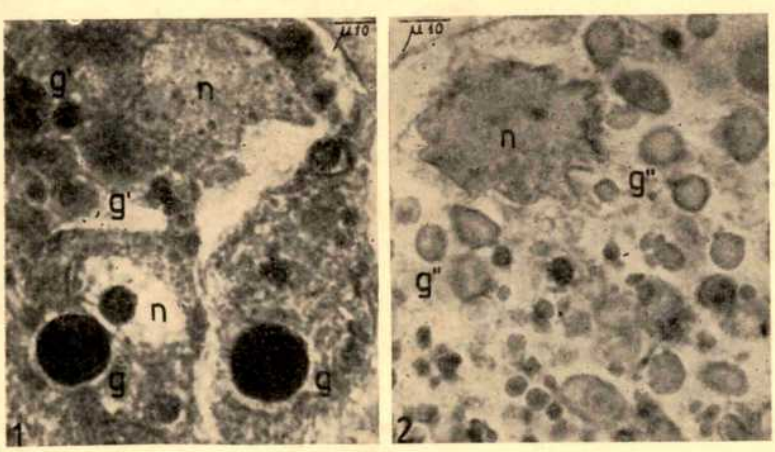


Fig. 1. Relatively young oocytes of *Aplysia depilans* in a preparation stained with Mallory's method. *g*, Initial yolk globules; *g′*, yolk globules in transformation; *n*, nuclei

Fig. 2. Portion of a fully developed oocyte of *Aplysia depilans* in a preparation stained as above; *g″*, final yolk globules; *n*, nucleus

Localization and Fractional Determination of Neutral Deoxyribonuclease Activity in Cat Serum

The biological role, biochemical and enzymatic characteristics and cellular localization of DNases in higher mammals are still little understood. The existence of numerous isozymes suggests that the DNase activity manifested by most tissues could be related to one or several molecular forms of one or several enzymes or their inhibitors. In this respect we present a preliminary investigation concerning the localization of DNase activity in the serum of the cat fractionated on gelose and starch gel, and the concentration and fractional determination of this activity by chromatography on substituted celluloses. When compared with various mammalian sera, the cat's serum has a very high DNase activity and therefore constitutes good material for examination[1]. Blood was obtained by cardiac puncture from animals anæsthetized with ether. The serum was obtained immediately after retraction of the clot and kept in the refrigerator. It was fractionated by starch-gel electrophoresis according to Smithies[2,3] and by agar-gel electrophoresis according to Grabar and Burtin[4].

Running a DNA gel (200 μg DNA/ml. 1·5 per cent gelose solution) over the electrophoresis plates makes it possible to reveal directly the DNase activity of fractions after incubation for 16 h at 37° C., by staining of the non-hydrolysed DNA by a methyl green solution (1 per 1,000 in a *tris* buffer 0·1 M, *p*H 7·4).

It is also possible to prepare an electrophoretic gelose support containing 100 μg DNA/ml., in which case the protein migration is not disturbed, and enzymatic activities are revealed immediately after incubation for 2 h at 37° C by a methyl green reaction. This method ensures more exact separation and selection of the active zones (Fig. 1).

Serum chromatography is carried out on DEAE cellulose in a continuous gradient, and on CM cellulose in a discontinuous gradient, by the classical procedure of Sober and Peterson[5,6].

Every chromatographic fraction is lyophilized and dissolved in 0·5 ml. *tris* buffer 0·1 M,

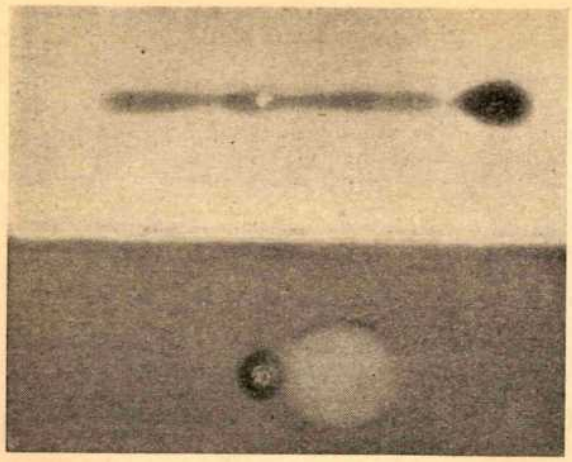


Fig. 1. Electrophoresis of serum of the cat on agar gel with DNA. Demonstration of activity of DNase by methyl green, as compared with protein migration

*p*H 7·4. DNase activity is examined and evaluated by a semi-quantitative method based on the proportionality between the enzyme concentration in a given medium and the importance of enzyme diffusion in an agar gel containing DNA[7]; its determination is effected by Kurnick's method[8]. Determination of the protein values is carried out by Lowry's method[9].

Figs. 2 and 3 show the fractionation of serum proteins on DEAE cellulose and on CM cellulose.

In the two cases the DNase activity is found in two separated fractions. Electrophoretic analysis of these fractions, whether on agar gel or on starch gel, leads to their identification as α_2-globulins; these electrophoretic media, however, do not allow their separation.

The recovery of protein is 81 per cent on DEAE cellulose and 87 per cent on CM cellulose.

The results obtained by the method of Lowry are identical to those obtained by ultra-violet absorption at 278 mμ.

Fractions IV, V and VI (DEAE cellulose) include 58 per cent of the total proteins; fractions I and II (CM cellulose) include 70 per cent of the total proteins.

The sum of the DNase activities in each fraction equals about 75 per cent of the initial DNase activity (DEAE and CM cellulose). Each of the peaks obtained was re-chromatographed; in all cases elution was effected under the same conditions in terms of *p*H and ionic strength as in the initial chromatography.

The detection and quantitative evaluation of DNases by diffusion are not disturbed by the presence of inhibitors or by physicochemical conditions of environments unfavourable to enzyme activity. This method by diffusion, therefore, is justified in the case of electrophoretic determinations in the presence of buffers with a borate, citrate or such similar base.

Our results can be compared with those of Becking *et al.*[10], who succeeded in separating two fractions of crystallized pancreatic DNase I by chromatography on CM cellulose; Baumgarten *et al.*[11] demonstrated that crystallized DNase I can be separated by paper electrophoresis into three well-defined components.

Our results are not in favour of the existence of organic DNase inhibitors in cat serum because the recovery of DNase activity on the basis of each fraction is satisfactory. Nevertheless, no definite conclusions can be formed in this respect except by detailed examination of the enzyme kinetics of each of the fractions obtained; this requires a higher degree of purification of the enzymes in question.

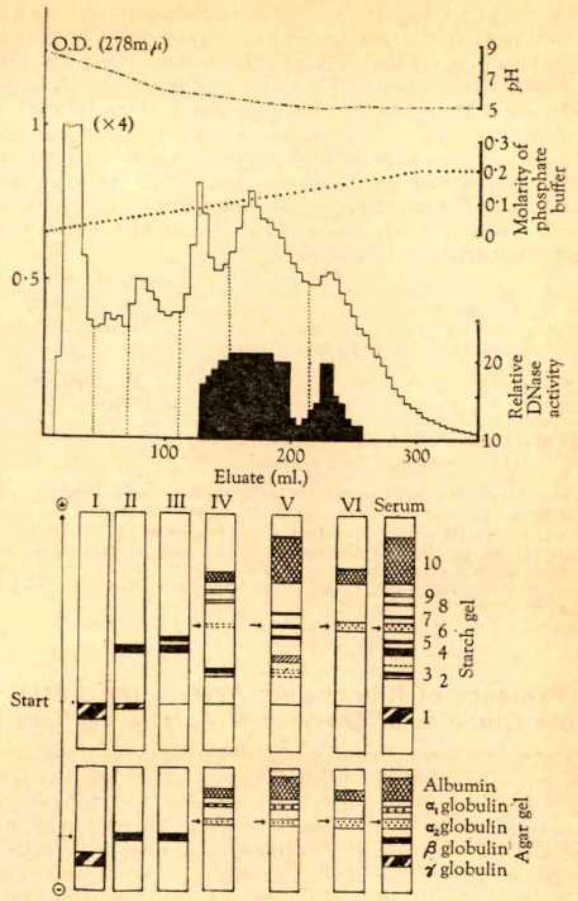


Fig. 2. Chromatography of serum of the cat on DEAE cellulose, with continuous gradient; 9 g DEAE cellulose, 4·5 ml. serum (proteins 67 mg/ml.; DNase activity 1·5 KU/ml.); in black, relative DNase activities. Arrow corresponds to a DNase activity in electrophoretic analysis of fraction electrophoresis

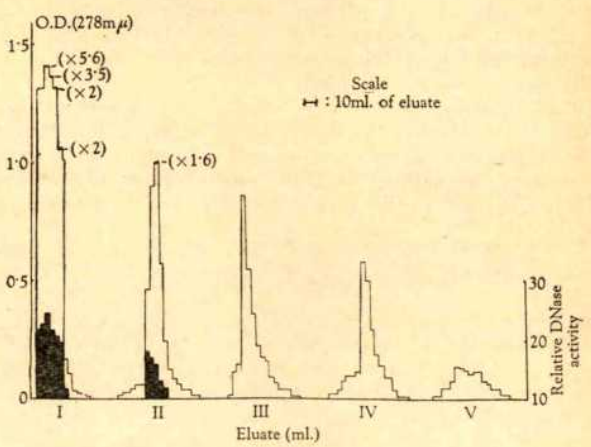


Fig. 3. Cat serum chromatography on CM cellulose, with discontinuous gradient; 3 g CM cellulose – 2 ml. serum (proteins 67 mg/ml., DNase activity 1·5 KU/ml.); in black, relative DNase activity. Buffers used: (I) sodium acetate 0·02 M, *p*H 4·6; (II) sodium acetate 0·08 M, *p*H 6·0; (III) sodium phosphate 0·1 M, *p*H 7·0; (IV) sodium phosphate 0·1 M and sodium chloride 0·5 M equal parts, *p*H 9·2; (V) sodium carbonate 0·3 M, *p*H 10·6

For similar reasons it is impossible to establish whether the two isolated fractions have identical enzymatic characteristics comparable to isozymes, or different characteristics[12].

Some preliminary tests have proved that, unlike cat serum (and mice sera), the serum in rabbits and rats shows

localization of the DNase activity in the electrophoretic range of post-albumin α_1-globulins.

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PHYSIOLOGY

Identification of Inhibitory Neurones in the Hippocampus

In their recent article in *Nature*, Andersen, Eccles and Løyning[1] claim to have unequivocally identified the basket cells of the hippocampus as the inhibitory neurones of this brain structure. They claim that "there is no alternative" to this interpretation and that it therefore "seems possible for the first time to give an example from the mammalian nervous system of a recurrent inhibitory pathway where both the inhibitory neurone and its synapses are histologically identifiable".

The conclusions reached by the authors are based on three main premises. The correctness of none of these is fully substantiated by the findings presented in their article.

Premise (1) postulates that the inhibitory neurone should possess "an axon with extensive ramifications . . . distributed to a large number of pyramidal cells". Quite apart from the fact that cells other than basket cells located in the stratum oriens fulfil these criteria (for example, the neurone No. 1 in Fig. 8 of Lorente de Nó's[2] paper), the observations made could just as easily be explained by assuming a rather large number of inhibitory neurones distributing their axons to a moderately small number of pyramidal cells.

It is, however, premise (2) which rests on the shakiest foundations. The authors assume that the synaptic terminals of the inhibitory neurone end on the soma of the pyramidal cell. So far as can be judged from the findings presented, this assumption is based on the laminar profiles of the responses evoked by commissural, septal and 'local' stimulation (Fig. 1*D* of ref. 1). This profile shows peak positivity of a late response component in the pyramidal layer. However, the same profile would result if excitatory post-synaptic potentials (EPSP's) were developed on the apical dendrites. In fact, this explanation would better agree with the laminar profile observed. As Fig. 1*F* of Andersen's *et al.* paper[1] correctly shows, a 'source' developed by a hyperpolarizing potential at the soma level should give rise to two 'sinks', one in the apical dendrites, the other near the alveus. The experimental findings, however, show only one 'sink' in the apical dendrites.

Furthermore, no evidence is given to indicate that the positive wave recorded extracellularly in the pyramidal layer is associated with inhibition of unit discharge and thus may safely be equated with the intracellularly recorded IPSP's. That the two are identical is an assumption for which there exists no proof. The authors themselves note a discrepancy in the duration of these two potentials which they try to resolve by assuming that the shorter duration of the extracellularly recorded potential is due to the fact that the extracellular current flow lasts for a shorter time than the intracellularly recorded hyperpolarization.

However, inhibitory potentials of the same duration as the intracellularly recorded inhibitory post-synaptic potentials (IPSP's) can be observed extracellularly with responses evoked by entorhinal stimulation, as we have shown in our own experiments[3,4]. These potentials are clearly associated with inhibition of pyramidal cell discharge (ref. 4, Figs. 8 and 9). The laminar profile of this extracellularly recorded inhibitory potential showed a peak in the apical dendritic layer (ref. 4, Fig. 5) whereas the cell layer often acted as a 'sink' (ref. 4, Figs. 8 and 9). The synapses producing this inhibitory potential therefore seem to be located predominantly on the apical dendrite and not on the soma. This excludes the basket cells from consideration and makes it likely that other hippocampal interneurons with axons ramifying mainly in the apical dendrites are responsible for these inhibitory potentials. Candidates for this role can be found in the stratum oriens as well as in the apical dendritic layer (for example, ref. 2, cell No. 5 in Fig. 6, cells No. 1, 3 and 6 in Fig. 7 and cell No. 1 in Fig. 8). We have, furthermore, given evidence suggesting that axo-somatic synapses on pyramidal cells may be excitatory rather than inhibitory[4], although we do not claim that our observations provide incontrovertible proof for this interpretation. Nevertheless, one can certainly say that the question whether the basket cells are excitatory or inhibitory neurones still remains unresolved.

Premise (3) assumes that the responses analysed are mediated by three separate inputs. This is very questionable. 'Local' stimulation must have activated the terminals of the commissural pathways and therefore we are dealing at the most with two different afferent inputs, rather than three as claimed.

There seems, therefore, to be no basis for the claim that experiences by Andersen *et al.*[1] have unequivocally identified the basket cells as the inhibitory neurones of the hippocampus. Such a conclusion is merely one among several possible, but unproved, interpretations to be considered, especially since all the known experimental evidence cannot be reconciled with this view.

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We welcome this critical comment by Dr. Gloor because it gives the opportunity of clearing up some misunderstandings and of refuting Dr. Gloor's statement that there is "no basis for the claim that experiences by Andersen *et al.* have unequivocally identified the basket cells as [the] inhibitory neurones of the hippocampus". The word [the] is in brackets because we made no claim of exclusiveness; otherwise we accept Dr. Gloor's formulation of our position.

We deny Dr. Gloor's statement that premise (2) "rests on the shakiest foundations". Any appearance of insecurity derives from a failure to appreciate the degree of assurance which can be attained, under favourable geometric conditions, when interpreting an assemblage of extracellular potentials together with the associated

intracellular potential. The favourable geometric situation is provided in the hippocampus by the arrangement of the somas of the pyramidal cells practically in a single layer parallel to the ventricular surface and with their apical dendrites perpendicular thereto. Dr. Gloor states that the extracellular 'laminar profile' of our Fig. 1*E* with a sharp peak of positivity at the soma could be due to an active synaptic depolarization generating EPSP's in the apical dendrites. We need not discuss in detail the merits of this alternative explanation of the 'laminar profile'; it is sufficient to recognize that from it arises the prediction that intracellular recording from the soma would discover a depolarization, the electrotonically transmitted EPSP; whereas, in fact, we reported that a large hyperpolarization was found in 76 of the 79 cells recorded from successfully. We submit that the laminar profile in conjunction with this intracellularly recorded hyperpolarization admits of no interpretation other than an active synaptic hyperpolarization of the somatic region of the pyramidal cells, that is, of inhibitory synaptic action concentrated on the soma. Such a location would give a source for current flowing extracellularly into two sinks, one in the region of the apical dendrites, the other in the opposite direction in the region of the basal dendrites, as was actually shown diagrammatically in our Fig. 1*F*. Dr. Gloor denies the existence of the latter sink on the irrelevant grounds that it is not negative to the indifferent earth electrode. Its existence is of course indubitably established by the slope of the extracellular laminar profile from the positive peak at the soma layer (about + 2 mV) to a relatively negative level in the stratum oriens (about + 0·5 mV).

It is strange that Dr. Gloor maintains that the extracellular positive wave at the pyramidal layer cannot be safely equated with the intracellularly recorded IPSP's unless it can be shown to be associated with inhibition of unit discharge. This was frequently observed, but not reported. Surely it is sufficient that the extracellular positivity is concurrent with the large hyperpolarization of the IPSP, or is Dr. Gloor doubting whether IPSP's are inhibitory? There is a precise correlation (within 1 msec) between the onsets of the IPSP's and of the positive potential recorded outside the soma in response to all three inputs. However, as we stated, the development of a later negative potential in the apical dendrites obscures the prolonged low hyperpolarizing current that would be expected for inhibitory synapses generating an IPSP with a half decay time of 50–80 msec in a membrane that has a time constant of about 10 msec[1].

It is irrelevant for criticism of our paper that Dr. Gloor cites his own evidence from extracellular observations that a source on apical dendrites with a sink on the soma is "an inhibitory potential" and that in the stratum oriens there are various neurones having axons projecting up to the apical dendrites. We made no statements for or against other inhibitory sites on the pyramidal cells. Our exclusive statement referred to the synapses responsible for the large IPSP's generated in the somas of pyramidal cells by the inputs used in our experiments. However, it will be appreciated from the foregoing discussion that intracellular recording is desirable before positive extracellular potentials are attributed to inhibitory synaptic action. It is a minor point that, in criticizing our claim to have used three separate inputs, Dr. Gloor states that the: "Local stimulation must have activated the terminals of the commissural pathways". This statement is incorrect. The commissural pathway projects to the hippocampal region under examination from a direction opposite to the site of the locally stimulating electrodes[2].

Having disposed of Dr. Gloor's criticisms we reassert that the amazing concentration of basket cell synapses on the pyramidal cell somas provides the only structures that could produce the powerful inhibitory synaptic action that is specifically exerted on these somas. In a parallel investigation on the cerebellum, it has likewise been shown that the concentration of basket cell synapses on Purkinje cell somas gives the structural basis of the powerful inhibitory action on Purkinje cells[3].

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Medullary Vagal Effects of *d*-Lysergic Acid Diethylamide in the Decerebrate Cat

Despite the fact that 2-brom-*d*-lysergic acid (BOL-148) and *d*-lysergic acid diethylamide (LSD-25) are both potent anti-metabolites of serotonin *in vitro*[1,2], they do not exhibit similar behavioural or pharmacological effects *in vivo*. LSD-25 has been shown to produce bradycardia, a decrease in respiratory rate and hypotension in the pento-barbitalized, intact cat[3]. This is in contrast to the hypertension and tachycardia produced in spinal animals. The explanation advanced for these effects has been that in the intact animal the hypertension and tachycardia, due to peripheral vasoconstriction, are masked by more potent effects of LSD-25 on higher centres. BOL-148 was also shown to have no effect on heart rate and a weak non-specific effect on blood pressure in the intact anæsthetized cat in doses up to 1 mg/kg (ref. 3). In the decerebrate cat, ergot alkaloids cause hypotension and bradycardia via medullary vagal centres[4,5]. Therefore, we attempted to study possible vagal effects of LSD-25 and BOL-148 in the decerebrate cat.

Forty cats, 2–4 kg, were used. Tracheal, arterial and venous cannulations and placement of subcutaneous electrodes were carried out under ether anæsthesia. Decerebration was accomplished by suction through the superior colliculi following ligation of the common carotid arteries and compression of the vertebral arteries. Adequacy of decerebration and intactness of the lower brain stem were judged by the presence of decerebrate rigidity and rhythmic spontaneous breathing. Following decerebration, at least 1 h elapsed before recording in order to eliminate the influence of the anæsthetic. Blood pressure was measured by means of a strain gauge. Respiration was recorded by a transducer attached to the side-arm of the tracheal cannula. Lead II of the standard electrocardiogram was routinely recorded with the animal supine. These parameters were recorded simultaneously on a Sanborn Poly-Viso. Drugs were dissolved in saline (1–2 mg/ml.) and administered intravenously within 30 sec.

Table 1. Summary of Pulse Rate, Blood Pressure and Electrocardiographic Findings in the Decerebrate Cat following LSD-25

No. of animals	Dose (mg/kg)	Pulse rate (heart rate/min) Control	Pulse rate (heart rate/min) Maximum	Blood pressure (mm/Hg) Control	Blood pressure (mm/Hg) Maximum	Abnormal rhythm No.	Abnormal rhythm Aver. duration
4	0·2	160 ± 12	108 ± 20	$\frac{160 \pm 20}{90 \pm 30}$	$\frac{120 \pm 22}{50 \pm 20}$	0/4	—
4	0·3	153 ± 45	110 ± 46	$\frac{140 \pm 40}{90 \pm 32}$	$\frac{103 \pm 10}{60 \pm 30}$	1/4	2 min
12	0·4	175 ± 40	93 ± 42	$\frac{135 \pm 30}{80 \pm 20}$	$\frac{90 \pm 15}{40 \pm 15}$	10/12	20 min

Table 1 summarizes the results obtained with LSD-25 (0·2, 0·3 and 0·4 mg/kg): bradycardia, hypotension and a decrease in respiratory rate were observed. In one of the 4 cats administered 0·3 mg/kg, transient auriculo-ventricular (A.V.) nodal rhythm was produced lasting 2 min. At 0·4 mg/kg dose level, 10 of the 12 animals developed

conduction disturbances. *P–R* prolongation followed by complete heart block or A.V. nodal rhythm with retrograde auricular conduction was observed, although the latter disturbance was seen more frequently. Fig. 1*A* is an electrocardiographic tracing illustrating this effect. The change in conduction lasted 6–28 min, averaging 20 min in duration. Artificial respiration was maintained prior to and following drug administration in 4 animals to rule out possible secondary effects due to anoxia. These cats all developed similar conduction abnormalities following the administration of LSD-25. An immediate return to regular sinus rhythm, and improvement in blood pressure and respiration were noted following either bilateral vagotomy or atropinization (1 mg/kg). (Fig. 1*B*).

In 4 cats the vagii were sectioned in the neck, and in 4 other animals atropine (4 mg/kg) was given 15 min prior to administration of LSD-25. In these animals, no conduction disturbances were observed. The changes in blood pressure and cardiac rate were not so marked as in those preparations without pre-treatment.

To rule out the carotid sinus mechanism as the mediator of the effects observed, 4 cats were subjected to bilateral excision of the carotid bifurcation. LSD-25 (0·4 mg/kg) produced effects similar to those observed in animals with carotid sinuses intact.

BOL-148 (0·4 mg/kg) produced mild, transient slowing of heart and mild hypotension lasting not more than 60 sec. The effects seen with 1·0 and 2·0 mg/kg were more pronounced and lasted 3–5 min. At the higher dose-levels QRS changes and *T* wave inversion were observed in the electrocardiogram (Fig. 1*C*). No A.V. nodal rhythm or second degree heart block with A.V. dissociation was produced even with the larger doses of BOL-148. Bilateral vagotomy resulted in an increase in heart rate if done at the height of these changes, but pre-vagotomized animals were not protected as they were with LSD-25.

Marked differences in the *in vivo* action of LSD-25 and BOL-148 are illustrated in this study. LSD-25 appears to have a medullary stimulating action, as contrasted with BOL-148 which probably has direct cardiac toxicity when administered in large doses. BOL-148 has little or no effect on medullary vagal centres. Both LSD-25 and BOL-148 appear to penetrate the brain with little difficulty[6], although selective uptake by specific brain sites cannot be ruled out from these studies. Serotonin antagonism cannot explain the differences observed since BOL-148 is as potent an antagonist to serotonin as LSD-25 (ref. 7). Cholinesterase inhibition does not appear to be the mechanism involved since LSD-25 and BOL-148 are only weak inhibitors of true cholinesterase, although they exhibit marked pseudo-cholinesterase inhibition[8]. It is interesting to note that the dose of LSD-25 required in this study to produce cardiac conduction disturbances in the decerebrate cat approximates the amount of LSD-25 needed to produce clear-cut behavioural effects in the unanæsthetized cat[9].

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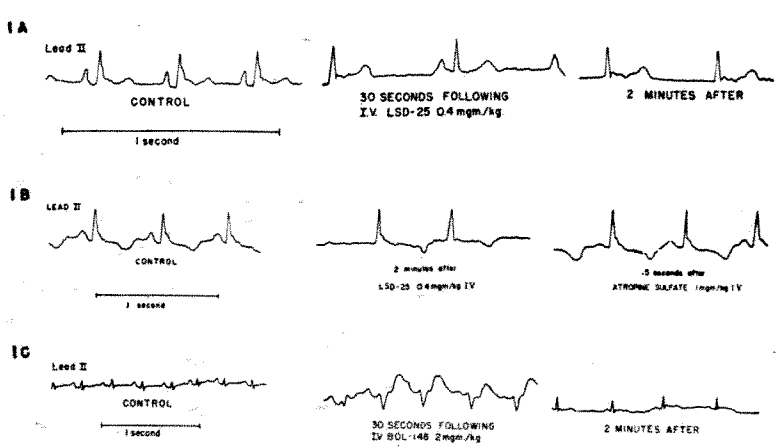


Fig. 1. *A*. Effects of LSD-25 on the electrocardiogram of the cat; *B*, effects of atropine sulphate on reversing the effects of LSD-25; note reappearance of *P* waves following atropinization; *C*, effect of BOL-148 on the electrocardiogram of the cat

Crab Axons showing a Prolonged Repolarization Phase

A FURTHER type of response from isolated crab axons has been recorded by a new technique. The method is to thread an isolated axon through two holes in a fine polythene tube. The axon outside the tube is treated with isotonic sucrose solution and then raised into paraffin oil. Inside the tube test solutions can be flowed over the axon membrane. The effect is to produce an isolated 'node' of excitable membrane. Non-polarizable potassium chloride wick electrodes provide stimulating and recording connexions on each side of the 'node'. This method gives the normal variety of responses from different fibres[1]. In one type of fibre, when bathed by normal sea-water, the action potential shows a very prolonged repolarization phase of 25 msec with two discrete segments of 1 msec and of 24 msec, and during the later segment a supernormality develops, that is, during the falling phase of the first action potential a second action potential can be elicited by a weaker stimulus. The absolute refractory period extends for a period of 2 msec, up to the end of the first phase of the repolarization. Following this for 3–5 msec a period of typical subnormality leads to the period of supernormality which can last as long as 30 msec (Figs. 1 and 2 *a* and *b*).

When the 'node' is stimulated by direct current there is a repetitive response. Before the first action potential a normal local potential develops, but the following action potentials arise at a level of depolarization greater than the amplitude of the first local potential. Therefore no pacemaker potentials can be seen, that is, the later action potentials grow out of the falling phase of the preceding action potential (Fig. 2 *c*). With strong direct currents the amplitude of subsequent action potentials is greatly influenced and oscillatory processes become noticeable.

If a second current pulse is applied during the second segment of the repolarization its shape and time relations can be altered, cathodal current lengthening and anodal current shortening the repolarization. During the repolarization phase and the refractory period the ratio of the 'inward' to 'outward' resistance, as measured by small current pulses, is smaller than in normal fibres. All the evidence at present available suggests that the later prolonged segment of the repolarization is due to a low level of increase of potassium permeability. If then the level of rectifica-

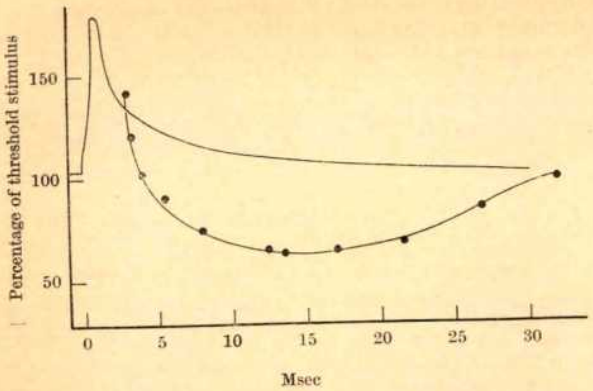


Fig. 1. Recovery curve (—●—) in relation to the form of the action potential (——). Each black spot represents the mean of a just effective stimulus and a just sub-threshold stimulus, plotted as a percentage of the initial threshold

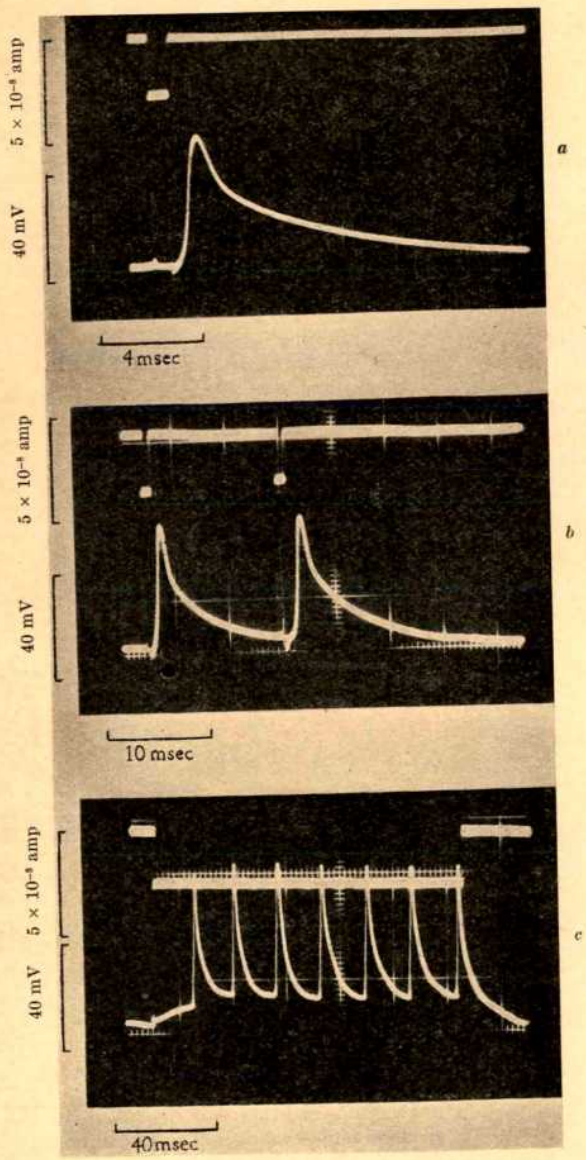


Fig. 2. Responses of an isolated 'node' from an opener motor axon of *Carcinus maenas*. *a*, A single action potential showing prolonged repolarization; *b*, dual shock showing supernormality of the refractory period; the first stimulus was at threshold; *c*, repetitive response of 'node' to direct current, illustrating absence of pacemaker potentials

tion is low and the 'node' is still depolarized, supernormality of the refractory period and the response to direct current become understandable.

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PHARMACOLOGY

Mechanism of the Antagonism by Tetraethylammonium of Neuromuscular Block due to *d*-Tubocurarine or Calcium Deficiency

Tetraethylammonium salts (TEA) can antagonize the neuromuscular block caused by either calcium insufficiency or by *d*-tubocurarine[1]. The release of acetylcholine from motor nerve endings appears to be dependent on the presence of calcium ions[2,3], and neuromuscular block produced by calcium deficiency would appear to be caused by a reduced transmitter output. In this way the block differs sharply from that produced by *d*-tubocurarine, which does not affect acetylcholine release[4]. On the basis of indirect evidence Stovner[5] suggested that the 'decurarizing' action of tetraethylammonium salts may be the result of an increased liberation of acetylcholine from the motor nerve endings. That tetraethylammonium salts may have such an action is supported by the results of Koketsu[6] and Frank[7]. The study reported here provides quantitative evidence to support this theory.

The isolated rat phrenic nerve diaphragm preparation has been used in this work; the method being essentially that of Burgen, Dickens and Zatman[8]. The acetylcholine released from one hemidiaphragm in the presence of eserine (10^{-5} g/ml.) has been assayed on the blood pressure of the anæsthetized rat or on the eserinized leech muscle. The nerve was stimulated by supramaximal rectangular pulses (10 per sec; 0·3 msec) for periods of 15 min, separated by 10 min rest periods. Control experiments indicated no significant difference in the output of acetylcholine between five such consecutive periods of stimulation. When the diaphragm was suspended in Krebs solution containing normal amounts of calcium, significant increases in the amount of acetylcholine accumulating were found in experiments in which tetraethylammonium salts

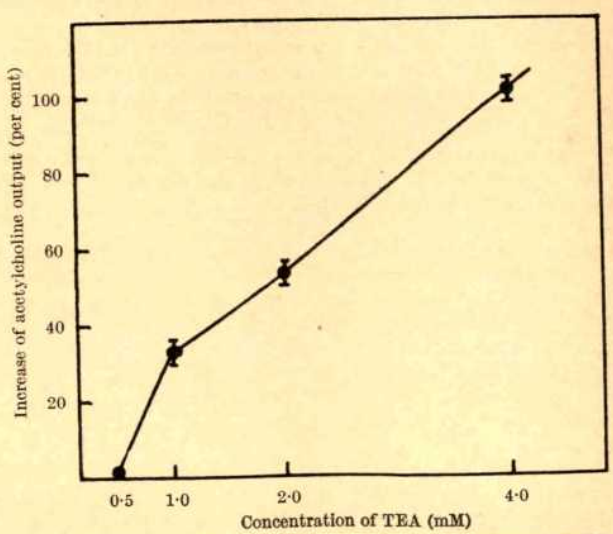


Fig. 1. Effect of varying concentrations of tetraethylammonium iodide (TEA) on the amount of acetylcholine recovered after stimulation of the rat phrenic nerve-diagram suspended in Krebs solution (10 pulses/sec; 15 min). Ordinate: acetylcholine recovered expressed as a percentage increase over control values (mean of five experiments ± *S.E.*). Abscissa: concentration of TEA (mM)

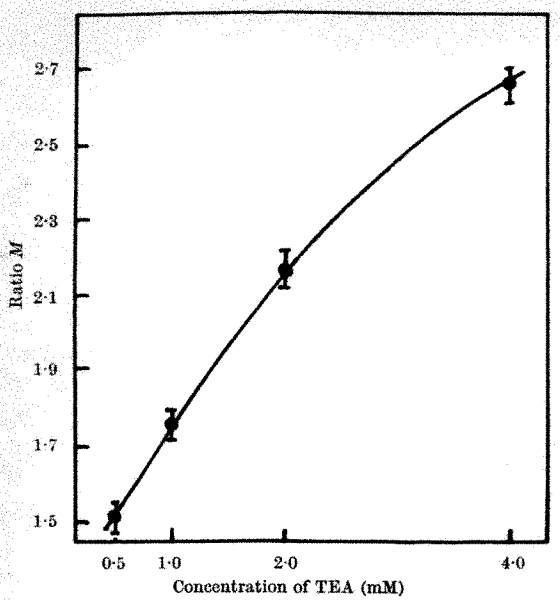


Fig. 2. Antagonistic effect of varying concentrations of TEA to *d*-tubocurarine. Ordinate: antagonistic effect as measured by ratio M (mean of five experiments ± *S.E.*).

$$M = \frac{\text{half-paralysing dose of tubocurarine in presence of TEA}}{\text{half-paralysing dose of tubocurarine in absence of TEA}}$$

Abscissa: concentration of TEA (mM)

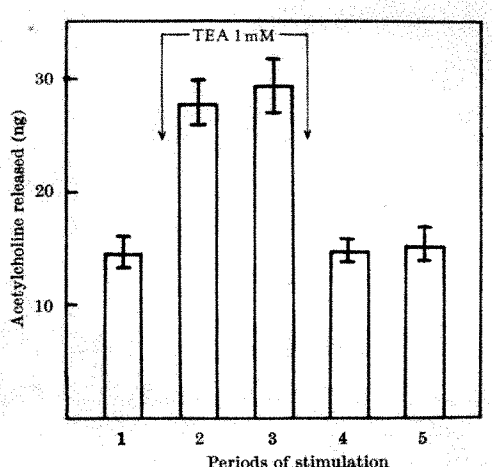


Fig. 3. Effect of 1 mM TEA on the amount of acetylcholine recovered after stimulation of the rat phrenic nerve-diaphragm suspended in low calcium Krebs solution. Ordinate: ng acetylcholine recovered (mean of five experiments ± *S.E.*). Abscissa: periods of stimulation (10 pulses/sec; 15 min). Each period of stimulation preceded by 10 min rest. TEA present during second and third periods

were present at concentrations of 1–4 mM, but not with lower concentrations. Considering the apparent lack of inhibitory effect of tetraethylammonium salts on cholinesterases, both *in vitro*[8] and *in vivo*[5], this increased accumulation of acetylcholine must be attributed to an increased liberation of transmitter. The magnitude of the effect was estimated by comparing the output of acetylcholine during the second and third periods of stimulation, when tetraethylammonium salts were present, with that of the first, fourth and fifth periods, when tetraethylammonium salts were absent. Fig. 1 shows the relationship between concentration of tetraethylammonium salts and the increased output of acetylcholine.

A measure of the antagonistic effect of tetraethylammonium salts to *d*-tubocurarine was obtained in other experiments using identical stimulation frequency. Antagonism was expressed as the ratio (M) of the concentration of tubocurarine producing 50 per cent reduction of contraction height in the presence of tetraethylammonium salts to the concentration producing a similar degree of paralysis in the absence of tetraethylammonium salts. Fig. 2 shows the relationship between concentration of tetraethylammonium salts and this ratio M.

There is a significant positive correlation between the increased amount of acetylcholine released and the anti-tubocurarine activity of tetraethylammonium salts ($r = 0{\cdot}957$, $P < 0{\cdot}001$).

It was found that calcium insufficiency resulted in a neuromuscular block which was associated with a reduced release of acetylcholine. This is consistent with the findings of Del Castillo and colleagues[2,3]. Tetraethylammonium salts, in a concentration which antagonizes the neuromuscular block caused by calcium deficiency, restored the output of acetylcholine to approximately normal values (Fig. 3).

This work therefore provides quantitative evidence to support the theory that the antagonistic action of tetraethylammonium salts to neuromuscular block caused by either *d*-tubocurarine or calcium insufficiency may be attributed to an increased release of acetylcholine from motor nerve terminals.

Attempts are now being made to elucidate this action of tetraethylammonium salts in greater detail.

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Absorption of Chlorhydroxyquinoline

There has been some uncertainty about the fate of halogenated derivatives of oxine in the body[1], but only a small part of an oral dose of chlorhydroxyquinoline could be detected in the urines of rats[1,2]. In further experiments, traces were found in the blood of rats but the amount could not be estimated.

More recently, investigations have been conducted in species other than the rat by means of the classic method for estimating phenols using Folin and Ciocalteau reagent or Gibbs's reagent. Substantial portions of oral doses have been found in the urines, as shown in the examples presented in Table 1.

Table 1. Urinary Levels following the Oral Administration of Chlorhydroxyquinoline

Species	Dose (mg)	Urines collected (h after dosing)	Amount in urine (mg) and % of dose
Rabbit	200	0–24	39 (19·5)
Rabbit	200	0–24	37 (18·5)
Rabbit	200	0–24	24 (12·0)
Calf	8,000	0–48	2,071 (25·9)
Man	250	0–8	26 (10·4)
Man	250	0–8	76 (30·4)
Man	250	0–8	89 (35·6)
Man	750	0–1·25	121
		1·25–2·25	31
		2·25–5	65
			217 (28·9)

The oral LD_{50} for mice has been found to be approximately 800 mg/kg and the intraperitoneal LD_{50} in this species was about 300 mg/kg. Other toxicity results are summarized in Table 2.

The gastro-intestinal tract of the guinea pig seems to be unusually susceptible to irritation by high concentrations of chlorhydroxyquinoline.

Partial absorption may be advantageous in an agent intended for the treatment of amœbic and bacillary

Table 2. Toxicology of Chlorhydroxyquinoline

Species	Dose (mg/kg) and route	Period of dosing (days)	Signs of toxicity
Rat	200 (oral)	97	Only moderate diarrhœa. Blood and other organs normal
Rabbit	300 (oral)	7	None
Rabbit	2·5–10 (i.v.)	4	None
Guinea pig	300 (oral)	3	7 of 8 died. Examination showed gastric lesions in 6 and enteritis in 1.
Guinea pig	150 (oral)	7	3 of 9 died. Examination showed gastric lesions in 2 and intestinal lesions in 1. All tests normal in survivors
Guinea pig	100 (oral)	5	None
Pig	200 (oral)	3	None
Pig	500 (oral)	7	None
Man	70 (oral)	10	None

dysentery, provided that high levels are attained in the large bowel.

Circulating levels of a drug like chlorhydroxyquinoline, which has a direct effect on *Entamoeba histolytica*[2,3], may protect the liver in amœbic dysentery. Furthermore, it would appear that in Africa *Shigellae* are being isolated from the blood of patients with bacillary dysentery sufficiently often to suggest that invasion of the blood stream is perhaps more common than is generally believed.

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HÆMATOLOGY

Hexokinase Activity as a Function of Age of the Human Erythrocyte

The human erythrocyte normally survives in the circulation approximately 120 days. The factors which limit the cell's survival are unknown. An important factor may be an age-related diminution in the activity of one or more enzymes vital to the cell[1]. A decrease in the activity of a key glycolytic enzyme, or enzymes, might be of particular importance because the mature human red cell derives virtually all its energy through glycolysis. Certain inherited enzymatic defects, both in the anaerobic glycolytic and in the pentose phosphate pathways of the human red cell, are associated with shortening of the life-span of the cell[2–5].

Many of the enzymes of the human red cell, both glycolytic and non-glycolytic, apparently decrease in activity as the cell ages *in vivo*[6–10]. The activities of two enzymes of glucose metabolism, glucose-6-phosphate dehydrogenase (G-6-PD) (ref. 7) and glyceraldehyde-3-phosphate dehydrogenase (GA-3-PD) (ref. 8) have been reported to decrease markedly. The decline in the activity of G-6-PD, which catalyses the initial step of the pentose phosphate pathway, is well documented[7,8,11]. Evidence concerning GA-3-PD, which catalyses a key step of anaerobic glycolysis, is conflicting. Data reported by Lohr and co-workers[8] suggest that the activity of GA-3-PD in the human red cell decreases strikingly as the cell ages *in vivo* and that the relative decrease in the activity of this enzyme is even more marked than that of G-6-PD. Our preliminary data with respect to GA-3-PD[11] have not supported the findings of Lohr *et al.* It has been suggested that the reaction catalysed by GA-3-PD may be a rate-limiting step of anaerobic glycolysis[10,12]. Available evidence, however, suggests that the initial phosphorylation of glucose, catalysed by hexokinase, is the rate-limiting step of overall glycolysis in erythrocytes of normal individuals[9,13,14].

We have carried out preliminary investigations of the relationship between the age of the human red cell and the activity of hexokinase. Erythrocytes from seven normal adult males were separated by centrifugation into fractions containing relatively young and relatively old cells. A slight modification of a previously described method[15] of repeated centrifugation was used: 450 ml. of whole blood, instead of 250 ml., were centrifuged initially, and four serial centrifugations, instead of three, were performed. Dialysed hæmolysates were prepared as described previously[16]. Hexokinase activity was assayed by a modification of the method of Slein, Cori, and Cori[17]. The assay, as applied to hæmolysates, is described in detail elsewhere[16]. The per cent reticulocytes in the fractions of red cells and the activity of G-6-PD (assayed by a modification of the method of Glock and McLean[18] similar to that used by Zinkham and Lenhard[19]) in the hæmolysates were utilized as indicators of the separation of red cells with respect to age. The effectiveness of centrifugation as a method of separating relatively young red cells, which are less dense than are more mature cells, from relatively old erythrocytes has been well documented[9,15,20–22]. Centrifugation yields samples of intact erythrocytes the fractions of which are processed similarly. We have examined other methods for separating young and old human red cells[21–23]. The method used in this work is the one we have found most satisfactory. Less than 1 per cent of the hexokinase activity in hæmolysates of any of the fractions of red cells obtained by this method of separation is attributable to contamination by leucocytes or platelets[24].

Table 1. Normal Human Erythrocytes separated by Differential Centrifugation into Relatively Young and Old Fractions

Subject	Hexokinase activity*		G-6-PD activity†		Reticulocytes (%)	
	Young cells	Old cells	Young cells	Old cells	Young cells	Old cells
1	17·3	13·7	259·0	181·1	1·9	0·1
2	18·0	14·5	196·1	161·7	6·9	0·9
3	14·9	12·8	157·2	95·3	6·2	0·5
4	29·6	13·8	401·7	211·1	5·4	0·7
5	13·8	10·0	283·4	205·1	3·3	0·0
6	13·6	8·6	284·9	159·2	5·0	0·3
7	14·4	10·6	260·0	177·6	9·3	0·1
Mean	17·4	12·0	263·2	170·2	5·4	0·4
Significance	$0·02 < P < 0·05$‡		$0·01 < P < 0·02$		$P < 0·001$	

* Hexokinase activity, μmoles glucose phosphorylated/g hæmoglobin/h.
† G-6-PD activity, μmoles nicotinamide-adenine dinucleotide phosphate reduced/g hæmoglobin/h.
‡ Evaluated by Student's *t*-test of Fisher (ref. 26).

Results are shown in Table 1. The differences in the reticulocyte counts and in the activities of G-6-PD indicate that significant separation of red cells with respect to age was achieved. The activity of hexokinase in hæmolysates of fractions enriched with relatively young cells was significantly greater than that in hæmolysates of fractions containing relatively old cells. The data indicate that the activity of hexokinase in the human red cell decreases markedly as the cell ages *in vivo*.

We have found that the activity of hexokinase in hæmolysates is increased during phlebotomy-induced reticulocytosis in normal individuals[25]. Increased activity of hexokinase persists after subsidence of the reticulocytosis, suggesting that not only reticulocytes but also young non-reticulated erythrocytes have activity of hexokinase greater than that of more mature cells.

Hexokinase occupies a strategic position in the metabolism of the human erythrocyte. A decrease in the activity of hexokinase may be of importance in senescence of the cell *in vivo*. Decreased activity of hexokinase may underlie certain of the other metabolic alterations[8,9,15] that occur during red cell ageing and may contribute to ultimate lysis of the effete cell.

Available methods for separating young and old human erythrocytes are not ideal. The potentially important role of hexokinase in contributing to senescence of the human

erythrocyte should be further evaluated as improved methods of separation are developed. Methods that would permit clear definition of changes occurring late during the red cell's life span would be of particular value.

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Production of Specific Anti-E Autoantibodies in Experimental Iso-immune Hæmolytic Anæmia in Man

IN 1961 we reported the results of studies on a case of acute, iso-immune hæmolytic anæmia experimentally induced in a $C^wDe/Ce(R_1{}^wR_1)$ volunteer transfused with plasma containing exceptional anti-CD antibodies[1]. Antibody sensitization of the recipient's erythrocytes was detected by the direct antiglobulin test (Coombs) up to day 133 following the plasma transfusion. Positive tests for antibody-coated red cells were obtained in the serum + albumin (a mixture of equal parts of human serum and 30 per cent bovine albumin) slide sensitization test through the 98th post-transfusion day. The study of the recipient's post-transfusion serum specimens for free, circulating antibodies in titration procedures employing serum + albumin revealed three distinct periods of antibody activity especially with the $cDe/ce(R_0R_0)$ primary test cells. The first of these appearing between the first hour and the ninth post-transfusion day was interpreted as surplus antibodies remaining from those transfused in the anti-CD plasma, the second between post-transfusion days 14 and 98 as indicative of eluted or released antibodies and the final between days 105 and 140 as possible auto-immune antibodies representing the product of active immunization by the recipient himself. The peak titres of the free, circulating antibodies in the second and third periods of increased antibody activity were definitely higher than those obtained in the first, immediate post-transfusion one. In view of the Rh genotype of the recipient (C^wDe/Ce), the serologic specificity of the transfused antibodies (anti-CD) and the observation that the antibodies in the third peak were reactive against the C and D antigens in the Rh system, no conclusive proof of the auto-antibody nature of these antibodies could be established on the basis of blood group specificity.

In an attempt to substantiate that specific Rh auto-antibodies could be produced by an antigenic stimulus provided by the Rh antigens of the host's own erythrocytes altered by their saturation with specific Rh iso-antibodies, additional transfusion experiments were performed. A large volume of sterile ACD plasma containing anti-CD antibodies from the same donor (*Bak*) used in the initial investigation was prepared from donations made following her fifth pregnancy and by plasmaphoresis during her sixth pregnancy. This latter pregnancy terminated again, as had the four preceding ones, in a full-term, macerated, stillborn fœtus. The titre and avidity of her anti-CD antibodies determined in the serologic characterization experiments against the cells of the recipients selected for these experiments were considerably higher than in the plasma used for the earlier investigation. The three volunteers in these experiments were selected primarily on the basis of their Rh genotypes. One belonged to the Rh group $cDE/cE(R_2R_2)$, another to $CDe/cE(R_1R_2)$ and the final to cde/cde (rr), a non-reactive control included to determine the survival of the transfused anti-CD antibodies. Individuals possessing the $cDE(R_2)$ chromosome were chosen on the premise of their being potential candidates for the production of anti-E auto-antibodies.

Antibody-coated red cells were detected in the cDE/cE (R_2R_2) recipient's circulation until post-transfusion day 168 by the direct antiglobulin test and through the 105th day in the slide serum + albumin sensitization test. The sera from this recipient's blood specimens collected in the immediate post-transfusion period contained free, circulating antibodies against his red cells from a pre-transfusion sample which had been preserved in glycerol at $-25°$ C and against test cells of Rh genotypes cDe/ce (R_0R_0) and Cde/cde (R′r). This finding, together with the strongly positive antibody-erythrocyte sensitization tests in the same period, indicated saturation of all his available red cell Rh antigen receptors with the transfused anti-CD antibodies.

Antibody eluates were prepared in human serum diluent by a modified heat elution technique from the red cells of blood samples collected from the cDE/cE (R_2R_2) recipient throughout the post-transfusion observation period. Examination of these eluates in titration procedures using serum + albumin as a diluent and test cell suspending medium revealed the presence of specific anti-E antibodies first appearing on the 70th post-transfusion day. This anti-E activity was detectable throughout the remainder of the observation period, which was terminated on post-transfusion day 229. That these eluted antibodies were indeed anti-E specific was shown by the fact that only test cells from three unrelated individuals of Rh genotype cdE/cde (R″r) were agglutinated. Test cells containing all the known major human blood group antigens other than the D antigen of the Rh system were consistently negative.

In the examination of these antibody eluates against the pre-transfusion specimen of cells from the recipient himself, activity was detected from the 1st hour post-transfusion through the last day 229. This was also found with test cells of the Rh genotype $cDe/ce(R_0R_0)$, indicating the presence of anti-D antibodies in addition to anti-E. The

activity of the eluted antibodies against his own cells was greatest in the period between post-transfusion days 126 and 229.

Thus, we believe this experiment has yielded evidence of the production of specific anti-E auto-antibodies as obtained in eluates prepared from the cells of a cDE/cE (R_2R_2) recipient transfused with exceptionally potent anti-CD antibodies. A more detailed report of these findings will be published elsewhere.

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PATHOLOGY

Ribosomes Active in Protein Synthesis in Human Reticulocytes: a Defect in Thalassæmia Major

The most prominent abnormality in thalassæmia major appears to be a decreased synthesis of hæmoglobin A[1,2]. Ingram and Stretton[3] have suggested that this defect in synthesis may be associated with an alteration in the amino-acid sequence of the hæmoglobin which does not alter the electrophoretic mobility of the tryptic peptides. However, no abnormalities have yet been detected in any of the types of hæmoglobin found in thalassæmia[4]. An alternative hypothesis[1–3] proposes that the defect involves only an altered rate of synthesis of hæmoglobin A.

In rabbit reticulocytes, protein synthesis appears to occur on a relatively stable ribosome-informational RNA complex[5]. These ribosomes, active in protein synthesis, are characterized by a sedimentation coefficient greater than 78*S*. Electron micrographs indicate that the active ribosomes are clusters of 2 or more 78*S* ribosomes[6,7]. The role of ribosomes in protein synthesis in human reticulocytes has now been examined in order to investigate the nature of the defect in synthesis of hæmoglobin in thalassæmia major. Compared with ribosomes isolated from reticulocytes of patients with sickle-cell anæmia and various non-hæmoglobinopathies, ribosomes of reticulocytes of thalassæmic subjects have a significantly decreased capacity to incorporate leucine into protein.

Blood was obtained from patients with a reticulocytosis following loss of blood, or with thalassæmia major, sickle-cell anæmia, or acquired hæmolytic anæmia. There was no significant difference in the reticulocyte count in the group of patients with thalassæmia major compared with the sickle-cell anæmia subjects (Table 1). The cells were separated from the plasma and incubated at 37° C in a modified Krebs–Ringer–bicarbonate medium[5] containing, as a radioactive amino-acid, uniformly labelled ^{14}C-leucine (1·51 mc./mmole) or tritiated-isoleucine (32·3 mc./mmole). At intervals up to 45 min, aliquots of the suspension were removed to determine counts incorporated into acid-insoluble material[5]. At 30 min, an aliquot of the cell suspension was removed, the cells recovered by centrifugation and then lysed by a short exposure to a hypotonic solution[5]. This procedure preserves the leucocytes and disrupts only the red cells. Ribosomes were recovered from the lysates by centrifuging at 105,000*g* and suspending in a solution of $1{\cdot}5 \times 10^{-3}$ M magnesium chloride, 5×10^{-2} M potassium chloride and 10^{-3} M *tris*, *p*H 7·5. The ribosomes were characterized by their sedimentation characteristics in sucrose density gradients[5,8]. Radioactivity incorporated on the ribosomes and into an acid-insoluble form was determined by techniques detailed elsewhere[5].

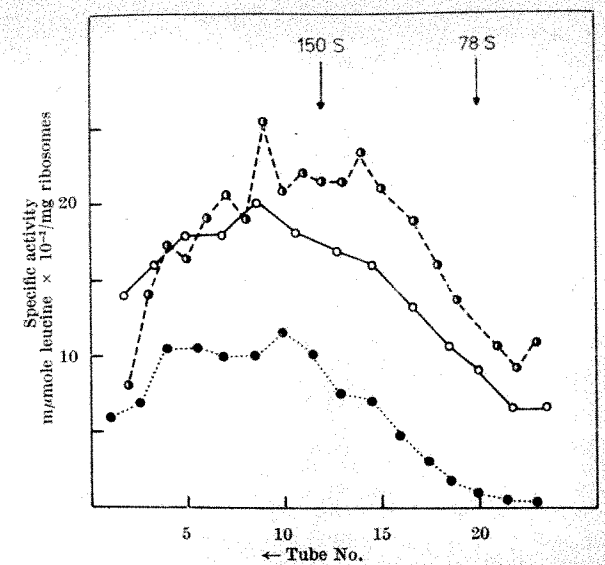


Fig. 1. Sucrose density gradient analysis of the distribution of ribosomes isolated from human reticulocytes incubated with ^{14}C-leucine as indicated in the text. The pattern of incorporation of isotope is similar in these three illustrative studies. (◑- - - ◑, sickle-cell anæmia; ●. . . . ●, thalassæmia; ○——○, congenital spherocytosis). Note that all classes of ribosomes of the reticulocytes of the thalassæmia major have a lower specific activity than the other two examples

The distribution of ^{14}C-leucine incorporated on ribosomes isolated from reticulocytes of thalassæmic and non-thalassæmic subjects was analysed by the sucrose density gradient technique. In all investigations, the ^{14}C-leucine was incorporated primarily in association with ribosomes with sedimentation coefficients greater than 78*S* (Fig. 1). In each group, the amount of ribosomes recovered was

Table 1. Human Reticulocyte Ribosome Content and Amino-Acid Incorporation

Group*	Reticulocytes %	Ribosome content		Leucine incorporation		Isoleucine incorporation
		Total (mg/10^{10} retic)	> 78*S*† %	Ribosomes	Soluble protein	Ribosomes
				mμM/mg ribosomes‡		
Thalassæmia major	6 ± 0·8§	1·10 ± 0·27	28 ± 3	0·57 ± 0·09	191 ± 45	0·05 ± 0·02
Sickle-cell anæmia	8 ± 0·8	0·70 ± 0·05	30 ± 5	1·93 ± 0·08	252 ± 55	0·01
Non-hæmoglobinopathy	17 ± 5·0	1·05 ± 0·80	30·5 ± 5	2·66 ± 0·57	262 ± 125	0·02 ± 0·01

* The values for ribosome content and leucine incorporation are based on studies of nine thalassæmia major, seven sickle-cell anæmia and seven non-hæmoglobinopathy subjects. The values for isoleucine incorporation are based on examination of four thalassæmia major, one sickle-cell anæmia and five non-hæmoglobinopathy subjects.

† Percentage of ribosomes sedimenting with a coefficient of sedimentation greater than 78*S* as estimated by analysis of sucrose density gradient patterns.

‡ Millimicromoles of amino-acid incorporated per milligram of ribosomes recovered.

§ Mean value ± *S.E.*

similar (Table 1). In addition, there was no significant difference in the proportion of the ribosomes which had sedimentation coefficients greater than 78*S*, a measure of the ribosomes which are clustered (Table 1). Thus, the defect in synthesis of hæmoglobin A in thalassæmia major does not reflect an alteration in the class of ribosomes active in protein synthesis or a deficiency in the proportion of ribosomes which are clustered.

It was found that there is a significantly lower incorporation of leucine on the ribosomes of reticulocytes from patients with thalassæmia major compared with that on the ribosomes of reticulocytes of non-thalassæmic subjects ($P < 0{\cdot}001$) (Table 1). This decreased level of leucine incorporated could not be attributed to a lower rate of incorporation of the amino-acid. In all instances, radioactivity associated with the ribosomes reached a plateau value in 5 min, while the incorporation of ^{14}C-leucine into supernatant protein continued to increase for the 45-min period of incubation. The rate of incorporation of ^{14}C-leucine into supernatant protein was somewhat lower with reticulocytes from thalassæmia major than with cells from the sickle cell anæmia ($P < 0{\cdot}05$) or the non-hæmoglobinopathy subjects, but this latter difference was not statistically significant.

Isoleucine is present in hæmoglobin F, but is not found in hæmoglobin A. If the biochemical defect in thalassæmia is related primarily to the synthesis of hæmoglobin A, it would be anticipated that isoleucine would be incorporated by reticulocyte ribosomes of thalassæmic subjects in amounts comparable to that found in non-thalassæmic subjects. The incorporation of tritiated-isoleucine on the ribosomes of reticulocytes from thalassæmic patients was generally greater than that on the reticulocyte ribosomes from non-thalassæmic subjects. Four patients with thalassæmia major were examined in this manner. In three, hæmoglobin F comprised more than 60 per cent of the total hæmoglobin, while the fourth had 2 per cent hæmoglobin F. The subject with the low level of hæmoglobin F had the second highest level of tritiated-isoleucine incorporated on the ribosomes.

The results recorded here indicate that there is a selective defect in the capacity of reticulocyte ribosomes of thalassæmic subjects to incorporate ^{14}C-leucine. It is likely that in normal subjects more than 90 per cent of the protein synthesized by human reticulocytes is hæmoglobin A (ref. 4). The present findings, therefore, suggest that thalassæmia major involves a selective decrease in the capacity of reticulocyte ribosomes to form hæmoglobin A. This defect may reflect an alteration in the RNA directing hæmoglobin synthesis (messenger RNA). This alteration could involve a decrease in the amount of messenger RNA associated with the ribosomes or a messenger RNA which is modified so as to lead to a decrease in the rate of hæmoglobin A synthesis[9].

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Effect of Liver Regeneration following Partial Hepatectomy on the Uptake of Tritiated Thymidine in the Pituitary Gland of the Rat

THE role of the pituitary gland in liver regeneration following partial hepatectomy is not clearly defined. It has been reported that hypophysectomy has no effect on rat liver regeneration following partial hepatectomy[1]. There are conflicting reports as to the effect of thyroidectomy on rat liver regeneration[1,2]. Variance of the foregoing reports suggested a necessity for further investigation of the inter-relationship existing between the regenerating liver, pituitary and thyroid glands. In another report from our laboratory, the thyroid gland demonstrated a marked increase in uptake of tritiated thymidine several days after peak incorporation in the regenerating liver following partial hepatectomy[3]. This communication is concerned with the inter-relation of the anterior pituitary gland and liver regeneration as studied by means of tritiated thymidine incorporation.

Twenty female Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.), weighing 126–154 g at the time of partial hepatectomy, were used. They were housed in individual cages in air-conditioned quarters and were fed Purina chow and water *ad libitum*. The animals were divided equally into experimental and control groups; the periods selected for study were from 0–21 days. The experimental animals were partially hepatectomized with removal of two-thirds of the liver by the method of Higgins and Anderson[4]. Control animals were sham-operated by opening the abdomen and manipulating the liver. An intraperitoneal injection of 1 μc. of tritiated thymidine (the tritiated thymidine had a specific activity of 1·9 c./mM and was purchased from Schwarz Bioresearch, Inc., Orangeburg, New York) per gram of animal body-weights was given 4 h before death. All animals were killed between 1 p.m. and 3 p.m. to minimize any variation in diurnal mitotic activity[5]. Intraperitoneal injection of tritiated thymidine was given to the zero-time animals 4 h prior to death. After death, the liver, thyroid and pituitary glands were excised, weighed, fixed in formalin, embedded in paraffin, cut at 5μ, and mounted on glass slides for autoradiography. Autoradiography, using Kodak *NTB*-2 emulsion, was performed according to the method of Messier and Leblond[6]. After a 4-week exposure, the autoradiograms were developed, fixed and stained with hæmatoxylin and eosin.

The degree of labelling in the liver cells was obtained by counting the number of labelled nuclei with 8 or more silver grains in 5 random fields in different areas of the tissue. At least 2,000 cells were counted in each field. The average number of labelled nuclei in the liver per 1,000 cells was obtained. The number of labelled thyroid parenchymal cells was obtained by counting labelled and unlabelled nuclei in 5-step sections of both the left and right thyroid lobes; there was little difference in the degree of labelling of the two lobes. The number of labelled nuclei with 8 or more silver grains in approximately 20,000 parenchymal cells was counted in the thyroid and pituitary gland sections and the values reported represent an average value for 10,000 cells.

Results are given in Table 1. Thymidine counts in the cell nuclei of the regenerating liver are found to increase between 12 and 24 h post-partial hepatectomy, reach a maximum at 24 h, and return to normal values by 6 days. The results are in general agreement with the recent autoradiographic study of regenerating liver by Grisham[7]. The counts in the anterior pituitary gland showed an increase from 6 days post-partial hepatectomy and reached a maximum on the 14th day with 324 labelled nuclei per 10,000 cells. By the 21st day post-partial hepatectomy, this value was reduced to near-normal values. The pituitary glands from the sham-operated animals were found to have no period of greatly increased counts of tritiated thymidine; however, there was a period of

slightly increased counts between 4 and 21 days which is probably the result of the recovery from the sham operation. The cells of the posterior pituitary gland, when observed, showed very few counts of tritiated thymidine. However, the study was not designed to include the posterior pituitary gland. Results of tritiated thymidine labelling in the thyroid (Table 1) show increased labelling between 10 and 21 days, with peak labelling of cell nuclei at 14 days following hepatectomy. The increased thymidine counts in the anterior pituitary gland begin at 6 days post-partial hepatectomy, whereas in the thyroid gland the increased thymidine counts are noted to begin 10 days post-partial hepatectomy.

Table 1 Effect of Partial Hepatectomy on the Tritiated Thymidine Uptake in the Liver, Pituitary Gland and Thyroid Gland of the Rat

Regeneration period	Liver (labelled cells/1,000 cells)		Pituitary gland (labelled cells/10,000 cells)		Thyroid gland (labelled cells/10,000 cells)	
	Control	Experimental	Control	Experimental	Control	Experimental
0	4	6	0	0	0	0
12 h	2	3	0	1	0	1
24 h	3	321	4	8	0	2
36 h	2	151	7	1	0	1
3 days	4	112	0	9	0	1
4 days	6	58	11	12	0	0
6 days	24	3	21	69	1	0
10 days	2	3	19	179	1	14
14 days	1	3	15	324	0	33
21 days	0	4	13	27	0	16

Thymidine is a specific precursor of DNA[8], the principal nucleic acid in the cell nuclei. Histological localization of the tritiated thymidine makes it possible to determine sites of DNA synthesis[9]. The results suggest peak DNA synthesis in the thyroid and pituitary glands occurs 14 days post-partial hepatectomy. The increased pituitary gland activity begins just before increased activity is noted in the thyroid gland. It is possible the increased thyroid activity may be secondary to stimulation from the pituitary gland. However, the effect of the pituitary gland on liver regeneration may not be mediated entirely through the thyroid gland. Carriere[10] has presented evidence that the pituitary gland alone plays some definite part in liver cell growth. Treatment of thyroidectomized rats with growth hormone produced growth and formation of increasing numbers of liver polyploid nuclei despite the absence of thyroid hormone. The increased tritiated thymidine incorporation in the pituitary and thyroid glands following the active phase of liver regeneration may be a recovery mechanism to restore normal body processes. Examination of hæmatoxylin and eosin sections of the anterior pituitary gland showed no detectable morphologic changes during the increased tritiated thymidine incorporation in either the sham-operated or experimental groups.

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Adenosine Triphosphate : an Inhibitor of Generation of Thromboplastin

In a series of experiments designed to investigate the possible existence of a relationship between energy metabolism and blood clotting mechanisms, the inactivation of human thromboplastin by adenosine triphosphate (ATP) has been noted (Table 1). The thromboplastin system is assayed for activity by determining the clotting time after adding 0·1 ml. of the reaction mixture, described in Table 1, to 0·1 ml. of 1 : 10 citrated (0·1 M trisodium citrate) substrate plasma. The substrate plasma had been recalcified exactly 15 sec earlier with 0·1 ml. calcium chloride. At maximum generation of thromboplastic activity, 0·08 ml. of ATP (0·1 M) or saline is added to the test system. This approach allows removal of 0·1 ml. of the reaction mixture for a clotting time assay before addition of ATP or saline. Concentration of ATP in the reaction mixture following removal of the 0·1 ml. aliquot is 0·0087 M.

Several assays of clotting time before addition of ATP are made possible by using separate control runs. At the specified intervals, subsequent to addition of ATP, an aliquot of the reaction mixture is tested for thromboplastic activity as previously indicated. Incubation was performed at 37° C. ATP was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. As a confirmatory check on purity, ATP was chromatographed on Whatman No. 1 paper with a solvent consisting of 100 ml. 0·1 M phosphate buffer, *p*H 6·8; 60 g ammonium sulphate; and 2·0 ml. *N*-propanol. The compound appeared free of contamination. The thromboplastin generating system, without ATP, had a 20·7-sec clotting time after 20 min of incubation. At that time, the system with ATP had been depressed to a clotting time of 107·5 sec (Table 1). It is evident from the progressive increase of the clotting time, as compared with the control, that an inactivation of thromboplastin activity is occurring which cannot be due to inherent instability of the thromboplastin molecule. A difference in *p*H as a cause of the effect of ATP was ruled out by maintenance of the same hydrogen ion concentration in the experimental and in the control. That the inactivation is not caused by a significant effect of ATP on thrombin or fibrinogen is demonstrated by a clotting time of 23·0 sec on addition of 0·2 unit of human thrombin to the test system at the 60 min incubation interval.

Table 1. Adenosine Triphosphate Inactivation of Generation of Human Thromboplastin

The thromboplastin generating (TPG) system consisted of 0·19 ml. human serum diluted 1 : 10 with 0·85 per cent sodium chloride, 0·19 ml. barium sulphate adsorbed plasma (100 mg barium sulphate per ml. of oxalated plasma) diluted 1 : 10 with saline, 0·19 ml. phospholipid as a platelet substitute (Azolectin, Associated Concentrates, Inc., Woodside, New York), 0·19 ml. calcium chloride (0·025 M), 0·14 ml. sodium chloride 0·85 per cent, and 0·04 ml. imidazole buffer (*p*H 7·2). Plasma and serum are centrifuged at 3,000 r.p.m. for 15 min for purpose of removing platelets. The results represent the mean of 12 experiments. Time of addition for ATP or saline to the system is indicated in the respective columns of data.

Incubation time (min)	Clotting time: TPG system (sec)	TPG system plus ATP (sec)	TPG system plus ATP (sec)
0	> 120	> 120	ATP
3	79·5	80·0	> 520
6	13·5	13·5	520
7	Saline	ATP	> 520
8	13·7	34·3	> 520
12	14·9	80·0	> 520
16	17·6	97·3	> 520
20	20·7	107·5	> 520

Prior to addition of thrombin, the system had shown a 112·8-sec clotting activity. A unit of thrombin is defined as that concentration per ml. which will give a 15-sec clotting time under standard conditions. ATP does not inactivate the clotting activity of pure thrombin. Addition of 0·3 unit of human prothrombin to the system did not alter the ATP-induced inactivation. Nor is it possible that the inactivation can be due to a calcium-chelating action of ATP because calcium concentration in

excess of the combined complexing capacity of ATP and citrate failed to prevent the effect of ATP. Since pyrophosphate (0·0087 M), a possible ATP derivative, failed to inhibit the reaction in presence of excess calcium ion and since adenosine monophosphate (0·0087 M) was essentially without activity, it must be concluded that the inactivation phenomenon is based on a function unique to the high-energy phosphate bonds of the adenosine molecule.

Although the inactivation of thromboplastin by the common currency of energy metabolism could be of considerable portent to an understanding of the physiological mechanisms controlling fluidity of the blood, the magnitude of the effect did not seem adequate to explain previous observations on the pronounced inhibitory action of ATP on the plasma recalcification time. Furthermore, a site of inadequate inactivation of an already formed thromboplastin molecule does not readily lend itself to an adequate physiological control mechanism. On the basis of these considerations, the effect of ATP on generation of thromboplastin was examined. Introduction of ATP as the first reactant in the system depressed thromboplastin generation to a minimum clotting time of 520 sec (Table 1). Other than the sequence of addition of ATP, all other conditions are similar to those of the previous investigation. A comparision of the magnitude of this effect on generation of thromboplastin to the maximum effect noted when ATP was introduced after the thromboplastin was at maximum activity strongly indicates the principal site of the ATP action to be on a thromboplastin precursor. The enhanced activity of ATP which is added to the system before thromboplastin generation cannot be due to increased destruction of the low levels of thromboplastin which are present during initial stages of generation. This was proved by experiment where the serial addition of small, but progressively increasing, increments of human thromboplastin to ATP gave the same picture as the middle column of Table 1. On completion of the serial addition of thromboplastin, a system identical in composition to the other systems had been achieved.

Reports from this laboratory have demonstrated an enhanced thromboplastin generation in the ageing subject who has been afflicted with a myocardial infarction[1,2]. These results have indicated the physiological mechanism for prevention or control of generation of thromboplastin to be located at an intracellular site[1]. It is, therefore, of some importance to note that the concentration of ATP used in these experiments is comparable with the level found in the normal cardiac cell by Fleckenstein, Janke and Gerlach[3].

If deposition and organization of fibrin or thrombi on the arterial wall were accepted as a cause of arteriosclerosis, as indicated more than a century ago by von Rokitansky[4] and more recently by work by Clark[5], Duguid[6], Harrison[7], Heard[8] and others, then it would seem possible for defective cellular energy metabolism to be of ætiological significance to an enhanced thromboplastin activity and arteriosclerotic sequelæ.

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IMMUNOLOGY

Adsorption of Rabbit Antibody by Rat Tissue Mast Cells and Other Cells *in vitro*

From observations obtained on guinea pig mesenteries dissected out from animals which had been passively sensitized *in vivo* with rabbit anti-ovalbumin, Humphrey and Mota[1] concluded that the antibody is reversibly adsorbed to the mast cell surface. When normal tissue mast cells isolated from the peritoneal fluid of albino rats were incubated with rabbit anti-human γ-globulin and afterwards treated with human γ-globulin characteristic morphological changes and release of some of the stored histamine occurred at the same time[2]. None of these authors has been able to provide direct evidence for the presence of cell-fixed antibody on mast cells by means of the fluorescent antibody technique. However, White[3], using this technique, demonstrated recently that some of the antibody-containing fractions from rabbit sera were indeed fixed to the mast cell surface in guinea pigs. The work reported here deals with quantitative aspects of antibody adsorption by rat mast cells as compared with the adsorptive capacity of other tissue cells.

Peritoneal mast cells were obtained from normal albino rats by differential centrifugation, as already described earlier[4]. After centrifugation these cells were washed 4 times with buffered physiological salt solution containing 2 per cent rat serum. The spleen, lymph nodes, and liver of the same animals were cut into fragments with scissors, immersed in physiological solution and then passed through a stainless steel sieve. The same washing procedure was used for both the mast cells and the cells passing through the sieve. Rabbit antiserum to human serum albumin, containing 'cytophilic antibody'[5], was prepared by repeated intravenous injections of human serum[5]. ^{131}I-human serum albumin (^{131}I-HSA) was obtained from the Radiochemical Centre, Amersham, England. The technique used for estimating cytophilic antibody fixed on to cells was the same as that of Boyden and Sorkin[5]. For each experiment one portion of a given type of cells was incubated with normal rabbit serum (control), another with rabbit anti-human serum.

Table 1. Adsorption of Antigen (^{131}I-HSA) by $5{\cdot}5 \times 10^6$ Normal Rat Mast Cells and Spleen Cells

The cells had been treated first with 70 per cent ethanol and then with 0·5 ml. rabbit antiserum or normal serum.

Type of cell	Treated with normal serum and washed four times (c.p.m.)	Treated with anti-serum and washed four times (c.p.m.)
Mast cells	1	456
Spleen cells	9	21
Spleen cells (0·5 ml. 20 per cent susp.)	34	2,648

0·5 μg ^{131}I-HSA was added to each tube (=5,365 c.p.m.)

Table 2. Adsorption of Antigen (^{131}I-HSA) by $2{\cdot}8 \times 10^6$ Normal Rat Mast Cells and Other Cells treated with 0·5 ml. Rabbit Antiserum or Normal Serum

Type of cell	Treated with normal serum and washed four times (c.p.m.)	Treated with anti-serum and washed four times (c.p.m.)
Mast cells	8	3,822
Spleen cells	4	263
Liver cells	5	4,820
Mesenteral lymph node cells	1	308
Red cells (0·1 ml. packed cells)	10	1,622

0·5 μg ^{131}I-HSA was added to each tube (=10,162 c.p.m.)

In the first series of experiments, restricted to spleen and mast cells, the capacity of cells to adsorb antibody was evaluated with cells which, after treatment with 70 per cent ethanol at room temperature for 10 min, had been incubated with rabbit anti-human serum at 0° C for 1 h. The results of a typical experiment of this type are listed in Table 1. The data obtained show that both spleen and mast cells treated *in vitro* with rabbit antiserum against HSA are capable of specifically adsorbing ^{131}I-HSA. In further experiments treatment with ethanol was omitted.

The results of such an experiment are recorded in Table 2. They show that liver and mast cells have a high capacity to adsorb cytophilic antibody and then specifically ^{131}I-HSA. Spleen, lymph node and red blood cells show distinctly lower values. The comparison of the results obtained with and without ethanol treatment reveals that unfixed antibody treated mast cells display a greater capacity to adsorb ^{131}I-HSA than cells treated with ethanol. The results demonstrate that mast cells, liver, spleen, lymph node and red blood cells of the albino rat treated *in vitro* with rabbit antiserum against human serum albumin are capable of specifically adsorbing ^{131}I-HSA. Moreover, the uptake of antibody by liver and mast cells was nearly ten times that of the other cells, which may at least partly be due to differences in cell size.

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Immunization against *Cryptococcus neoformans* by Capsular Polysaccharide

It has been reported[1] that crude *Cryptococcus neoformans* capsular polysaccharide when injected by the subcutaneous route was incapable of protecting mice against a lethal challenge dose of the parent organism. Purified polysaccharide, on the other hand, elicited a minimal protective response which was markedly increased by coupling to an ion exchange resin adjuvant. Both polysaccharides were obtained by direct recovery from cell-free culture medium. More recently it was shown indirectly that the protective antigen appeared to be concentrated in an area of the capsule immediately surrounding the cell wall[2]. It was therefore of interest to determine if fractionation of the capsule by repeated grinding would provide antigens with increased stimulatory properties.

C. neoformans, isolated *BRI*, was grown in asparagine medium at 28° for 4 days. The cells were killed (0·5 per cent formalin for 24 h), separated from the culture medium by centrifugation, and washed five times with saline solution. Afterwards, the cells were ground in a ball mill (flint balls) at 4° C for 24 h, washed free of loosely bound polysaccharide and reground. A total of five such procedures were carried out. The cells after the last grinding were approximately 90 per cent decapsulated when examined microscopically in india ink. Less than 1 per cent of the cells appeared to be ruptured. Polysaccharide was recovered from each fraction and purified by procedures described earlier[1].

The results presented in Table 1 substantiated the earlier reported indirect evidence for the existence of an immunizing antigen in close proximity to the cell wall of this organism. When the same immunization regimen was used in a second series of animals (Table 2), it was established that the specificity of the most active fraction (Cap-5) appeared to be species specific, but not strain specific.

In a third series the polysaccharide was administered as before with the exception that spleen weights and serum hæmagglutination titres were monitored at intervals. The antibody determinations were made by a modification of the technique described by Landy *et al.*[3]. The results of this experiment (Table 3) complement the protection studies by demonstrating that antibody stimulation as reflected by these parameters appeared to be responsible, at least in part, for the observed protective effect. Whether this finding was due entirely to a qualitatively different antigen or represented merely a concentration effect is beyond the scope of this communication.

Table 1. Effect of Mechanically-Fractionated *Cryptococcus neoformans BRI* Capsular Polysaccharide Administration on Survival to Homologous Challenge

Group	Polysaccharide from	Survivors on post-Challenge days 10	20	30	$P <$
1	Original CFF	13/20	0/20	0/20	
2	Cap-1	18/20	4/20	0/20	
3	Cap-2	18/20	5/20	0/20	
4	Cap-3	20/20	8/20	2/20	
5	Cap-4	20/20	9/20	4/20	0·05
6	Cap-5	20/20	16/20	11/20	0·03
7	Control	12/20	0/20	0/20	

Groups consisted of white, male (12–14 gm), *ICR* mice. Each animal received a single 600 μg dose of polysaccharide intravenously (control = 0·2 ml. saline). All mice were challenged with 10^3 cells of *C. neoformans BRI* seven days after antigen dose. Data are the arithmetic means of duplicate experiments.
Abbreviations: CFF, cell-free filtrate; Cap-1, supernatant from first grinding; Cap-2, supernatant from second grinding; etc.

Table 2. Effect of *Cryptococcus neoformans BRI* Cap-5 Polysaccharide Administration on Survival to Heterologous Challenge

Group	Challenge organism	Dose	Survivors on post-Challenge days 10	20	30	$P <$
1	*C. neoformans* TRE		20/20	18/20	13/20	0·05
2	Control	$10^{4.5}$	15/20	2/20	0/20	
3	*C. neoformans* BRY	$10^{5.2}$	20/20	18/20	14/20	0·05
4	Control		16/20	3/20	0/20	
5	*Staphylococcus aureus* KMG	Approx.	9/20	0/20	0/20	
6	Control	10^8	9/20	0/20	0/20	
7	*Klebsiella pneumoniae* LIM	Approx.	30/20	0/20	0/20	
8	Control	10^7	2/20	0/20	0/20	

Groups consisted of white, male (12–14 gm), *ICR* mice. Immunization and challenge schedule, same as Table 1. Data are the arithmetic means of duplicate experiments. All organisms are isolates from human cases.

Table 3. Primary Response to Cap-1 and Cap-5 Polysaccharides from *Cryptococcus neoformans BRI* as Reflected by Spleen Weights and Hæmagglutination Titres

Day after antigen dose	Cap-1 Mean spleen weight (per cent difference from control $\pm\sigma$)	Cap-1 Reciprocal of hæmagglutination titre (pool)	Cap-5 Mean spleen weight (per cent difference from control $\pm\sigma$)	Cap-5 Reciprocal of hæmagglutination titre (pool)
1	− 0	< 10	0	10
3	0	< 10	0	10
5	0	< 10	0	10
7	0	< 10	+ 1·7 ± 0·45	20
10	+ 0·8 ± 0·15	< 10	+ 7·8 ± 0·30	40
14	+ 1·2 ± 0·29	10	+ 12·2 ± 1·1	40

Groups consisted of twenty-five white, male (12–14 g), *ICR* mice inoculated with 600 μg of polysaccharide. At intervals animals were bled from the abdominal aorta (ether anæsthetic) for serum and killed to obtain spleens.
Abbreviations: Cap-1, first grinding; Cap-5, fifth grinding; σ, standard deviation.

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ANATOMY

Olfactory Relationships of the Diencephalon

The dominant part played by olfaction in the behaviour of lower mammals has long been appreciated, but it is only comparatively recently that the underlying physiological mechanisms have begun to be elucidated. There is now a considerable body of evidence implicating cer-

tain of the accepted olfactory projection sites, such as the amygdala and the pyriform cortex in emotional and reproductive behaviour[1,2], and more recent work has shown how directly the more peripheral parts of the olfactory pathway may regulate reproductive function[3,4]. It has generally been assumed that the anatomical link between the olfactory system and the hypothalamus is by way of the well-defined efferent pathway from the amygdala, the stria terminalis. However, in the past few years a number of experimental neuroanatomical studies have cast doubt on the essential place of the amygdala in the olfactory system, and have led us to re-examine the primary and secondary connexions of the olfactory bulb.

In the first group of experiments the olfactory bulb of one side was removed in several rats to determine the total olfactory projection on the telencephalon. Sections of these brains stained by the Nauta and Glees methods show severe degeneration in the anterior olfactory nucleus, the lateral olfactory tract and in the anterior limb of the anterior commissure. The lateral olfactory tract distributes fibres to the anterolateral part of the olfactory tubercle and to the superficial layers of the pyriform cortex back to the level of the caudal end of the amygdala. The amount of degeneration overlying the amygdala becomes progressively reduced towards the cortical amygdaloid nucleus, and it appears that few, if any, fibres reach medially as far as the medial nucleus of the amygdala. The degenerating fibres in the anterior limb of the anterior commissure can be traced to the deep layers of the pyriform cortex of the same side and to the opposite olfactory bulb. No evidence has been found for a projection to the bed nucleus of the stria terminalis, or its apparent continuation, the central nucleus of the amygdala. This failure to find a projection to the amygdala by way of the anterior limb of the anterior commissure is in agreement with two recent studies using the Nauta technique[5,6]; earlier reports of such a connexion were based on Glees-stained material, which, as is now known, commonly shows pseudo-degeneration bilaterally in these sites[7].

Despite the fact that the pyriform cortex represents the major site of projection of the olfactory bulb, comparatively little is known of its further connexions[8,9]. In an attempt to define the efferent projection of this area superficial lesions restricted to the pyriform cortex were placed in a second group of rats. In all these experiments heavy fibre degeneration has been found extending medially into the lateral preoptic region. After lesions of the rostral part of the pyriform cortex degeneration passes back into this region, but in addition some fibres can be traced further caudally into the basal and lateral amygdaloid nuclei and into the posterior parts of the pyriform cortex by both a superficial and deep pathway. From lesions in this caudal part of the pyriform cortex degenerating fibres extend forwards through the lateral and basal amygdaloid nuclei (in which some fibres are clearly terminating) and through the anterior amygdaloid area, to reach the lateral preoptic region. From this part of the preoptic region fibres are distributed rostrally to the olfactory tubercle, the horizontal limb of the diagonal band nucleus and to the cortex below and in front of the genu of the corpus callosum; and caudally, through the medial forebrain bundle, to the lateral hypothalamic area as far as the mid-tuberal level, and, mainly by way of the inferior thalamic peduncle, to the dorsomedial and lateral habenular nuclei of the thalamus. The projection to the thalamus is bilateral, a small proportion of the fibres crossing to the opposite side. In addition to these sub-cortical projections, efferent fibres also pass caudally to the entorhinal area, and forwards, in close relation to the lateral olfactory tract to the ipsilateral olfactory bulb as recently described[10], and commissural fibres cross to the opposite side in the posterior limb of the anterior commissure. Control experiments with lesions involving the cortex immediately above the rhinal sulcus (including the temporal and insular regions) give a quite different pattern of degeneration, and indicate that the degeneration described here is not consequent on the interruption of fibres simply passing through the pyriform area. In view of the close similarity in the distribution of degeneration in these experiments with that previously described after lesions of the amygdala[10], it should also be stressed that our pyriform lesions have never penetrated deeper than the subcortical white matter, and in each case care was taken to exclude possible injury to the amygdala.

Lesions of the amygdala itself (with or without direct involvement of the pyriform cortex) give rise to degeneration in two distinct subcortical projection systems. The first of these, commonly known as the longitudinal association bundle or ventral amygdalo-fugal pathway, has exactly the same course and distribution as the subcortical projection fibres of the pyriform cortex outlined here. Whether the degeneration seen after these lesions is entirely due to the interruption of fibres arising in the pyriform cortex and passing through the amygdala, or whether the amygdaloid nuclei themselves also contribute to this projection system cannot be determined from the present material. But clearly any degeneration found within this system after amygdaloid or preoptic lesions must take into account the involvement of efferent fibres from the pyriform area. Indeed all accounts of afferents to the dorsomedial nucleus from the basal forebrain areas could be explained on this basis because the descriptions of the course of such afferents correspond to that described here for the fibres from the pyriform cortex[11,12]. The second efferent pathway from the amygdala is the well-known stria terminalis; this bundle, which always shows severe degeneration when the baso-lateral group of nuclei is damaged, distributes fibres to its own bed nucleus, and to the medial preoptic and anterior hypothalamic areas of the same side. This description of the distribution of the stria terminalis agrees closely with that of Nauta[10], and like him we have been unable to find evidence for the bilateral projection to the ventromedial hypothalamic nuclei described by Adey *et al.*[13,14].

Experiments with lesions in the preoptic region effectively interrupt fibres from both the rostral and caudal parts of the pyriform area (together with other fibres in the medial forebrain bundle) and consequently show particularly heavy degeneration in their terminal projection sites; but, in addition to this involvement of the efferent fibres, considerable numbers of degenerating fibres are seen running caudally in the stria terminalis and in the longitudinal association bundles. The fibres in both pathways can be traced to the basal, lateral and medial amygdaloid nuclei. The description of caudally directed fibres in the stria terminalis is in agreement with other reports[15–17], but the ventral pathway, which does not appear to have been recognized as a source of afferents to the amygdala, is probably more important from a functional point of view as it contains considerably more fibres. Analysis of a large number of other experiments makes it clear that most, if not all, of these fibres arise from within the field of distribution of the medial forebrain bundle, and especially from the hypothalamus and preoptic areas. The reciprocal relationship between the amygdaloid region and the hypothalamus suggests that the ventral pathway is essentially a laterally directed extension of the medial forebrain bundle, and that the stria terminalis should be regarded as a dorsal component of this bundle which has become separated from the main part of the medial forebrain bundle by the development of the internal capsule.

Perhaps the most significant finding of this investigation is that the pyriform cortex sends a substantial projection to the hypothalamus and to the dorsomedial and lateral habenular nuclei of the thalamus. The importance of the first of these observations is that it provides evidence

for a direct relationship between the olfactory system and the rostral hypothalamus, with only two synapses interposed between the olfactory receptors and the cells of the hypothalamus. Physiological investigations have clearly indicated that there must be a close connexion between the sensory systems and the hypothalamus, but hitherto no unequivocal neuroanatomical evidence for a considerable projection to the hypothalamus from a major sensory system has been forthcoming. As the pyriform cortex represents the principal site of termination of secondary olfactory fibres, a corollary of this finding is that the hypothalamus must be considered as one of the main sites of induence of the olfactory system. At the same time it may provide an anatomical basis for the known relationship between olfaction and reproductive behaviour, and in this context it is relevant to note that the pyriform cortex projects primarily on the anterior hypothalamus, which appears to be the critical area involved in several aspects of the hypothalamic control of reproduction[18].

The observation that the pyriform cortex projects to the dorsomedial nucleus of the thalamus is of interest for three reasons. First, it shows that the thalamus is related to the olfactory system in much the same way as it is to other sensory pathways, and from this it follows that part, at least, of the orbito-frontal area of the cortex may be thought of as a sensory projection field for olfaction—although clearly not in the same way as the primary sensory areas. Secondly, in recent years afferents to the dorsomedial nucleus have been described from a number of basal forebrain areas (including the amygdala). As the pathway taken by the fibres from these various sources is identical with that described here for the projection of the pyriform cortex on the dorsomedial nucleus, and as the projection fibres from the pyriform cortex either pass through or in close relation to these other sites, it seems likely that the degeneration found in the dorsomedial nucleus in previous studies can be attributed, in part at least, to the interruption of fibres arising in the pyriform cortex. The question whether the amygdala and other such structures also contribute afferents to the dorsomedial nucleus cannot be resolved at present.

The third point of interest arising out of the present findings is their implication for our understanding of the phylogenetic development and functional significance of both the dorsomedial nucleus and the frontal cortex. As is well known, the dorso-medial nucleus and the frontal cortex increase considerably in the primate brain while at the same time the pyriform cortex undergoes a relative regression. As it is probable, however, that the part of the dorsomedial nucleus which is in receipt of afferents from the pyriform cortex is homologous with only the medial, magnocellular element of this nucleus in the primate, the apparent discrepancy between the increase in the development of the dorso-medial nucleus as a whole and the frontal cortex in ascending phylogeny, in contrast with the marked decrease of the olfactory system, presents no real problem. While perhaps unexpected, the finding of a direct projection of the pyriform cortex to the dorso-medial nucleus of the thalamus is not really surprising in view of the direct connexion of the hippocampus with the anterior thalamic nuclei. There is in fact an interesting similarity in the whole diencephalic projection of these two phylogenetically old cortical areas. Not only do they both send efferent fibres directly to the thalamus, but both also project to the hypothalamus, the pyriform cortex principally to its rostral part and the hippocampus to the mammillary bodies in its caudal part. While there is clearly some overlap in the hypothalamic distribution of these two projections, it may be useful for our present understanding of the organization of the afferent connexions to the hypothalamus to make this generalization. At the same time it should be pointed out that the pyriform cortex is connected with the hippocampus so that at their origin there is a close relationship between these two hypothalamic afferent systems.

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BIOLOGY

An Attempt to demonstrate *in vivo* Recognition of Ectopically Placed Autologous Tissues

The organization and control of growth of the normal tissues of the body has been related by Green[1] to the presence on the surface of normal cells of specific reactive sites, which are thought to be lipoproteins[2]. These he called tissue specific antigens, and these antigens were postulated to undergo a modification when cells become displaced outside their normal environment[3], and thereby alert the disposal mechanisms of the body which prevent the growth of ectopically placed autologous tissues. An attempt has therefore been made to design a system to demonstrate an immune response to ectopically placed autologous tissues.

Small portions of Wistar rat liver were excised, and after enclosing in 'Millipore' chambers of 0·45μ pore-size, were re-inserted intraperitoneally into the donor rats. The animals were killed 8–12 days later, and cell suspensions were made, after the method of Branster and Morton[4], of the spleens and livers. In some cases cell suspensions were made of the kidneys also, to act as controls. In other cases the controls were cell suspensions made from normal unsensitized spleens from animals of the same strain as the experimental rats.

After counting the cell suspensions in the improved ruling Neubaur counting chamber, the sensitized and control spleen suspensions were adjusted to equality, and a 3:1 volume of sensitized spleen suspension to liver suspension was injected subcutaneously into the flank of a normal guinea pig. The opposite flank of the same guinea pig received a similar suspension of normal unsensitized spleen and liver. The guinea pigs were killed on the third day, the skin at the site of injection was reflected, and the area of the flare excised and photographed. Guinea pigs which showed no flare at all, on either or both sides, were eliminated from the experiment.

A further experiment was designed to control the sensitization of the spleen by the contents of the 'Millipore' chamber. Here the experimental side received liver sensitized spleen and liver as before, while the control side was injected with liver sensitized spleen and autologous kidney suspension. The spleen in another experiment was

'sensitized' to an empty 'Millipore' chamber, and 'Millipore' sensitized spleen cells and liver were compared with autologous liver sensitized spleen and liver. The 'Millipore' chamber itself did not appear to activate the spleen.

Table 1

Experimental side: flares larger	Control side: flares larger	Flares equal on both sides	No. of guinea pigs used
18	3	10	31

Cells 1·5 million spleen cells, and 0·5 million antigen cells.

Adding the results of these three experiments together, the sensitized spleen and the antigen on the experimental side, and on the control side, either the sensitized spleen and a different antigen, or normal spleen and the same tissue as was used as antigen on the experimental side, the experimental side gave a greater number of larger flares than the control side.

In this group of experiments an attempt has been made to demonstrate a recognition system of ectopically placed autologous liver in a 'Millipore' chamber. It has been suggested that tissue organization and growth are partly controlled by tissue-specific antigens which undergo modification when placed outside their normal site, and that this modification is capable of activating the immune mechanism. It is believed that the prior sensitization of the spleen, which appears to elicit a larger flare in the guinea pig when injected with the sensitizing agent, as compared with the control side using unsensitized spleen, is suggestive that some such mechanism of recognition may indeed take place. It is thought that this recognition is specific and not merely a non-specific activation of the spleen, and that this was shown when larger flares were obtained when liver sensitized spleen and liver were injected and compared to liver sensitized spleen and autologous kidney.

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Inability of the Kidney of the Hagfish to secrete Phenol Red

The kidney of the hagfish (*Myxine glutinosa* L.) consists of about 70 extraordinarily large Malpighian corpuscles, which through short ducts are connected with the ureters. The latter extend from the level of the heart to the cloaca. It is assumed that two ureters (Wolffian ducts) carry out functions which in higher vertebrates are performed by kidney tubules. This theory is based on an alleged histological resemblance of the hagfish ureters to renal tubules[1]. However, it has not yet been confirmed electron-microscopically that the resemblance is a real one and not only superficial.

One of the well-known properties of renal tubules is their ability of active transport of certain organic molecules. This kind of secretion has been demonstrated both in whole kidneys and isolated tubules from various vertebrates[2,3]. A similar kind of secretion is accomplished by the liver during the formation of the bile[4,5].

In order to determine whether or not the ureters of the hagfish are able to function as substitutes for tubules, living specimens of *Myxine glutinosa* were injected subcutaneously with 1 ml. sea-water containing chlorophenol red in a contraction of 10^{-4} g/ml. The animals were kept in aquaria with running sea-water, and investigated 3–48 h after the injection.

It is found that the hagfishes after 1–2 days had eliminated most of the injected dye from their tissues. Not at any time during this period did chlorophenol red appear in the urine. In most of the animals, however, the liver had a slightly red colour, and the gall bladder contained bile mixed with chlorophenol red at a concentration higher than that of the injected solution. Similar experiments with fluorescein in a concentration of 10^{-4} g/ml. also demonstrated a secretion exclusively by the liver. No fluorescein was discovered at any time after injection in the ureters by examination under ultra-violet light.

It can be concluded that in *Myxine* the ureter epithelium does not react to handle chlorophenol red or fluorescein. This is probably true also for a series of other organic compounds. Nevertheless, in some respects the ureter epithelium shows similarities with ordinary renal tubular epithelium. Thus alkaline phosphatase activity can be demonstrated[6]. This enzyme may take part in a mechanism for re-absorption of glucose. The liver of the hagfish is built like a tubular gland[7], and probably the hagfish liver tubules secrete chlorophenol red and other compounds by a transport mechanism similar to those active in the renal tubules and the liver of higher vertebrates.

Presumably the secretion of phenol red, etc., by kidneys of many animals reflects an ability to transport certain naturally occurring substances like hippuric acid[2]. If this is true, such products in the hagfish have to be eliminated by the liver and not by the kidney. The inability of the hagfish kidney to transport chlorophenol red and fluorescein seems to be a consequence of its 'atubular' condition. In hagfish urine seems to be formed without interference of secretory processes, but further data are needed to verify this theory.

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Polymorphism in Living and Pleistocene Populations of the African Land Snail, *Limicolaria martensiana*

Diver[1] has shown that polymorphism in colour and pattern in living populations of the land snail, *Cepaea*, may also be distinguished in Pleistocene fossils, and although there may have been changes in the relative frequency of the colour forms, the polymorphism has persisted for thousands of years.

Examination of fossil shells of the land snail, *Limicolaria martensiana* (Sm.), obtained from volcanic ash deposits in the Katwe explosion area in the Western Rift of Uganda reveals that here also polymorphism has been maintained for a long time: radio carbon dating and surface morphology suggest that the Katwe ashes are 8,000–10,000 years old[2]. Although the *Limicolaria* shells are somewhat faded (not all the possible colour forms would be distinguishable), heavily streaked and pallid forms occur in the same horizons and their relative frequency can be compared with that of modern populations living in the same areas. As shown in Table 1, at one locality, Equator Road, streaked shells comprise 67·9 per cent of the fossil population and only 42·7 per cent of the living population. This is a statistically significant difference:

$\chi^2_{(1)} = 30{\cdot}20$ and $P < 0{\cdot}001$. In addition, the semi-streaked form is absent from the fossil population, but comprises 32·5 per cent of the modern population. About 22 miles east of Equator Road, at Kichwamba, 90·0 per cent of the fossils and 42·0 per cent of the living snails are streaked. This difference is statistically significant, $\chi^2_{(1)} = 24{\cdot}11$ and $P < 0{\cdot}001$ (Yates's correction applied). The semi-streaked form is absent from both the living and the fossils at Kichwamba. But within the living snails grouped together as pallid in Table 1, three forms are present: these would not be distinguishable in the fossil pallid snails as the differences between them, though constant, are slight.

Table 1. Relative Frequency of Colour Forms in Fossil and Living Populations of *Limicolaria martensiana*

	N	Streaked	Percentage Semi-streaked	Pallid*
Equator Road				
Fossil	165	67·9	—	32·1
Living	422	42·7	32·5	24·8
Kichwamba				
Fossil	30	90·0	—	10·0
Living	405	42·0	—	58·0

* These colour forms are figured in an earlier communication (ref. 3).

At Equator Road, living adult snails average 15 per cent less in length than fossils and the presence at high relative frequency of a colour form not found in the fossils suggests that the area has been re-populated from elsewhere. Alternatively, an evolutionary change could have occurred in which the snails became smaller and a new colour form spread into the population. Equator Road fossils are in fact more similar in size and coloration to both the living and the fossil snails from Kichwamba. At Kichwamba, the lower relative frequency of the streaked form in the living than in the fossil population could represent an evolutionary change. On the other hand, samples of living snails collected within a mile of the fossil site indicate that there may be marked variation in the relative frequency of the colour forms; but in no area have streaked snails been found to comprise more than 60 per cent of the population.

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Effects of Puromycin on Morphogenesis in Amphibian Eggs and *Acetabularia mediterranea*

The effects of actinomycin *D*, an antibiotic which is a specific inhibitor of the enzyme DNA primed-RNA polymerase, on morphogenesis in amphibian eggs and in the alga *Acetabularia mediterranea* have been presented in a preceding communication[1]. It was shown that inhibition of nuclear RNA synthesis (presumably messenger-RNA synthesis) results in the inhibition of cephalo-caudal and dorso-ventral differentiation, in amphibian embryos.

Further, still unpublished, experiments by Denis on combined explants of *Pleurodeles* gastrulæ clearly show that actinomycin *D* treatment inhibits, in less than 1 h, both the inducing power of the organizer and the competence of the reacting ectoderm: RNA synthesis is thus an absolute prerequisite for successful neural induction.

In *Acetabularia*, regeneration of anucleate fragments is much less inhibited by actinomycin *D* than that of nucleate ones; this fact has been repeatedly confirmed in more recent experiments: although synthesis of nuclear RNA is required for morphogenesis (cap formation), anucleate fragments of the alga apparently contain enough informational RNA, at the beginning of the experiment, to permit the initiation of cap formation even in the presence of actinomycin *D*.

But a cap, in *Acetabularia*, is not made of RNA alone: nuclear RNA is nothing but an intermediary between the genes and the phenotypic character; its task is to carry from the nucleus to the tip of the stalk the 'information' needed for the synthesis of specific proteins, required for cap formation. It was therefore of interest to compare the effects of puromycin, a protein synthesis inhibitor, with those of actinomycin *D*, a nuclear RNA synthesis inhibitor. As is well known, puromycin is an analogue of the terminal chain of transfer RNA; in its presence, ribosomes form incomplete polypeptide chains instead of normal proteins[2].

Puromycin (10–30 μg/ml.) was tried on *Pleurodeles* and *Rana temporaria* eggs: no effect was observed until the late gastrula stage, at which time the membranes surrounding the embryos were removed. This lack of effect is probably due to poor permeability of the antibiotic, since cleavage stops very quickly when dissociated morula cells are treated with puromycin (Tencer and Bieliavsky, unpublished observations). But neurulation is definitely inhibited: the nervous system is markedly reduced, microcephaly is the rule, and the elongation of the body is reduced (Fig. 1, *a* and *b*). By and large, puromycin-treated embryos resemble actinomycin-treated ones, as one would expect.

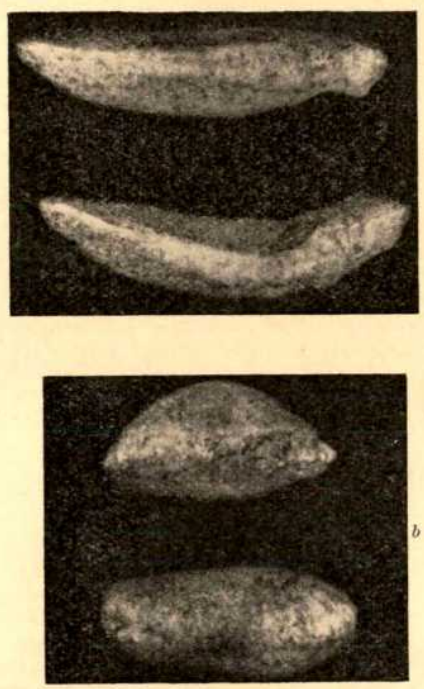


Fig. 1. *a*, Control *Pleurodeles* embryos of 4 days; *b*, *Pleurodeles* embryos treated for 4 days with 30 μg puromycin/ml.

At the same concentrations, puromycin very quickly inhibits growth in *Acetabularia*. The inhibition is almost irreversible if anucleate fragments are treated in the dark; in the light, after a 2-week treatment, very few of these fragments produce whorls or small, abnormal caps. The effect of puromycin on anucleate halves is thus much stronger and less reversible than that of actinomycin *D*. In the case of nucleate fragments or whole algæ, growth is generally resumed if the algae are returned to normal medium; after a lag period, the length of which varies with the severity of the treatment, a high proportion of malformations (heteromorphoses) is found in the algae. At low concentrations (5 μg/ml.), abnormal caps are formed (Fig. 2). At higher concentrations (10–30 μg/ml.), duplication or triplication of the apical end of the stalk can often be observed (in almost 50 per cent of the algae in one experimental series which dealt with nucleate halves

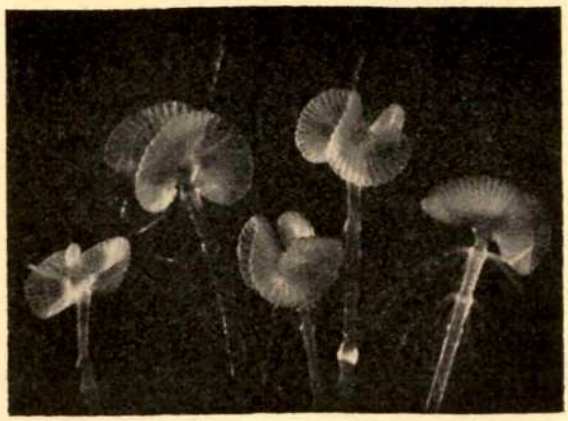


Fig. 2. Abnormal caps produced after a 4-week treatment of whole *Acetabularia* algæ with puromycin (5 μg/ml.)

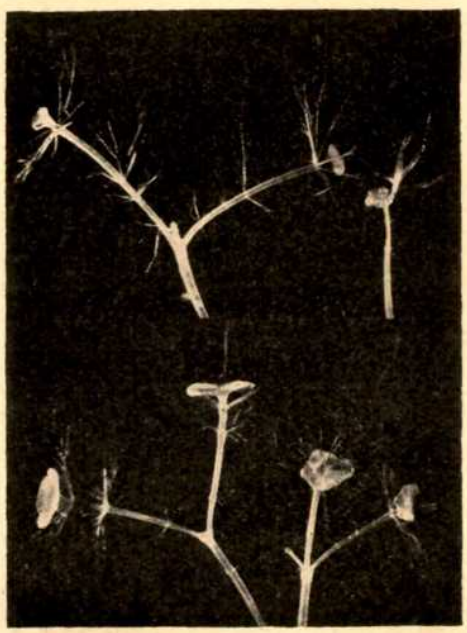


Fig. 3. Duplications of the apical end of the stalk in nucleate fragments of *Acetabularia* after a 1-week treatment with puromycin (20 μg/ml.) and a 3-week culture in normal sea-water

(Fig. 3)). In such cases, dominance of one or two of the stalks on the others is the rule, so that one never obtains more than two caps in a single alga. Less frequently, the growth of the stalk may be completely arrested and a new stalk, which ultimately forms whorls and a cap, shoots at the proximity of the rhizoid.

Inhibition of protein synthesis by puromycin therefore strongly affects the tip of the alga, where particular proteins are accumulated[3] and where incorporation of labelled methionine is considerable[4].

The orderly synthesis of these proteins, which is upset by puromycin in nucleate fragments and which is little affected by actinomycin in anucleate ones, is thus a prerequisite for normal morphogenesis.

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Effect of Time of Picking on the Ethylene Production of Apples

Composite samples of Cox's Orange Pippin apples were picked from 15 trees at weekly intervals during September 11–November 21, 1962, for storage experiments. The maturity of the fruit was tested by following the course of the respiration 'climacteric' at 12° C by a simplified method previously described[1].

From the weight of the fruit, the fall in percentage oxygen, and the rise in ethylene concentration, it is possible to calculate approximate values for the rate of absorption of oxygen, and the rate of production of ethylene. Curves for these values are shown in Fig. 1. There were 11 picks in all; but for the sake of clarity alternate picks have been omitted and only 6 pairs of curves are shown. The other 5 were intermediate.

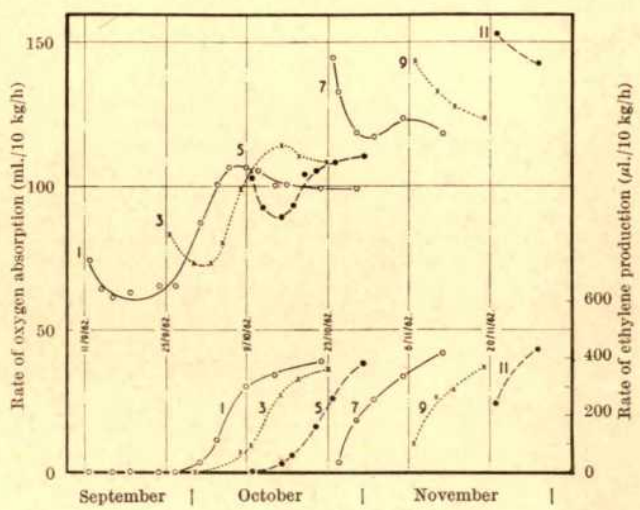


Fig. 1. Rate of oxygen absorption and of ethylene production by apples picked at fortnightly intervals

The general picture of the effect of date of picking on respiration (including the high values for very late picks) is consistent with that found by Hulme *et al.*[2] for other Cox's Pippin apples in the same season.

As regards ethylene the main points of interest are as follows:

(1) The rise in rate of ethylene production of the apples of picks 1 and 3 followed the rise in rate of oxygen absorption in the manner usually shown for apples. (In 1962 the normal picking date for Cox was considered to be about October 1.)

(2) By pick 7, oxygen absorption was at a rate associated with the peak of the 'climacteric', and there was still no substantial amount of ethylene being produced, although the rise in the rate of oxygen absorption must have begun many days earlier. Moreover, for the first few days in the laboratory, ethylene production was rising while oxygen absorption was falling. The same was true of picks 9 and 11.

(3) In picks 1–7 large amounts of ethylene were not evolved until after the fruit had been picked. (It is realized that small amounts of ethylene may have been produced much earlier[3].) However, extrapolation of the ethylene curves for picks 9 and 11 suggest that by that time considerable amounts of ethylene were being produced by the fruit on the trees.

Curves similar to 1–7 were also obtained for Cox's Orange Pippin apples from another orchard in 1961; but in that season the fruit did not remain on the trees as long as in 1962.

In recent years, doubts have been cast on the validity of the earlier concept of the coincidence of the 'climacteric' and the evolution of ethylene[4]. Evidence for the

change of view included observations that in the fruits of cherimoya and feijoa the beginning of the ethylene production lagged behind the rise of respiration by 2–4 days[5]. The results recorded here suggest that in apples (in which the 'climacteric' and ethylene production are usually considered to be coincident) a separation in time between the 'climacteric' and the rise in rate of ethylene production can be brought about by delaying picking until the 'climacteric' has been commenced on the tree.

The trees and fruit were made available by the East Malling Research Station as part of a joint experiment on the effect of picking date on storage behaviour. Dr. D. F. Meigh measured the amounts of ethylene by gas chromatography.

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Penetration of Seeds by Acetone Solutes

During an investigation of the toxicity of organic solvents to seeds it was found that those of higher plants could be soaked in acetone for periods of up to six months and still germinate readily, provided that the solvent was removed before the seeds were soaked in water. There was no reduction in viability, in contrast to that reported when dehydrated moss was soaked in ethanol or acetone for 1 h[1].

Seeds of a number of plant species were soaked in 'AnalaR' grade acetone for periods up to three months, dried at 35° C for 12 h, and then germinated. Results in Table 1 show that acetone treatment had no inhibitory effect on germination nor was any reduction in the size of the plants observed. Seeds of wheat could also be germinated after ten days' soaking in benzene, chloroform or ether.

It was found that water-insoluble, but acetone-soluble, compounds applied in acetone solutions penetrated seeds and could be recovered from the seedlings. Some preliminary results on the effects of DDT applied to barley, wheat, oat and pea seeds by this method are given. DDT was a suitable choice, since it is detectable in minute amounts, and since some data on its absorption into plants were already available.

2 g of seeds of 'Rika' and 'Proctor' barley, 'Blenda' oats, 'Svenno' wheat and 'Alaska' pea were soaked with 1 ml. of a 3 per cent (w/v) solution of DDT in acetone, which was allowed to evaporate at 20° C after the seeds had been incubated in stoppered bottles at 20° C for 24 h. After ten days' germination in soil at 22° C the first leaves were cut 1 cm above ground-level, macerated, dried and extracted with hexane. DDT was measured by gas/liquid chromatography[2] using the conditions set out by Goodwin *et al.*[3].

Table 1. Effect of Three Months' Soaking in Acetone at 2 0°C on the Germination of Ten Species of Seed, Samples of 200

The apparent variability of some values was exaggerated because the count of the number of seedlings was made after 10 days. There was no noticeable difference between the viability of the two sets

Species	Percentage germination	
	Control	Acetone treatment
Barley	97	45
Linseed	94	96
Mung bean	84	92
Mustard	85	96
Oat	90	80
Pea	80	76
Ryegrass	56	65
Sugar beet	62	76
Tomato	47	91
Wheat	35	63
Mean	73	78

Table 2. Recovery of *pp'*-DDT from the Shoots of 10-day-old Barley, Oat, Pea and Wheat Seedlings

2 g of seed were soaked with 1 ml. of 3 per cent DDT in acetone which was allowed to evaporate after 24 h.

		μg DDT in shoots	μg DDT/g fresh weight
Barley var.	'Rika'	9·5	1·32
	'Proctor'	6·5	0·83
Oat	'Blenda'	3·5	2·92
Pea	'Alaska'	2·6	1·51
Wheat	'Svenno'	3·5	1·67

No DDT was found in control plants of any variety

The results are shown in Table 2. The 1·51 μg DDT/g recovered from pea plants the seeds of which had been soaked in acetone solutions can be compared with Lichtenstein's results[4], who found less than 0·01 μg/g shoot fresh weight in pea plants which had been grown in sand containing 30 μg DDT/g. This was probably due to its low water solubility (1·2 parts per billion[5]). Less than 0·05 μg DDT/g was recovered from pea plants grown under the conditions described by Lichtenstein. The presence of DDT in the extracts of the first leaves of cereals could not have been due to surface contamination as the seeds were covered with 2 cm of soil and the coleoptile completely encloses the first leaf until the tip of the coleoptile comes above ground. Nor were toxicity symptoms observed in oat seedlings grown from 2 g of seeds which had been dusted with 30 mg DDT.

The toxicity of DDT sprays to young barley plants of a number of varieties has been shown by Hayes[6] to depend on the genotype of the variety and to follow a simple resistant or sensitive pattern. These experiments were repeated with both spray-sensitive ('Freja' and 'Rika') and spray-resistant ('Proctor') varieties. Although there was a marked difference in the response of the seedlings to DDT sprays, all strains were unaffected when dosages of up to 0·6 mg DDT per seed were applied in acetone as described. Similar amounts of DDT were recovered from

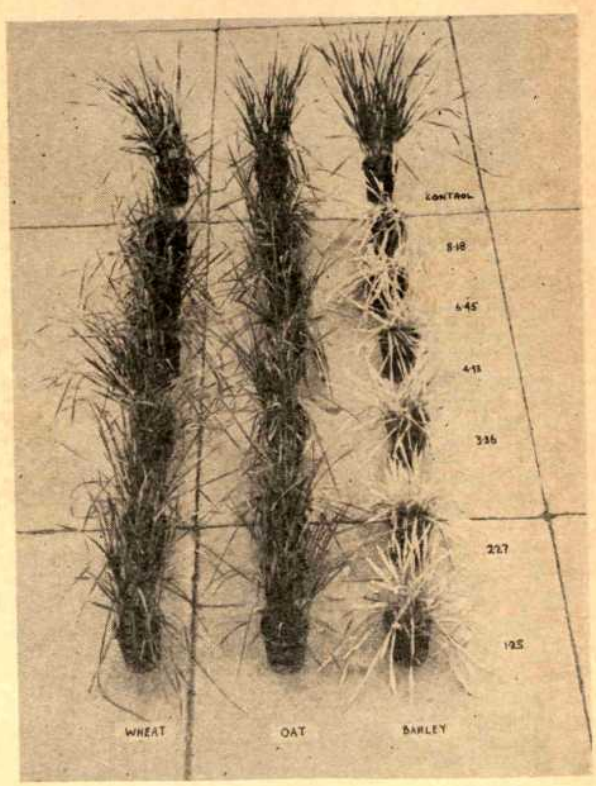


Fig. 1. Severe chlorosis of a spray-sensitive barley sprayed with a DDT emulsion at rates between 8·18 and 1·25 mg DDT/dm². Seedlings of 'Svenno' wheat and 'Rika' barley were treated after 10 days. 'Blenda' oats after 12 days' growth; all were photographed 10 days later

the shoots of spray-sensitive and spray-resistant seedlings (Table 2). Seedlings of oats ('Condor' and 'Blenda') and of wheat ('Svenno' and 'Capelle') were unaffected by DDT sprays (Fig. 1); when DDT was applied to the seeds as a 3 per cent acetone solution the oat seedlings produced were stunted and showed a characteristic mottled chlorosis, but wheat was unaffected. The difference in response of oats and barley to DDT applied as a spray or as an acetone solution affords a striking example of how the method of application can affect the response of plants to a compound.

As acetone solutions are capable of penetrating seeds it should also be possible to remove some compounds, such as germination inhibitors, from seeds using this technique.

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MICROBIOLOGY

Trace Metal Control of Bacterial Flagellation

Specific alterations in the normal battery of biosynthetic processes often occur when moderate changes are made in the balance of metallic ions in the environment. In microbial systems, for example, the quantities synthesized of organic acids, porphyrins, vitamins, antibiotics, toxins, enzymes, lipids, and polysaccharides are controlled by precise balances of metallic ions that permit, but are not necessarily optimal for, vegetative growth. Likewise, the execution of such physiological processes as cell division; sporulation; germination; plasmodia and vesicle formation; and adsorption, induction, and synthesis of bacterial viruses depends on concentrations of specific metallic ions that differ from the minimum quantities needed for synthesis of protoplasm. Indeed, the tolerance of normal vegetative growth to fluctuation of the metallic ion environment is considerably greater than the fluctuation tolerated by a specific biosynthetic or physiological process[1].

The synthesis of bacterial flagella is influenced by the organic constituents and the *p*H of the culture medium, the temperature of incubation, and the age of the culture[2]. Oddly, the role of specific metallic ions has not been studied systematically, and the literature contains only a few fragmentary reports. For example, 10^{-5} M calcium is required for the functioning but not for the synthesis of flagella of *Rhodospirillum*[3], whereas 10^{-2} M calcium or aluminium suppresses motility of *Azotobacter*[4]. In the latter report, no results were presented concerning possible suppression of viability by such high concentrations of the cations. With *Chromobacterium* grown in a complex medium, the addition of various trace elements, unspecified as to kind and concentration, was reported to have no effect on flagella synthesis[5].

In the work recorded here, partially and completely flagellated strains of *Bacillus megaterium* and *B. subtilis* were grown (at 25° and 37° C) in a shaken liquid medium containing (μg/ml.) glucose, 2,000; NH_4NO_3, 1,000; Na_2HPO_4, 1,300; KH_2PO_4, 800; $MgSO_4$, 250; $FeCl_2$, 0·25; Na_2EDTA, 1·0; *p*H 7·0. Partial flagellation indicates that, in this medium, 40–60 per cent of the cells in the middle of the logarithmic phase of growth possessed flagella; complete flagellation denotes more than 90 per cent flagellated cells. The cultures were examined at various times for number of cells, per cent of flagellated cells, number of flagella per cell, and *p*H of culture medium. These results were compared with those obtained with cultures grown in the basal medium plus one of 13 metallic ions (added as salts of sodium or of chloride) or one of three metal-binding agents. The final concentrations of the 13 ions ranged in each case from 10^{-6} M to the maximum sub-bacteriostatic quantity listed in parenthesis: Cu^{+2}, Zn^{+2} (2×10^{-6}); Co^{+2}, Ni^{+2} (2×10^{-5}); Ca^{+2}, Sr^{+2}, V^{+5}, Mo^{+6}, Mn^{+2}, Fe^{+2}, B^{+3} (6×10^{-4}); Al^{+3}, Se^{-2} (12×10^{-4}). The concentrations of the metal binding agents (Na_2EDTA, L-cysteine, and L-histidine) varied from 10^{-5} to 3×10^{-4} M.

Of the 16 added substances, only manganese, iron, and aluminium affected flagellar synthesis. The qualitative effects to be described were obtained with both species of *Bacillus* at each temperature of incubation; the quantitative results were obtained with *B. megaterium* at 25° C. With partially flagellated strains, manganese in some experiments caused a moderate increase in per cent of flagellated cells (in one experiment, for example, from 45 per cent in basal medium to 60 per cent in medium enriched with 6×10^{-4} M manganese); however, this effect was not obtained consistently. With either partially or completely flagellated strains, iron and aluminium had a marked and consistent ability to suppress flagella formation.

The results of a typical experiment with a completely flagellated strain of *B. megaterium* are summarized in Table 1. It can be seen that the percentage of flagellated cells was reduced not only in cultures grown in the presence of added iron or aluminium but also in cultures which were exposed to either metallic ion after initial growth had occurred. On the other hand, cells grown for 8 h (middle of logarithmic phase of growth) in the presence of either iron or aluminium and then washed and resuspended in basal medium for 14 h did not fully regain the ability to produce flagella.

Table 1. Suppression of Flagella Formation by Ferrous or Aluminium Chloride

Age of culture (h)	Growth medium		Percentage of flagellated cells
8	Basal		97
	plus Fe		49
	plus Al		10
14	First 8 h	Next 6 h	
	Basal	Basal	90
	Basal	plus Fe	54
	Basal	plus Al	31
	plus Fe	Basal	67
	plus Al	Basal	55
22	First 8 h	Next 14 h	
	Basal	Basal	94
	Basal	plus Fe	25
	Basal	plus Al	5
	plus Fe	Basal	50
	plus Al	Basal	41

Concentration of Fe: 6×10^{-4} M; concentration of Al: 12×10^{-4} M. Percentage of flagellated cells observed by staining with Leifson's flagella stain (ref. 2).

Cultures in which the percentage of flagellated cells was suppressed by iron or aluminium or enhanced by manganese were similar to control cultures with respect to cell viability; generation time; *p*H of culture medium; and size, shape, and number (2–8) of flagella per cell. A single notable exception occurred in cultures of *B. subtilis* grown in basal medium enriched with 6×10^{-4} M each of manganese and aluminium; these cultures contained less than 10 per cent flagellated cells, but the length of the flagella was increased as much as ten-fold over normal flagella.

After these studies were completed, a report was published describing a complex medium which can be used to suppress flagella synthesis of strains of *B. cereus*, *Proteus vulgaris*, and *P. mirabilis*[6]. The active ingredient in this medium was found to be 9×10^{-4} M iron; other metallic ions were not tested. This finding, plus those described in the present report, suggest that persons engaged in studying bacterial flagella should make certain that the quantities of iron and aluminium in their culture media are sufficiently low to permit maximum flagellation in addition to adequate vegetative growth.

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CYTOLOGY

A Simple Method for the Display of Chromosomes from Cultures of White Blood Cells with Special Reference to the Ox

The intensive examination of human chromosomes during the past two years has led to important discoveries in the understanding of certain disorders. Similar studies on domestic animals have barely begun. Before this can be carried out on a large scale, however, it is necessary to develop techniques which can be readily applied in the field since the standard methods used for the display of human chromosomes from cultures of the white blood cells are not necessarily directly applicable to the domestic animals. In this communication we describe the technique we have developed as a result of our interest in the chromosomes of the domestic ox.

To display the chromosomes from cultures of human white cells the following three steps are involved[1]: (1) separation of the white cells from a sample of blood; (2) culture of these isolated white cells for a period sufficient to generate enough cells for observation and also under such conditions that promote their rapid multiplication; (3) preparation of smears suitable for examination under the oil-immersion objective of the microscope.

In the past the white blood cells have been isolated by one of three methods. These are by the promotion of agglutination which hastens sedimentation of the red blood cells, by flotation methods which exploit the fact that the red blood cells are denser than the white cells, and by lysing the red blood cells, leaving the white cells intact. (For references on the methods commonly used see Christlieb *et al.*[2].) Investigators who have isolated white cells in order to subject them to tissue culture have usually favoured the first method. In some species, such as the horse, agglutination is so spontaneous that the red cells settle very quickly and leave the so-called buffy coat of white blood cells above them.

During investigations of the domestic cow, problems are encountered particularly in the separation stage. This is because of the well-known fact that the red blood cells of the cow sediment very slowly since they show little tendency for rouleaux formation. Human red blood cells, however, have some tendency to agglutinate spontaneously and this can be hastened by the addition of phytohæmagglutinin to the blood. However, this substance, in our hands, seems to have little effect on the red blood cells of the cow and therefore cannot be used to hasten sedimentation.

Other methods used for producing the same effect are, for example, the addition of dextran and fibrinogen, both of which cause red blood cells to clump. The addition of fibrinogen to bovine blood will certainly promote agglutination and can be used for the separation of white blood cells. However, it has to be added in such a high concentration that it is difficult to sterilize the fibrinogen solution in order that tissue culture methods can afterwards be used. The flotation method has been used in the past by workers who are concerned with isolating white blood cells but not necessarily concerned with their tissue culture. Consequently, problems of sterility were not intimately involved.

White blood cells can also be isolated by placing a sample of blood over a solution, for example, of bovine plasma albumin[3] or gum acacia[4], whose specific gravity lies between that of the red blood cell and the white blood cell. When the tube containing these materials is centrifuged at low speed, the red blood cells will sink through the medium and the white blood cells will stay at the interface between the plasma and the denser medium. Unfortunately, the concentrations of these solutions are very high. Albumin, for example, has to be used in a concentration of 35 per cent. Gum acacia also has to be used in high concentration and involves an elaborate series of steps in the preparation of its solution. Recently we have found that a substance 'Ficoll' (Pharmacia, Uppsala, Sweden), which is a highly polymerized form of sucrose, can provide a very dense solution which can be used for the separation of red blood cells from white blood cells. This material has sufficiently high molecular weight that only a 26 per cent solution is required to give a specific gravity of 1·094, which we have found to be optimal. This material can be dissolved in a standard tissue culture medium, such as Eagle's medium containing 10 per cent calf serum, and the resulting solution can be readily sterilized by passing it through a 'Millipore' filter. Experiments have shown that recovery of white blood cells with this method is as follows: sheep, 69·5 per cent, *S.E.* 1·67 (*D.F.* 4); cow, 69·9 per cent, *S.E.* 4·2 (*D.F.* 6). We therefore use this method routinely in our studies on the chromosomes of the sheep and the cow.

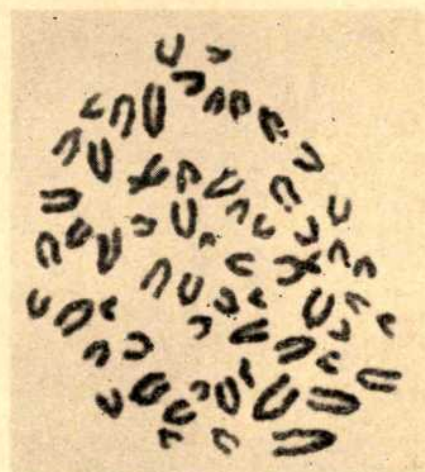


Fig. 1. Chromosomes of a female Santa Gertrudis cow. ($2n = 60$) (ref. 6)

Our method is as follows: 3 ml. of the tissue culture medium containing 26 per cent 'Ficoll' (specific gravity 1·094) is placed in a 15-ml. centrifuge tube. Two ml. of the blood sample is then placed on top of this material and the tube is centrifuged at roughly 500 r.p.m. for 5 min. The upper layer of plasma containing the white cells is then placed in a tissue culture bottle and the requisite amount of Eagle's medium containing 10 per cent calf serum is then added. Phytohæmagglutinin is also added to the bottle to promote cell division. The air in the bottle is displaced with 5 per cent carbon dioxide in air and the bottle tightly stoppered. It is then incubated at 37° C for 3 days. At the end of this period 0·05 μg/ml. of 'Colcemide' (Ciba, Summit, New Jersey) is added and the bottle incubated for a further 3 h. The cells are then removed from the bottle using trypsin and are then prepared for histological examination using an air-drying technique[5]. The smears are stained with Giemsa stain.

If the blood sample is collected at some distance from the laboratory, the blood is immediately chilled by placing

the tubes in a bowl of ice. We have found that the sample can be stored in this way for some time before use without prejudicing the tissue culture part of the experiment.

An example of the results obtained by this method is shown in Fig. 1, demonstrating the chromosomes of a cow of the Santa Gertrudis breed.

This method has also been successfully applied to blood samples from the dog, the horse, and from man; but it would appear that there is no advantage over simpler methods used in these species. Although the separation of the white cells from the erythrocytes is not complete, it should be appreciated that it is not necessary to remove all red blood cells from the sample in order to obtain successful tissue culture. Therefore, the insistence on an exact technique for preparation of pure white cell suspensions is superfluous. Another advantage of the method is that satisfactory suspensions of white cells in terms of number can be prepared from fairly small blood samples, for example, 2 ml. This is considerably less than the amount of blood usually drawn in standard work with human beings. Although this point is unimportant for the larger animals, it is very important when dealing with animals such as the cat, where the routine sample of 10 c.c. would be quite impossible to obtain.

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Preparation of Bovine Chromosomes

Certain problems have induced us to prepare the chromosomes of bovine leucocytes. Using the methods of other authors[1–3] the following technique was adopted:

20·0–40·0 ml. blood were collected from the jugular vein of an ox, to which was added 2·0–4·0 ml. heparin solution (200 mg heparin, 100 mg streptomycin, 10,000 units penicillin, 100·0 ml. of a 0·85 per cent saline solution) or an appropriate amount of 'Vetren' or 'Thrombovetren' (Promonta, Hamburg). This was put in a vessel containing 0·12–0·24 ml. phytohæmagglutinin *P* or 0·4–0·8 ml. phytohæmagglutinin *M* (Difco), well mixed, stored for 20 min in a refrigerator, centrifuged afterwards for 10 min at about 900*g* and the leucocyte-layer obtained. (Bovine erythrocytes were not agglutinated by phytohæmagglutinin.)

A leucocyte-suspension of 1,000–1,200 cells/mm^3 was prepared in 20 per cent homologous plasma and 80 per cent '*TC* medium' 199 (Difco). 10·0 ml. of the suspension were filled in each of 3 culture vessels and incubated at 37° C. 48–72 h later 1·0 ml. of a 0·04 per cent colcemid solution was added to the culture with the highest colour deviation towards yellow, kept for another 3 h in the incubator, cells centrifuged off, and put in a 0·95 per cent sodium citrate solution at 37° C and maintained for 20 min at 37° C. After this the cells are again centrifuged off, five times washed with a mixture of 1 part glacial acetic acid and 3 parts absolute alcohol (fixative) and the cells in sufficient density suspended in the fixative. One drop of this was placed on an iced moistened slide, air dried for 30 min, dyed in a 2 per cent orcein-solution and solidly covered.

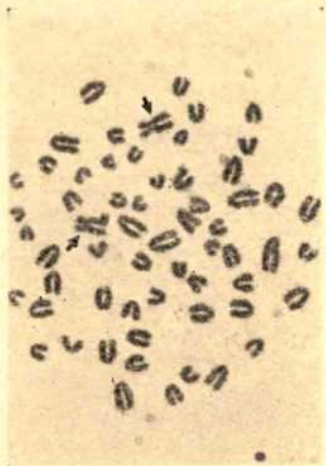


Fig. 1. Chromosomes of a female ox

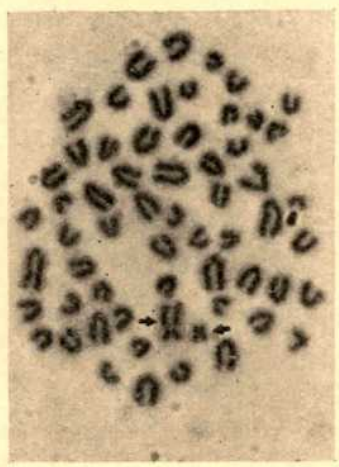


Fig. 2. Chromosomes of a male ox

Leucocytes of 4 males and 7 females were examined and 20 complete mitoses were counted of each animal. Figs. 1 and 2 show the normal diploid chromosomal rate in bovine leucocytes. Accordingly the ox cell has 60 chromosomes, 58 of which are acrocentric and obviously differ only by length of chromomeres, while 2 chromosomes are metacentric. The sex-chromosomes are marked by arrows.

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GENETICS

Genetic Basis of Stem Rust Resistance in Wheat

Present-day theory postulates a gene-for-gene basis for the host–parasite relationship between *Triticum aestivum* and *Puccinia graminis tritici* Eriks. and Henn. This emanates from the work of Flor[1] on flax rust as corroborated by Person[2] for stem rust in wheat.

In its ultimate extension this theory demands as many genes for rust resistance in the wheat plant as there are genes for pathogenicity in the rust fungus. However, some genes in wheat mediate resistance to a spectrum of

stem rust races[3]. Nevertheless, the number of identified genes for stem rust resistance in wheat is so small[4], in relation to the known number of physiological races of stem rust, as to raise serious doubts on the general validity of Flor's concept when applied to stem rust in wheat. Moreover, the fact that resistant plants can be predisposed to become susceptible in the absence of the pathogen[5,6] undermines the theory's basic tenet of a 'complementarity of fit' as an essential to successful parasitism[7].

If it be assumed that specificity in this form of obligate parasitism is dependent on the host supplying some essential nutrient for which the parasite is heterotrophic[8], a more satisfactory genetic theory for rust resistance can be based on present-day theory of cell metabolism[9,10]. Resistance genes in the wheat host can then be postulated to be regulator genes which repress the biosynthesis of such metabolites specifically essential to particular biotypes of the parasite. Thermolability of resistance or induction of susceptibility by high temperature previous to infection would then depend on inactivation of the repressor protein by inductor metabolites which are formed excessively in the host during high temperatures.

On this view the genetic control of rust resistance depends on a functional system in which all three elements of regulator, operator and inductor are important.

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Repressors, Cryptic Enzymes and Structural Genes

The Multiple Alleles at the MZ Locus. The fermentation of melezitose[1] by *Saccharomyces* is controlled by a gene which generates a single enzyme capable of hydrolysing the five α-glucosides, namely, turanose, maltose, sucrose, α-methyl-glucoside and melezitose[2]. The gene exists in a series of multiple alleles which differ from wild-type by the loss of capacity to react adaptively to one or more of the five α-glucosidic inductors[3,4]. Only seven (of 32 theoretically possible) alleles are known: *TMSGZ*, *TMSG-*, *TMS-Z*, *TMS- -*, *TM- - -*, *T- - - -* and *- - - - -*. (Possibly only six real alleles of *MZ* exist; the putative *TMS-Z* allele may be a double mutant—*TMSGZ* together with a gene generating a repressor of *G*.) Each of the capital letters indicates ability to respond adaptively to the inductor indicated. Each of the mutant genotypes is stable on vegetative transfer and retains its characteristics without recombination in hybrids, thus establishing each mutant as a genuine allele[5]. The adaptive enzyme generated by *MZ* by exposure to each of the different substrates is identical irrespective of the allele or the inductor; the negatives fail to respond adaptively to the inductors indicated. Cell-free preparations and whole living cells, in which the enzyme has been induced, split all five substrates at characteristically similar rates[6], indicating that permeability is not a factor in differentiating the different phenotypes; it has been inferred that the enzyme appears quickly after induction in the nucleoprotein layer[7].

The Receptor Hypothesis. The fact that fewer than the expected number of alleles exist suggests a non-random organization of the locus of the gene. for example, six or seven different patterns capable of fitting the various combinations of inductor glucosides. It has been inferred that the site of mutation is a receptor[8,9]—a folded protein capable of accommodating the specified inductors—and that an effective fitting of receptor and inductor leads to the induction of a single adaptive enzyme specified by the structural DNA component of the gene. The sensitivity of the gene to mutation suggested that the different elements forming the patterns are differentially sensitive to injury and that injury to any given element (except *G*) rendered the distal elements incapable of fitting substrate, thus producing the limited series of multiple alleles. These inferences were supported by numerous experiments on the mutagenic effects of ultra-violet radiation and X-rays and the demonstration that defective mutants reacted mutagenetically to substrate to restore the normal wild-type allele[3,5].

Repression of MZ by MG. The discovery of a 'mutant' with the apparent phenotype *T-SGZ*[6] was difficult to reconcile with these concepts. Cell-free preparations of this 'mutant' which had been exposed to sucrose hydrolysed all five substrates at the characteristic rates, indicating that the *MZ* locus was the active agent. Outcrosses to the *- - - - -* allele demonstrated that the culture carried *MG*. When *MG* was separated from *MZ* the genotypes were revealed, and the *MZ* allele was found to be *TMS-Z*; the recombinant lacking the *MG* gene (unlike the parental culture) responded adaptively to maltose. Thus, a mating of two maltose-negative parents produced segregants, one-fourth of which were maltose-positive (Table 1).

Table 1. Parental Combinations

Genotype	Phenotypes
TMS-Z MG	maltose-negative (repressed by *MG*) *amg*-positive; melezitose-positive
- - - - - mg	maltose-negative; *amg*-negative; melezitose-negative
	Recombinants
TMS-Z mg	maltose-positive; *amg*-negative; melezitose-positive
- - - - - MG	maltose-negative; *amg*-positive; melezitose-negative

Table 2. Some Interactions between Different Genes controlling Saccharidase Induction

Inductor	Gene	Gene-product	Biological function	End-product
Maltose	*MA*	Maltase	Hydrolyses maltose	Glucose (inductor of *SU*)
Maltose or α-methyl-glucoside	*MG*	A protein called 'α-methyl-glucosidase'	Represses *SU*	
Glucose	*SU*	β-Fructo-furranosid-ase	Hydrolyses sucrose and raffinose	Fructose, melibiose and glucose (inductor of *SU*)
Maltose	?	A protein called 'gluco-sucrase'	Represses *SU*	

It was inferred that *MG* generated a repressor which prevented this particular *TMS-Z* allele from responding adaptively to maltose, although it responded adaptively to turanose, sucrose and melezitose and, after induction, produced an α-glucosidase which split maltose as well as the other four saccharides. This particular allele of *MZ* was different from the alleles of *MZ* which had previously been studied, for *MG* did not inhibit the inducibility of the other alleles of *MZ* by maltose[6].

Inhibition of Genes by Non-homologous Substrates. When *TM - - -* is exposed to mixtures of maltose and sucrose, the inducibility of *TM - - -* by maltose is inhibited by the presence of sucrose[2]. It is inferred that the receptor of *TM - - -* permitted sucrose to fit its surface, without effecting the release of messenger RNA, but prevented effective contact of maltose with the surface of the receptor. Hence, non-inducing sucrose can block the inducible action of maltose.

Other similar examples[10] of substrate inhibitions of yeast fermentation are: (*a*) glucose inhibits the response of the genes *MA* and *MG* to their respective substrates; (*b*) α-methyl-glucoside inhibits the response of the gene *MA* to maltose.

Biological Function of Repressors. The *MG* gene was recognized (prior to demonstration of its repressor activity) by its capacity to generate the inducible 'enzyme' α-methyl-glucosidase[11,12] which cannot split maltose but

can split α-methyl-glucoside, a substance unknown in Nature. The gene *MA* produces maltase which can split maltose but not α-methyl-glucoside. Exposure of the double dominant *MA MG* to maltose simultaneously elicits both maltase and α-methyl-glucosidase[13]. This phenomenon suggests that 'α-methyl-glucosidase' is not elicited in Nature by α-methyl-glucoside but by maltose, but only when both *MA* and *MG* are jointly present. The simultaneous, adaptive elicitation of both proteins is interpreted to mean: (*a*) that the protein, maltase, acts to hydrolyse maltose; (*b*) that the protein 'α-methyl-glucosidase' represses a competing synthesis, thus preventing nucleotides and amino-acids from being appropriated by the competing gene-system. The hypothesis asserts that repressors[14] are full-fledged proteins controlled by a complete, separate structural gene, although in some instances a single receptor may act for both repressor and enzyme.

The *SU* gene, which produces an enormous excess of sucrase in the yeast cell, responds adaptively to glucose rather than to either sucrose or raffinose with the production of a β-fructofuranosidase. This view has recently been supported by experiments on protoplasts[7,15]. On the receptor hypothesis, all genes are inducible and act only under the influence of an inductor; if the inductor is a large molecule permeating the membrane very slowly, the natural inductor may be a smaller, more readily permeable, molecule. A gene (such as *SU*) which responds to an inductor (glucose) which is nearly always present (and which is an end-product of maltase activity) may appear to produce a constitutive enzyme although the enzyme is actually always induced. *SU* would pre-empt the nucleotides and the amino-acids necessary for maltase synthesis and, hence, a repressor of *SU* would have a real survival value by permitting the inducibility of maltase. It is proposed that *MG* controls the adaptive generation of a protein which can act catalytically *in vitro* to hydrolyse α-methyl-glucoside, but which functions biologically (and adaptively) in Nature to repress the excessive production of sucrase during adaptation to maltose. It is proposed, further, that the 'α-methyl-glucosidase' repressor protein fits the receptor of *SU* to preclude the excitation of the receptor by glucose.

Identity of Repressor Proteins and Cryptic 'Enzymes'. Living maltose- or α-methyl-glucoside-positive yeast cells, which were incapable of fermenting sucrose, were found after exposure to maltose or α-methyl-glucoside, respectively, to contain a 'covert' (cryptic) enzyme identified as a gluco-sucrase, that is, a sucrase which cannot split raffinose[10]. This gluco-sucrase appeared only in frozen cell preparations. Since the living cells, previous to freezing, could not ferment sucrose and since sucrose cannot pass through the plasma membrane, the cryptic enzyme must not have the biological function of catalysing the hydrolysis of sucrose *in vivo*, although it can catalyse the hydrolysis of sucrose *in vitro*. It is inferred, therefore, that this cryptic 'enzyme' and all other cryptic 'enzymes' are induced by repressor genes simultaneously with the enzyme generated by the principal structural gene (and by the same inductor). The function of cryptic repressor-proteins is to inhibit competing syntheses.

Receptor- and the Operon-Hypothesis. On the receptor-hypothesis, *vis a vis* the operon-hypothesis[14], the significance of repressors and repressive genes is quite different. On the receptor-hypothesis, all genes are inducible and generate enzyme only in response to contact of the receptor with the proper inductor. Genes which appear to be constitutive are induced, not by the substrate of the enzyme, but by an inductor different from it, for example, glucose acts as an inductor of β-fructo-furanosidase and galactose acts as inductor of melibiase[16]. In the latter instance, the double dominant, *ME GA*, when exposed to galactose generated both melibiase and galactokinase. If melibiose is also present, both the galactose and the glucose produced by splitting melibiose are consumed. However, an *ME ga* culture, exposed only to galactose, generates melibiase but no galactokinase. Since the only sugar available is galactose, on which melibiase cannot act, the induced enzyme has no value to the organism. This example of induction indicates: (*a*) that there is no necessary relationship between the inducer and the enzyme induced; (*b*) that the 'end-product' of enzyme action (cf., glucose for sucrase) may be an inducer of the enzyme; (*c*) that a repressor may confer a decided biological advantage.

Repressors in the Diplophase. The repressors of β-galactosidase induction are diffusible substances effective in the heterozygote[14]. The repressor generated by *MG*[17] is also effective in the diploid; one *MG* gene in the homozygous diploid, *MZ/MZ*, represses the induction of two repressible mutant *MZ* alleles by maltose.

Significance of Repressors in Ontogeny and Evolution. On the receptor-hypothesis, the original member occurring in the evolution of a gene-system is an inducible gene responsive directly to a specific substrate by the generation of an enzyme, and repressors are evolved subsequently to the original inducible gene. Gene-systems without regulator genes may exist, for regulator genes are secondary rather than primary items in evolution. The example of the repressible *MZ* alleles shows that mutations in the protein receptor may make only a single pattern (for example, the *M*-pattern) of the receptor sensitive to a specific repressor, thus increasing the possible variety of manifestations of a gene without changing the enzyme induced by the gene. Mutations of the receptor (rather than the structural DNA component of a gene) might be very important in ontogeny, and in other adaptations, because changes in the conformation of a receptor may be effected without necessitating changes in the nucleotide sequences which are so much more stable than protein conformations.

It is not possible to conclude this article without a tribute to my dear friend, the late Shlomo Hestrin, whose critical and painstaking work on induced glucosidases in yeast, both 'covert and overt', made data available more than a decade ago which could only be interpreted in the light of more recent knowledge.

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VIROLOGY

Antibacterial Activity of Herpes Simplex Virus grown in Tissue Culture

ANTIBACTERIAL substances have been recovered from bacteria, actinomycetes, fungi, lichens and seed plants[1,2] but not from viruses. Gan and Warsa[3] observed that the allantoic fluid of embryonated eggs infected with influenza

virus possessed lytic activity against a variety of micro-organisms, but concluded that this lytic factor was distinct from the virus itself. In the light of the observation that herpes simplex lesions around the nose and mouth rarely, if ever, become infected despite their heavy exposure to pathogenic strains of staphylococci, a study was undertaken to determine whether herpes simplex virus exerts any inhibitory effect on the growth of staphylococci and other bacteria.

Six tissue culture adapted herpes simplex virus strains were used, 1 stock strain isolated from a patient, 4 obtained from Dr. T. F. MacNair Scott of the Children's Hospital in Philadelphia, and 1 from Dr. Stephen Millian of the laboratories of the New York City Department of Health. Bacterial strains tested were those freshly isolated from clinical specimens received at the Diagnostic Bacteriology Laboratory. Petri dishes containing equal parts of melted 1 per cent trypticase in 2 per cent agar and monkey kidney *B* medium 'Melnick' were seeded, after setting, with 0·2 ml. of a standardized inoculum of a 6-h culture of a coagulase positive strain of *Staphylococcus aureus* and spread evenly over the surface. After drying, a piece of sterile thick filter paper, 1 cm square, was dropped on to the plate and impregnated with 0·1 ml. of a tissue culture suspension of the stock herpes simplex virus strain prepared as follows. A rabbit kidney cell tissue culture tube suspended in antibiotic free monkey kidney *B* medium 'Melnick' was inoculated with virus and incubated for 2–3 days at 37° C until distinct cytopathogenicity was evident, after which the tube was frozen at −20° C overnight. The viral suspension was then thawed and dropped on the filter paper with a capillary pipette. A rabbit kidney cell tissue culture tube uninoculated with virus, but treated in a similar manner, was used as a control. Plates were incubated in an inverted position for 24 h at 37° C and observed for zones of bacterial inhibition.

As illustrated in Fig. 1, a distinct zone of inhibition was produced around the filter square impregnated with virus-tissue culture suspension as contrasted to uninoculated control tissue culture suspension where no such zone was evident. Nine other coagulase positive *Staphylococcus aureus* strains as well as a number of strains belonging to other bacterial species were similarly tested with the results noted in Table 1. As may be observed from Table 1, with the exception of nearly all strains of *Streptococcus fecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*, growth of most strains belonging to the other bacterial species tested were inhibited by the herpes simplex virus. Five additional herpes simplex strains tested for antistaphylococcal activity all produced an inhibitory zone. When the 6 herpes simplex strains were grown in serially propagated cell lines such as human embryonic lung (*WI*–26), human amnion (*Wish*) and mouse embryo fibroblasts (MEF) instead of in the primary rabbit kidney cell line, staphylococcal inhibition also ensued.

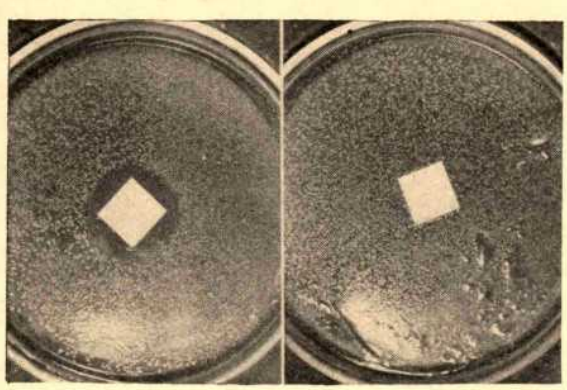


Fig. 1. Left, Inhibition of *Staphylococcus* by herpes simplex virus grown in monkey kidney cell tissue culture; right, absence of *Staphylococcus* inhibition with monkey kidney cell tissue culture, uninoculated with virus

Table 1. Effect of Herpes Simplex Virus on Growth of Different Bacterial Species

Organism	No. of strains inhibited/ No. of strains tested	Inhibitory zone (average diam. (mm))
Staphylococcus aureus	7/10	25
Staphylococcus albus	3/3	32
Diplococcus pneumoniae	5/8	27
Streptococcus pyogenes	5/5	21
Streptococcus viridans	3/4	15
Streptococcus fecalis	0/5	—
Escherichia coli	1/4	23
Aerobacter aerogenes	3/5	22
B. proteus	5/5	20
Pseudomonas aeruginosa	0/5	—

Two of the 6 herpes simplex virus strains were inoculated into the chorioallantoic membrane of 9-day-old chick embryos and intracerebrally into 2-day-old suckling mice. Chorioallantoic membrane and allantoic fluid were collected after 96 h. The mouse brain suspension, obtained at the onset of convulsions, which usually occurred 96–112 h after injection, was frozen, suspended in approximately 2 c.c. antibiotic free culture medium and ground in a mortar and pestle. On impregnation of the filter paper squares with these specimens, no zone of bacterial inhibition resulted although all demonstrated distinct cytopathogenicity when re-inoculated into fresh rabbit kidney cells. Lack of inhibition with virus derived from these sources may, however, be due to insufficient virus production in these sources since previous experiments with tissue culture suspensions revealed that bacterial inhibition occurred only when the virus titre was 10^{-7}, measured by tissue cell cytopathogenicity, whereas a one log decrease failed to elicit the inhibitory effect. Titre of mouse brain suspensions, measured by mouse pathogenicity, proved to be 10^{-3}.

Contamination of various viral strains or tissue culture materials with pleuropneumonia-like organisms or bacteriophage were considered as possible causative factors for the observed zone of bacterial inhibition. Sub-culture of all viruses and tissue culture materials on special PPLO medium resulted in consistently negative isolates. Inoculation of broth seeded with the test staphylococcus strain with the same materials and incubation over a period of a week failed to demonstrate bacterial clearing either visually or photoelectrically or any evidence of lytic plaque formation when they were applied to a surface culture of the test staphylococcus. Furthermore, the diverse spectrum of antibacterial activity observed and the characteristic high degree of specificity possessed by bacteriophages for specific bacterial species would exclude bacteriophage as a possible causative factor. The possibility that intracellular material of the tissue culture cells released as a result of their disruption by virus might have exerted an antibacterial effect was also entertained. A heavy suspension of freshly obtained, trypsinized rabbit kidney cells suspended in the same antibiotic free tissue culture medium as was utilized for the tissue cultures was disrupted by freezing at −20° C and thawing, three times, and then centrifuging at 16,000 r.p.m. for 1 h in a refrigerated centrifuge. Both the supernate and sediment of the destroyed cells material failed to produce a zone of staphylococcal inhibition when applied to filter paper squares.

Whether the observed bacterial inhibition is due to the action of the virus itself or to some product of virus-tissue culture cell interaction remains to be established.

S. Stanley Schneierson
Bella Shore

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Mount Sinai Hospital,
New York.

[1] Waksman, S. A., *Microbial Antagonisms and Antibiotic Substances* (N.Y. Commonwealth Fund, 1945).

[2] Florey, H. W., Chair, E., Heatley, M. A., Jennings, A. G., Sanders, A. G., Abraham, E. P., and Florey, M. E., *Antibiotics* (Oxford Medical Publications, 1949).

[3] Gan, K. H., and Warsa, R., *Amer. J. Hyg.*, **69**, 83 (1959).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

BIOCHEMIST (preferably with some experience of simple statistics, immunology and work with animals), to assist with routine and research work concerned with gonadotropins and gross hormone in children—The Chemical Pathologist, The Hospital for Sick Children, Great Ormond Street, London, W.C.1 (August 22).

LECTURER (with a thorough knowledge of laboratory organization and techniques, preferably with a science degree, and normally a national of the United Kingdom or the Republic of Ireland) IN PRACTICAL SCIENCE AND LABORATORY TECHNIQUES at the Technical Teacher Training College, Lyallpur, West Pakistan—The Council for Technical Education and Training for Overseas Countries, 12 Lincoln's Inn Fields, London, W.C.2 (August 23).

ASSISTANT LECTURER (preferably with some specialization in metal forming) IN PRODUCTION ENGINEERING—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref. 7/63/6 (August 24).

ASSISTANT LECTURER (with medical qualifications registrable in the United Kingdom) IN ANATOMY—The Registrar, The University, Manchester 13 (August 24).

GRADUATE (or with equivalent qualifications, and preferably experience in microtomy or instrumentation) IN THE DEPARTMENT OF ZOOLOGY, for a technical post—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (August 24).

LECTURER (with a medical or dental qualification, or non-medically qualified) IN THE DEPARTMENT OF ANATOMY—The Registrar, The University, Liverpool, quoting Ref. No. CV/376/N (August 24).

SENIOR RESEARCH ASSISTANT (with a Ph.D. in physics or equivalent research experience) IN THE DEPARTMENT OF PHYSICS, to work on the electrical and optical properties of low mobility solids—The Registrar, The University, Sheffield 10 (August 24).

VETERINARY RESEARCH OFFICER (Bacteriology) (member of the Royal College of Veterinary Surgeons, or equivalent, a diploma in bacteriology and at least six years experience in veterinary research work) IN THE MINISTRY OF ANIMAL RESOURCES, Republic of the Sudan—The Sudan Embassy, Appointments Section, 3 Cleveland Row, London, S.W.1, quoting Ref. 4/2206 (August 25).

RESEARCH SCHOLARS (2) (graduates in either microbiology or biochemistry) IN THE DEPARTMENT OF BOTANY AND MICROBIOLOGY, for research in certain aspects of petroleum microbiology—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (August 26).

SENIOR LECTURER (university graduate, preferably with a higher degree, experience of teaching to final degree level, and some research experience) IN PHYSICS—Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (August 26).

SENIOR LECTURER OR READER IN PHYSICS—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (August 27).

LECTURER (interested in at least one of the following: (*a*) vertebrate zoology; (*b*) ecology; (*c*) genetics; or (*d*) physiology) IN ZOOLOGY at the University College of Cape Coast, Ghana—The Assistant Registrar (Overseas), University College of Cape Coast, 15 Gordon Square, London, W.C.1 (August 28).

LECTURER (with an honours, or higher degree in zoology, evidence of competence and interest in research, and preferably experience in university teaching) IN ZOOLOGY at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 30).

ORGANIC CHEMIST (recently qualified graduate), for synthetic work on fungicides and systemic fungicides; and a BIOCHEMIST OR PLANT PHYSIOLOGIST (recently qualified graduate), to study the effects of plant hormones on the enzyme activity of plants in the Unit of Plant Growth Substances and Systemic Fungicides—Prof. R. L. Wain, F.R.S., Wye College, near Ashford, Kent (August 30).

PHYSICIST (with a B.Sc. degree, or equivalent, and preferably a higher degree and experience in the application of radioactive isotope techniques to medical practice) IN THE RADIOTHERAPY DEPARTMENT, Auckland Hospital, New Zealand, for radioactive isotope work—The High Commissioner for New Zealand, New Zealand House, The Haymarket, London, S.W.1 (New Zealand, August 30).

PHYSICS TECHNICIAN (experienced in electronic/mechanical workshop practice) IN THE RADIOTHERAPY DEPARTMENT, Auckland Hospital, Auckland, New Zealand, to be mainly concerned with the maintenance of the 4 MeV Linear Accelerator Unit—The High Commissioner for New Zealand, New Zealand House, The Haymarket, London, S.W.1 (New Zealand, August 30).

TECHNICIAN (male or female, F.I.M.L.T. preferably with extensive experience in histological techniques) IN THE DIAGNOSTIC SECTION, Ruakura Animal Research Station, Agriculture Department, New Zealand, to take charge of the Histological Laboratory—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B.13/2/20/1960 (August 30).

LECTURER IN REFRACTORIES AND CERAMIC TECHNOLOGY—The Registrar, The University, Sheffield (August 31).

LECTURER OR ASSISTANT (preferably with a special interest in moral philosophy) IN PHILOSOPHY—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (August 31).

READER OR SENIOR LECTURER (graduate in physics, chemistry, biochemistry, or engineering, with experience of research in food processing) IN FOOD SCIENCE IN THE DEPARTMENT OF AGRICULTURAL SCIENCES at the School of Agriculture, Sutton Bonington, to be responsible for the organization of the food-processing laboratories and for the co-ordination of the lecture course in food science—The Registrar, The University, Nottingham (August 31).

RESEARCH ASSISTANT (preferably honours graduate in mathematics who wishes to co-operate with engineers in studying the dynamic behaviour of fluid power transmission equipment) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—Mr. J. D. Stringer, Department of Mechanical Engineering, The University, St. George's Square, Sheffield 1 (August 31).

ASSISTANTS OR ASSISTANT LECTURERS (2) (with a first- or second-class honours degree in agricultural science, chemistry, biochemistry or physics, and preferably suitable postgraduate experience) IN SOIL SCIENCE IN THE DEPARTMENT OF AGRICULTURAL CHEMISTRY AND SOIL SCIENCE, to assist in the teaching of undergraduate students and research—The Secretary and Bursar, University College, Dublin, Republic of Ireland (September 1).

LECTURER IN PHYSICS—The Registrar and Secretary, The University of Durham, 38 North Bailey, Durham (September 2).

LECTURER IN ANIMAL PATHOLOGY, for duties which include teaching and research—Dr. T. D. Kellaway, Secretary of the Appointments Committee, Department of Pathology, The University, Tennis Court Road, Cambridge (September 6).

LECTURER IN PHYSICS at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (September 7).

LECTURER IN THE DEPARTMENT OF SOCIOLOGY—The Secretary, The University, Edinburgh (September 7).

RESEARCH OFFICER OR SENIOR RESEARCH OFFICER (Pasture Agronomist) (with a Ph.D. in an appropriate field or postgraduate research experience of an equivalent standard and duration, supported by satisfactory evidence of research ability) at the Cunningham Laboratory, Division of Tropical Pastures, C.S.I.R.O., Brisbane, Australia, to join a research team working on both plant and soil aspects of the nitrogen economy of tropical pastures—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 850/194 (September 7).

LECTURER (with a good honours degree in metallurgy or chemistry or equivalent qualifications, and experience or special interests in one of the following fields: extraction metallurgy, alloy thermodynamics, electrochemistry, high temperature chemistry or structural inorganic chemistry) IN CHEMICAL METALLURGY IN THE DEPARTMENT OF METALLURGY of the Faculty of Science—The Registrar, The University, Manchester 13 (September 9).

CHAIR OF CLINICAL MEDICINE IN THE DEPARTMENT OF MEDICINE of the University of Cape Town—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (September 14).

LECTURER IN GEOCHEMISTRY at Victoria University of New Zealand, Wellington—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 15).

SENIOR LECTURER IN SPECTROSCOPY at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 15).

LECTURER OR ASSISTANT LECTURER IN MATHEMATICS—The Secretary, The University, Aberdeen (September 16).

DEPUTY LIBRARIAN (chartered librarian, and preferably with a university degree and experience of decimal classification work)—The Vice-Principal, Lanchester College of Technology, Priory Street, Coventry (September 20).

JUNIOR RESEARCH FELLOW (with a good first in zoology) IN LOCUST PHYSIOLOGY at the University College of Rhodesia and Nyasaland, for work on the water relations of locusts—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 20).

CHAIR OF THEORETICAL PHYSICS at the University of Cape Town—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (September 25).

ASSISTANT LECTURER (preferably with special training in either mathematics or psychopharmacology) IN EXPERIMENTAL PSYCHOLOGY—The Registrar, The University, Manchester 13 (September 30).

CHAIR OF AGRICULTURAL MARKETING—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (September 30).

KELVIN CHAIR OF NATURAL PHILOSOPHY—The Secretary of University Court, The University, Glasgow (September 30).

PROFESSOR OF EXPERIMENTAL PHYSICS at the University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

PROFESSOR OF INORGANIC CHEMISTRY at the University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

PROFESSOR OF PSYCHOLOGY at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

CHALLIS CHAIR OF PHILOSOPHY at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 1).

ASSISTANT EXPERIMENTAL OFFICER (interested in biochemical analysis, with a pass degree or H.N.C. or, if under 22, G.C.E. "A" level, O.N.C., or equivalent) IN THE CHEMISTRY DEPARTMENT, to help with research work on the synthesis of milk constituents and the rate of milk secretion—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 63/12.

ASSISTANT to help a research worker with a project on nucleic acid and protein metabolism supported by the British Empire Cancer Campaign—The Secretary, Department of Radiotherapeutics, University of Cambridge, Hills Road, Cambridge.

ASSISTANT WARDEN (male, single, with a university degree in zoology, an interest in ornithology, and preferably some research and/or teaching experience)—The Warden, Flatford Mill Field Centre, East Bergholt, near Colchester, Essex.

BIOCHEMIST OR BIOLOGIST, to work on problems connected with human gonadotrophins—The Director, Department of Clinical Endocrinology, Women's Hospital, Birmingham 11.

CHEMIST (male, with an honours degree in chemistry or equivalent qualification, and not less than two years postgraduate experience or training) IN THE DEPARTMENT OF AGRICULTURE, Tanganyika, for research work on coffee, including soil and plant analysis connected with nutritional problems and soil survey work—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting C1/RC213/145/05.

D.S.I.R. RESEARCH STUDENTS (with a first- or upper second-class honours degree in chemistry or an equivalent qualification, for example, G.R.I.C. or Dip.Tech.) IN THE DEPARTMENT OF CHEMISTRY, for work on the chemistry of clay minerals—Dr. A. B. Meggy, Department of Chemistry, Plymouth College of Technology, Tavistock Road, Plymouth.

EXPERIMENTAL OFFICER (with a degree, or equivalent, in physics or electrical engineering), to assist in research in radiation chemistry using a 3 MeV van de Graaff electron accelerator—Prof. F. S. Dainton, F.R.S., School of Chemistry, The University, Leeds 2.

POST-DOCTORAL RE-EARCH FELLOW IN THE CHEMISTRY DEPARTMENT, for work in collaboration with Prof. R. C. Cookson on applications of ultra-violet

spectroscopy and circular dichroism in organic chemistry—The Secretary and Registrar, The University, Southampton.

Research Assistant (chemist, with an interest in protein chemistry or the preparation of organometallic compounds suitable for crystallization with proteins) in the Crystallography Laboratory to work in a section dealing with protein crystallography—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

Research Assistant (with either a good degree in chemistry or Grad. R.I.C., and prepared to work for a higher degree) in Organic Chemistry—The Registrar, Administrative Offices, Portsmouth College of Technology, 9 Burnaby Road, Portsmouth, Hampshire.

Research Fellow (with a higher degree in science or engineering, experience in research, and preferably plant experience) in Process Engineering Metallurgy in the Department of Metallurgy, to prosecute research in his own account and join in running a Group concerned with basic research into the transfer of mass, heat and momentum under conditions important in metallurgical processes—Prof. F. D. Richardson, Department of Metallurgy, Imperial College of Science and Technology, London, S.W.7.

Technical Assistant (preferably with experience in steroid and other hormone estimations and/or a knowledge of histochemical techniques), for work related to cancer research—Dr. K. Lederis, Department of Pharmacology, Medical School, University Walk, Bristol.

Technician (Associate of the I.M.L.T. or equivalent, and preferably an interest in tissue culture or related fields, virology, etc.) with a Cytogenetic Group working on human chromosomes—The Secretary, Pediatric Research Unit, Guy's Hospital, London Bridge, London, S.E.1.

Technician (expert in preparing thin and polished sections and competent to take charge of other technical duties) in the Department of Geology—The Chairman, Geology Department, University of Ottawa, Ottawa, Ontario, Canada.

Technician (with technical experience in histology or an interest in helping with general animal experimental work), to assist with research in the Henry Dale Laboratory of Experimental Surgery at the Royal National Orthopædic Hospital, Stanmore—The Secretary, Institute of Orthopædics, 234 Great Portland Street, London, W.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Electrical Research Association. Technical Report Z/TI32: The Planetary Nebulae: Stellar Spiral and Barred Spiral Nebulae. By Dr. C. E. R. Bruce. Pp. 7+6 figures. (Leatherhead: Electrical Research Association, 1960.) 7*s.* 6*d.* [216

The Basis of Pacifist Conviction. By R. J. Billington. Pp. 16. (New Malden, Surrey: Fellowship of Reconciliation, 9 Coombe Road, 1963.) 1*s.* [216

University of Nottingham. Report of the School of Agriculture, 1962. Pp. 140. (Sutton Bonington, Loughborough: University of Nottingham School of Agriculture, 1963.) [216

Tin Research Institute. Tinplate Handbook: Facts about Tinplate for Buyers and Users. By Dr. W. E. Hoare. Fourth (revised) edition. Pp. 58+4 plates. (Greenford, Middx.: Tin Research Institute, 1963.) [216

Department of Scientific and Industrial Research: National Chemical Laboratory. The Determination of Uranium and Thorium—Handbook of Chemical Methods for their Determination in Minerals and Ores. Pp. vii+43. (London: H.M. Stationery Office, 1963.) 3*s.* 6*d.* net. [216

Journal of the Association of Public Analysts, Vol. 1, No. 1. Pp. 1–24. Published quarterly. Annual subscription 30*s.* (London: Association of Public Analysts, 1963.) [216

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Soil Survey Research Board. Soil Survey of Great Britain. Report No. 15, 1962. Pp. iv+48. (London: Agricultural Research Council, 1963. Obtainable from H.M. Stationery Office.) 5*s.* net. [216

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Department of Scientific and Industrial Research: National Physical Laboratory. Notes on Applied Science, No. 33: Turbulence Measurements with Hot Wire Anemometers. By P. Bradshaw and R. F. Johnson. Pp. 68+4 plates. (London: H.M. Stationery Office, 1963.) 5*s.* net. [216

Department of Scientific and Industrial Research. Building Research 1962: The Report of the Building Research Board with the Report of the Director of Building Research. Pp. v+102+16 plates. (London: H.M. Stationery Office, 1963.) 8*s* 6*d.* net. [216

The Finance of Education: Report of a Conference organized by the 1963 Campaign for Education. Pp. ii+34. (London: 1963 Campaign for Education, c/o Hamilton House, Mabledon Place, W.C.1, 1963.) 2*s.* 6*d.* [216

The Wellcome Trust. Fourth Report covering the period 1960–1962. Pp. 72+6 plates. (London: The Wellcome Trust, 1963.) [266

Commonwealth Mycological Institute, Kew. Mycological Papers, No. 89: Rust Fungi 1: On Acanthaceae. By Geoffrey F. Laundon. Pp. 89. 22*s.* 6*d.* Mycological Papers, No. 90: Beltrania and Related Genera. By K. A. Pirozynski. Pp. 37+2 plates. 10*s.* (Kew: Commonwealth Mycological Institute, 1963.) [266

The International Nickel Company (Mond), Ltd. Facts About Ni-Resist Austenitic Cast Irons. Pp. 8. (London: The International Nickel Company (Mond), Ltd., 1963.) [266

The Royal Institute of Chemistry. Register 1962. Pp. xxii+679. (London: The Royal Institute of Chemistry, 1963.) [266

Institute of Economic Affairs. Hobart Paper, No. 21: Freedom for Fuel. By Georg Tugendhat. Pp. 72. (London: Institute of Economic Affairs, 1963.) 7*s.* 6*d.* [266

Report of Symposium on Television in the Medical School. (Symposium arranged by the Association for the Study of Medical Education and the Scientific Film Association at the London Hospital Medical College, London, E.1. 10–11 May, 1961.) Edited by Charles Engel and John Ellis. Pp. v+73. (London: Association for the Study of Medical Education, 1963.) [266

Science in Sixth-Form General Education. (Science and Education, Part 2, Section 3. A Section of Part 2 of the Science and Education Report issued by the Association for Science Education (formerly the Science Masters' Association and the Association of Women Science Teachers). To be read in conjunction with the Policy Statement and the other three sections of Part 1, on Biology, Chemistry and Physics in the Grammar School Science Course.) Pp. 62. (London: John Murray, 1963. Published for the Association for Science Education.) 2*s.* net. [266

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New Zealand. Air Department: N.Z. Meteorological Service. Misc. Pub. 115 (1): The Climate and Weather of the Bay of Plenty Region. By J. F. de Lisle and I. S. Kerr. Pp. 12. (Wellington: Government Printer, 1963.) [216

United States Department of the Interior: Geological Survey. Geophysical Abstracts, No. 195, April, 1963. By James W. Clarke, Dorothy Vitaliano, Virginia S. Neuschel and others. Pp. iii+287–382. 35 cents. Geophysical Abstracts, No. 196, May, 1963. By James W. Clarke, Dorothy V. Vitaliano, Virginia S. Neuschel and others. Pp. iii+383–476. 35 cents. (Washington, D.C.: Government Printing Office, 1963.) [216

New Zealand Forest Service: Forest Research Institute. Forestry Research Notes. No. 33: The Effect of Certain Water-Borne Preservatives on the Use of a Resistance-Type Moisture Meter. By J. A. Kininmonth. Pp. 12. No. 34: The Effect of Pruning on the Resinification of Knots on Radiata Pine. By J. Maddern Harris. Pp. 7. Report of the Forest Research Institute, Rotorua, for the period January 1 to December 31, 1962. Pp. 86+9 plates. (Wellington: Government Printer, 1962 and 1963.) [216

Struktur und Dynamik von Auenwäldern. Von Hans Heller. (Pflanzengeographische Kommission der Schweizerischen Naturforschenden Gesellschaft. Beiträge zur Geobotanischen Landesaufnahme der Schweiz, Heft 44.) Pp. 75. (Bern: Verlag Hans Huber, 1963.) 15 Sw. francs; 15 D.M. [216

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U.S. Department of Commerce: National Bureau of Standards. Technical Note No. 185: Lectures on Ion-Atom Collisions. By M. R. C. McDowell. Pp. iv+66. (Washington, D.C.: Government Printing Office, 1963.) 40 cents. [266

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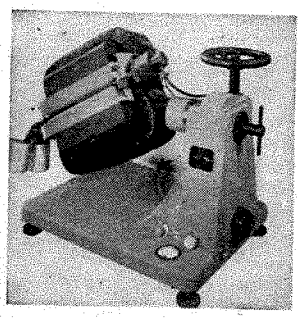
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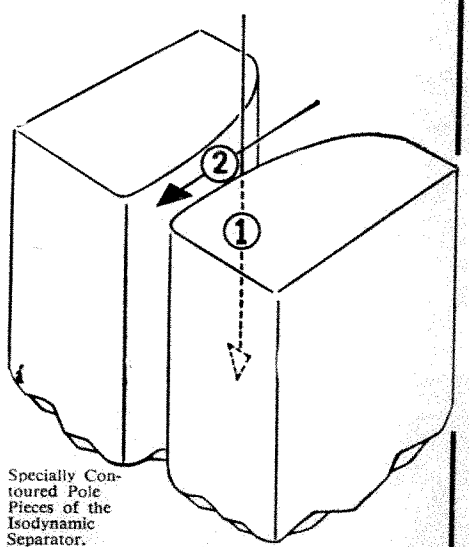
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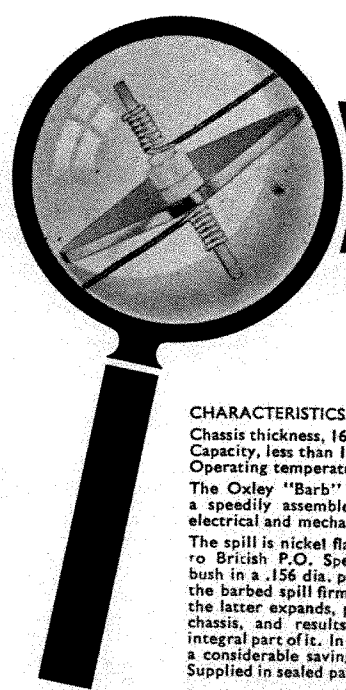
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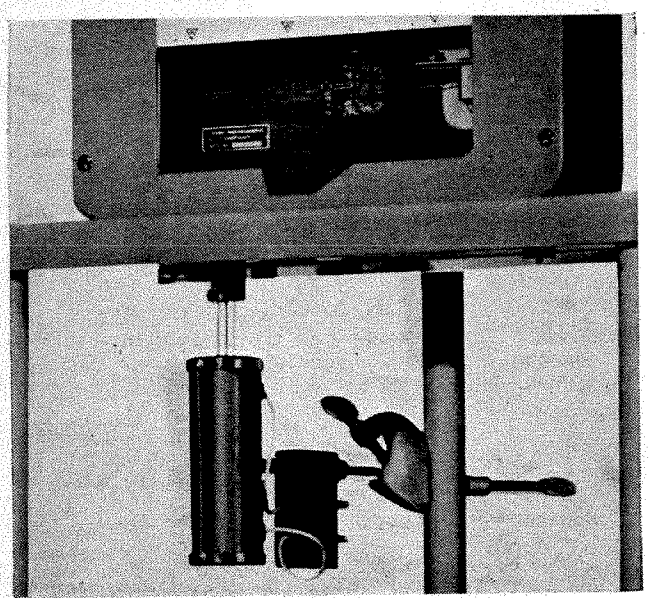
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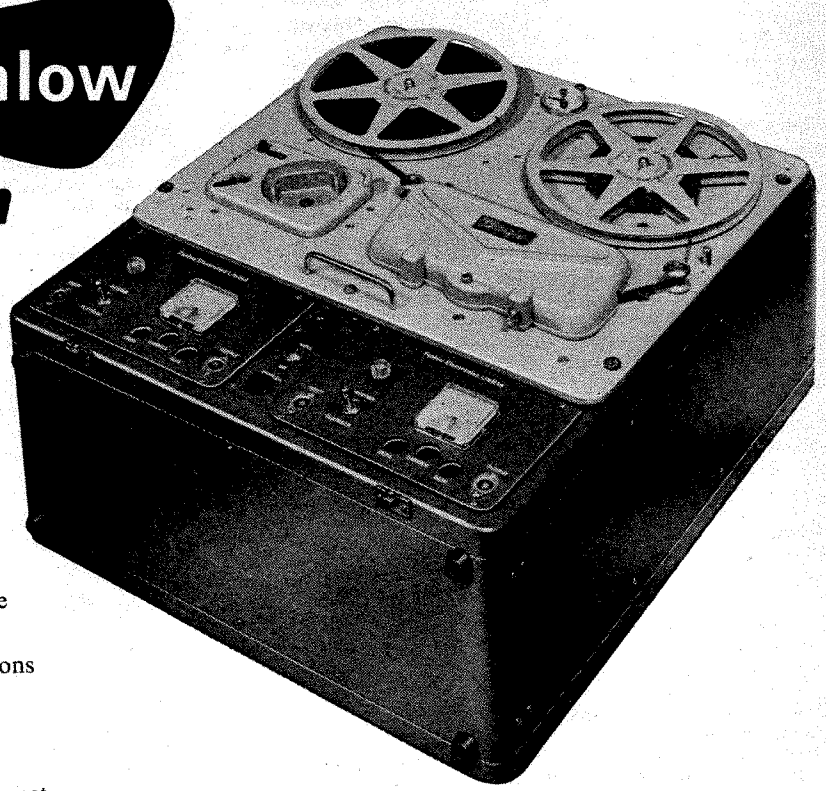
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ENCOURAGEMENT OF SCIENTIFIC RESEARCH IN BRITAIN

THE debate in Committee on Supply on the Vote for the Office of the Minister for Science in the House of Commons on July 15 covered a wide range of topics and brought some important statements from the Parliamentary Secretary for Science, Mr. D. Freeth. There were, however, three main subjects: the loss of scientific manpower by emigration; the stimulation of industrial research; and the Government organization for the development and control of research. All three were raised by Mr. R. H. S. Crossman in opening the debate, but neither he nor subsequent speakers, who referred to the recent report on the first subject by the Royal Society, added anything substantial to that report. Mr. Crossman urged that the important thing was to rectify any deficiencies in our universities and system of higher education which encouraged excessive emigration—it was never disputed that some emigration and immigration were desirable, and he claimed that an average increase in net expenditure on research in the universities over the next three years of not more than £30–35 million would suffice to create a completely new climate. Of this amount, £17 million would be for capital and £7 million for current expenditure and £8 million on grants to the research councils.

On this question of research expenditure, after pointing out that expenditure on university building had increased ten-fold in the past six or seven years, Mr. Freeth announced that in the next academic year the Research Councils would add to their postgraduate awards to students undergoing research training an annual sum of £200 per student, payable to the university department concerned, as a contribution to incidental training costs, such as provision of equipment. This annual sum of about £800,000 would, he believed, considerably relieve the shortage of funds for such purposes in the science departments. Mr. Freeth said that the Government was also considering extending the existing arrangements which enable certain scientists to take doctorates and who wish to pursue research abroad for a year or two, to apply first for certain appointments, such as research fellowships in the United Kingdom so that these can be taken up when they return from overseas. He believed that an extension of the present practice might well make scientists less inclined to accept offers of permanent employment when they were overseas. He also pointed out that last year the joint interviewing board of the Atomic Energy Authority and the Scientific Civil Service in North America interviewed nearly 250 people, and 86 appointments were filled compared with 56 in 1961.

That the unsatisfactory conditions available for research in Britain, and the uncertainty of resources or opportunity rather than salaries are the determining factor in the emigration of scientists was fully recognized in the debate, and this is confirmed by a report on chemists' salaries in the United States which Dr. H. Braunsberg has contributed to the July issue of the *Journal of the Royal Institute of Chemistry*. Dr. Braunsberg starts from the Royal Society's report and from a survey made by the National Science Foundation. He points out that while remuneration is an important factor so far as chemists are concerned, a man earning £1,000 or less in the United Kingdom and holding a similar position in the United States would find his increased net income largely absorbed by the higher cost of living and of medical care and insurance. At all levels, however, a man with no dependants is likely to be able to afford a higher standard of living than in Britain. As his income increases, so the potential net income in the United States over and above such expenses increases at a considerable rate. This, of course, applies particularly to the abler men whose loss causes the most disquiet, and the improvements in position, status and facilities add further incentive to leave Britain.

Nevertheless, the Government action is on the right lines, though whether it is swift enough or on a sufficient scale may be a matter of opinion. Dr. Braunsberg himself remarks that the cost of narrowing the gap between the standards of living attainable on both sides of the Atlantic sufficiently to make emigration less attractive may not be as large as is often supposed, but with any such action must go improvement in the facilities and opportunities available in Britain. It is at this stage that, as Mr. Crossman recognized, the problem is linked with the scale and organization of Britain's research effort generally, and Mr. Crossman strongly supported the granting of full university status to the colleges of advanced technology, for example. He also wanted more Government stimulus to research in industry.

Mr. Freeth, in this connexion, pointed out that national expenditure on research and development had risen from £300 million in 1955–56 to £634 million in 1961–62, or from 1·7 per cent to 2·7 per cent of the gross national product. Expenditure on research financed by Government civil departments and the Atomic Energy Authority had risen in the past three years from £64 million to just over £110 million; that financed by the research councils from just under £18 million in 1958–59 to just over £29 million in 1961–62, and expenditure on research in the universities and technical colleges from just over £23 million to just over £32 million. He also referred to the opening on July 12 at the Building Research Station of a new physics laboratory, costing more than £400,000, which would permit, *inter alia*, increased research on the problem of noise in buildings, for which purpose it includes a unit of adjoining anechoic and reverberant chambers unique in Western Europe.

Mr. Freeth then mentioned the work of the research associations, but while he agreed with the comment in the report of a Working Party of the Federation of British Industries, *Civil Research Policy**, that some amalgamations of research associations might be desirable, he made no specific reference to the report of the Industrial Grants Committee of the Department of Scientific and Industrial Research included in *Research for Industry 1962*, published in the previous week. He announced the merger of the Whiting Research Association and the Chalk, Lime and Allied Industries Research Association, and suggested that an alternative line of development of the smaller associations could be seen in the efforts of the British Hat and Allied Felt Makers and the Gelatine and Glue Research Associations to develop in international centres of technological research. He also saw a future for research associations of focal points of research in existing fields.

All this indicates a loosening of organization and a flexibility which could facilitate a re-shaping of research

* *Civil Research Policy: a Report of an F.B.I. Working Party.* Pp. iii + 11. (London: Federation of British Industries, 1963.) 2*s.* 6*d.*

associations by technologies rather than by industries, though no speaker in the debate referred to the suggestion which Mr. Holliday made to the British Association in Manchester last September. Nor is the suggestion noted in the report of the Industrial Grants Committee, though that report, and also some of the progress reports from the research associations the terms of grant of which come under review during the year, contains evidence of new thinking and a readiness to consider reorganization. The merging of the British Whiting Federation and the Chalk, Lime and Allied Industries is forecast by the Industrial Grants Committee in a section of the report entitled *Amalgamation of Research Associations*, which also describes the formation of the British Ship Research Association out of the British Shipbuilding Research Association and the Parsons and Marine Engineering Turbine Research and Development Research Association. There is also a reference to a new arrangement designed to ensure closer co-operation among the eight associations concerned with research on textiles.

Nevertheless, there seems to be considerable opportunity in this direction. Of the nine associations the terms of grant of which were renewed during the year, only one, the British Iron and Steel Research Association, has an income exceeding £1 million, and of the others only that of the British Coal Utilization Research Association and the British Ship Research Association exceed £400,000. The British Ceramic Research Association falls just short of £300,000, four are between £100,000 and £200,000, and one less than £20,000. It is scarcely surprising that with efforts on such scale the value of the research associations is questioned and the need for co-operation or for fundamental reshaping of the basis of organization pressed.

To this issue the Industrial Grants Committee in this report gives no further attention, though it remarks of the scheme for special assistance to industry, set up in 1959 for five years to help research associations increase the efficiency with which the results of their research were passed to industry and used, that it seems to have increased awareness in many sections of industry of the value of co-operative research and its application to industrial practice.

If *Research for Industry 1962* was ignored in the debate, the Federation of British Industries report, *Civil Research Policy*, was fully quoted in reference to the research associations. Mr. Freeth said that he agreed with much that appeared in the section of the report dealing with research associations, though he was concerned that the report appeared to overlook the use which was already being made in the civil field of development contracts, three of which the Department of Scientific and Industrial Research had already been authorized to conclude. Addressing industry, the Working Party expressed the view that industry in general and the medium and smaller firms in particular had still much to gain from the proper use and development of research, but while sympathizing with the smaller firm in not being able to afford much, if any, scientific staff of its own, they regret that so many seem to fail to use the help available in the research associations. It stressed the importance of all heads of companies becoming fully research minded and according adequate status to those responsible for research and development in their business.

The greater part of the report, however, is specifically addressed to the Government, and here the report comments on the slender resources of some research associations, suggesting that methods of financing may need recasting. It admits that the opinion in industry is not universally favourable to research associations and suggests that the time may be ripe for a critical reappraisal. Besides possible amalgamations, it suggests, rather on the lines of the Zuckerman report, grouping a number on a campus to facilitate sharing of services and costly equipment. It does not, however, regard the research associations as promising media for a large increase in civil applied research and development.

Apart from recommending the wider use of development contracts, the Working Party also suggests that there are possible advantages in rationalizing the work of Government research establishments so as to concentrate civil work in fewer centres. It commends the relations with industry developed by the Royal Aircraft Establishment as promoting invaluable cross-fertilization of ideas and mutual confidence, and suggests that there are possibilities of developing comparable methods on the part of other research establishments. The fundamental problem is to identify the general areas or industries offering the most promising prospects and then setting teams to work, partly in Government research establishments and partly in private companies. The method of payment it suggests is by the allocation of grants-in-aid towards research and development projects in the selected fields, and while some special methods of monitoring may have to be devised, and the contractors should expect to bear some share of the cost, the Government should be ever sensitive about possible advantages which might accrue to the company.

The Working Party's objective in making these proposals is to encourage the new technologies, and it urges that the Government must face the possibly invidious task of selecting the most suitable firm on the basis of the best chance of success scientifically and commercially. It visualizes in all a contribution of some £50 million from State funds to secure the research and development effort needed, with another £50 million from industry, this representing altogether a 25 per cent increase on industry's current spending over perhaps three or five years. This figure seemed to be accepted as reasonable in the debate, but although, as already noted, the proposals made in this section of the Working Party's report were welcomed by the Minister for Science, there was little detailed consideration of them in the debate.

There were indeed one or two further suggestions. Mr. A. Albu, who, like Mr. Crossman, referred to the importance of making wider use of the defence research establishments as expenditure on defence research fell, thought that some industrial research establishments might be used in this way and that such development contracts might be a better method of supporting such associations, particularly those covering a number of industries. Mr. Albu also suggested some development contracts might go to one or two of the colleges of advanced technology, particularly when they were located on the perimeter of an industrial estate. Mr. Aubrey Jones went even further and suggested that some research contracts might go to the universities. Most university research was already concerned with application and he thought that without disturbing the pattern of university research from outside it should be possible to make the connexion with application a little closer and more direct.

Much of Mr. Aubrey Jones's stimulating speech, however, was concerned with the organization of Britain's research effort and, like Mr. Albu, he made two or three suggestions which should be seriously considered. Mr.

Freeth admitted the importance of this aspect of the subject, which was also discussed by the Working Party of the Federation of British Industries. He pointed out, however, that the Government was still awaiting the report of the Committee sitting under the chairmanship of Sir Burke Trend. When this Committee reported in the autumn, the Government also expected to have before it the report of the Robbins Committee on Higher Education, and with the report of the Fielden Committee on engineering design expected later in July, after the debate, the Government would be better placed to consider the deployment of Britain's scientific effort and the appropriate improvement in its own organization.

Meanwhile, Mr. Freeth expressed concern at the insufficient number of technologists trained in Britain. In spite of an overall shortage of university places, the majority of the vacancies each October were in the engineering departments, and the percentage of failures in engineering was higher than in other subjects. This was a disturbing situation and the Department of Scientific and Industrial Research had this year offered an additional 100 research studentships in technology, while in the academic year beginning in October the number of awards for advanced technological training would be increased by 190, at a cost in a full year of £100,000.

Admittedly, the delay in the appearance of the report of the Trend Committee was a severe handicap to the debate, and neither Mr. Freeth nor Mr. Carr, for the Government, could have been expected to comment in detail either on the proposals advanced by Mr. Albu and Mr. Aubrey Jones, or on the Federation of British Industries report regarding the realignment of the responsibilities of the Ministry for Science, or the general organization of civil research under the Government. Much that was suggested develops ideas foreshadowed in the Zuckerman report, but nevertheless Sir Harry Legge-Bourke was not unreasonable in suggesting some acceleration of the appearance of the Trend Committee's report. Meanwhile, particularly in respect of the research associations, there seem to be specific opportunities which should be explored and suggestions which could usefully be examined in advance of that report. No excuse should be afforded for further delay in action once that report appears; and the debate on July 15 did not establish confidence that the Government is sufficiently awake to the seriousness of the whole situation and its urgency and prepared for early action once the full information it is seeking is laid before it.

CATALYSIS QUANTIFIED

Advances in Catalysis and Related Subjects
Vol. 13. Edited by D. D. Eley, P. W. Selwood and Paul B. Weisz. Pp. xiv+458. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 107*s*. 6*d*.

THE first volume of this series appeared soon after the close of the Second World War by which time it was apparent that a world-wide sprinkling of frustrated physical chemists had not only read the signs in the physics of the 'thirties, but also had attacked the central problems of heterogeneous catalysis anew. This very proper exercise directed attention to the properties of the formerly neglected partner in heterogeneous catalysis—the solid—and quickly procured the adoption of the notions of solid-state theory.

In a preface to Volume 13 of *Advances in Catalysis and Related Subjects*, P. B. Weisz remarks that catalysis, dealing with the re-arrangements of many electron atoms, is co-extensive with all chemistry and unlikely to be understood with one-electron theories devised primarily to describe the motion of electrons within solids. It is noticeable that even physicists, to force advances in metal physics with the aid of semi-empirical models, have recently resorted to the chemical ideas originated by Pauling in 1938.

Catalysis, including chemisorption, remains obstinately within the domain of chemistry and no one, outside of industrial chemistry, was more aware of this than the late Prof. W. E. Garner, to whom D. D. Eley here pays a short tribute. Garner, in all his widespread interests, remained uncommitted to schools; in time he overlapped pre- and post-quantum chemistry; in disposition and training he stood between empiricism and rationalism, while at Bristol his colleagues included on one hand Travers and Maxted, and on the other Mott and the physicists of the famous solid-state group.

F. S. Stone, who was a pupil of Garner's, contributes from Bristol a valuable article (50 p.) on the chemisorbing and catalytic properties of the oxides of the transitional metals of the first long period; acidic oxides such as alumina and the silica-aluminas are not included. He directs special attention to the thermochemistry of chemisorption, an important, experimentally difficult but recently much neglected topic, and provides a timely introduction to the more important work on photosorptive effects. The subject is developed, practice and theory, from the chemical attitudes of the 'thirties through the more physical approaches ('the electronic factor') of the forties and fifties and concludes with a section, again in more chemical vein, linking photosorption with crystal-field theory. It is to be hoped that this cycle generates an ascending helix rather than a vicious circle. Certainly the post-war period effected the belated juxtaposition with solid-state physics and an associated extension of technique and concept, but the boundary-layer theory, with its consequent subdivision of adsorbates and reactions into 'donors' and 'acceptors' of electrons, and other formalized theories are seriously lacking in chemical content. Stone brings out their value in the systematization of the surface chemistry of simple reactions involving strongly electronegative species such as oxygen but, as he implies, much more is needed to provide an understanding of processes as complex as, say, the ammoxidation of olefines. Whether ligand-field theories, the theoretical bases of which are at present undergoing a careful re-examination, will assist remains to be seen.

The effects of radiation on the catalytic activity of solids are not only of great technological importance, their study may also illuminate the crux of catalysis by solids—the nature of the interplay between valencies and defects in surfaces. R. Coekelbergs, A. Crucq and A. Frennet provide a painstaking account (79 pp.), based largely on their own work, of systems in which both the microporous solid and the gaseous reactants are simultaneously irradiated with either uranium fission fragments, gamma rays or beta rays. It is difficult to present a coherent report of this piecemeal and still infant subject, but the authors have knitted the fragments together with a short review of results by other workers and a longer survey of the generation and the characteristics of the changes produced in solids by various radiations.

"Radiation catalysis" has been demonstrated but, as the authors are aware, the uncertainty in the G values make further substantiation desirable, for example, by a detailed study of the effect of variation of surface area for one reaction. The high efficiency with which secondary electrons are formed by absorbed γ-radiation suggests that these electrons might also be included as a means of transfer of energy from solid to gas.

Random distributions of surface defects are undoubtedly important in surface chemistry, but L. H. Germer reminds us ("A New Electron Diffraction Technique, Potentially Applicable to Research in Catalysis", 9 pp.) of the power of an old technique in new guise—the diffraction of slow (0–500) electrons—to detect the presence of regular arrays of surface atoms and adatoms. Germer provides examples which should stimulate a wider interest in this erstwhile difficult technique, recently used so successfully by Farnsworth at Brown University.

"Radiation catalysis" tackles the complexity of the solid catalyst, "Polyfunctional Heterogeneous Catalysis", by P. B. Weisz, attacks the complexity of the reaction catalysed, and in 52 pages contributes a paper which is, to the catalyst designer, the most valuable in the volume; it should be compulsory reading for industrial chemists. The notions of the virtual mechanism and the polyfunctional catalyst are, of course, not new. They presume that (*a*) a complex process can be reduced to a limited set of reactions of known types, taken in appropriate sequence, (*b*) solids exist possessing the capacity to speed each reaction of the set, and (*c*) a mixture of these solids, properly compounded, will catalyse the overall process. After the mechanism has been chosen (*a*) the secret of success lies in (*c*), and it is here that Weisz and his colleagues, following other American workers concerned with new petroleum-reforming processes, have done much to provide scientific albeit qualitative, rules and practical demonstrations of them. The important advance lies in the realization and rough quantification of the part played by the diffusion of intermediates between the microporous phases of different specificity and the relation of this diffusion rate to overall and partial reaction rates. Together with our knowledge of the variation of catalytic activity in the Periodic Table, this model rationalizes the behaviour of complex catalytic processes and permits a comprehensive description of them which, as Weisz shows, may be extended to include biochemical reactions.

J. Wei and C. D. Prater have pushed the formal study of such complicated processes still further in a lengthy (186 pp.) mathematical contribution on "The Structure and Analysis of Complex Reaction Systems". The subject is introduced with a solution to the difficult practical problem of deriving accurate rate constants from the usual composition-time data for a reaction involving a group of coupled, reversible monomolecular reactions between species A_i. It is shown that the differential equations representing the rate of change of concentrations with time can be transformed into an equivalent set representing a group of uncoupled monomolecular reactions based on a set of hypothetical species B_i. Moreover, the transformation from a composition in the A system to one in the B system can be found from appropriate data so that the rate constants for the B system can be determined and then converted to the rate constants for the A system by the same transforms. These methods are afterwards extended to irreversible monomolecular systems and to certain other types of catalysed reaction; great care is taken to emphasize the practical value of the analysis in the treatment of data, and worked examples are given in detail.

Matrix algebra is used throughout and although this is interpreted, where possible, with geometric constructions and supported by four appendixes, it makes heavy going for the chemist. Yet the principles should be mastered and require only practice to become familiar as a numerical method. The deeper levels, those sections which expose the non-thermodynamic aspects of the equilibrium point and seek links with irreversible thermodynamics through Liapunov functions, require intensive study.

This is a valuable analysis of a subject which is beginning to stimulate interest again and chemists are lucky to have such a full account at their disposal. It is something of a relief to turn to chemistry with A. Farkas and G. A. Mills, who conclude this volume with an able, 50-page review "Catalytic Effects in Isocyanate Reactions". This is homogeneous, solution chemistry and it deals with the polymerization of ioscyanates and with the catalysed and uncatalysed reactions of isocyanates with compounds containing active hydrogen: these reactions are essential to an understanding of the rapidly expanding technology of polyurethane foams. In a period when the similarities between homogeneous and heterogeneous catalysis are becoming clearer, and the essential mechanism of one may ultimately be shown to apply to the other, 'all is grist to the mill' of the catalytic chemist.

This volume retains the original format and is as pleasant to handle and to read as its precursors. The scientific content remains high and will satisfy diverse interests although it presents an increasingly difficult task to the reviewer.

D. A. Dowden

THE UPPER ATMOSPHERE

Physics of the Upper Atmosphere

Edited by J. A. Ratcliffe. Pp. xi+586. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 14.50 dollars.

Proceedings of the International Conference on the Ionosphere 1962

Held at Imperial College. Pp. ix+528. (Published by: The Institute of Physics and the Physical Society; Distributed by: London: Chapman and Hall Ltd., 1963.) £5 5s.

THREE years ago there was published in New York and London (Academic Press Ltd.) a volume entitled *Physics of the Upper Atmosphere*, edited by Mr. J. A. Ratcliffe, who was then at the Cavendish Laboratory, University of Cambridge, where a school of scientific research on the ionosphere was established by Sir Edward Appleton some forty years ago. This book is essentially a collection of eleven monographs written by authors of international repute in the various branches of the subject in which they have worked. As the chapter headings indicate, the subjects dealt with cover the upper and outermost portions of the Earth's atmosphere with particular reference to the ionosphere; the influence of the Sun's ionizing radiations; the investigation of airglow, aurora and meteors; and the relationship between the upper atmosphere and geomagnetism.

As stated in the preface, the physics of the upper atmosphere was being intensively examined during the period (July 1957–December 1958) of the International Geophysical Year, when the book was being written. A twelfth chapter was therefore added to enable individual authors to supplement their accounts by the inclusion of information available up to December 1959, when the book went to press. With the passage of time, the value of the book is in no way diminished, and it forms an authoritative guide for research workers engaged in any field of study related to upper atmospheric physics.

Those who require more advanced and up-to-date knowledge of the ionosphere will find excellent material in the second volume under review. This book comprises some seventy-five individual papers presented and discussed at an International Conference on the Ionosphere held at the Imperial College of Science and Technology, London, during July 2–6, 1962, under the auspices of the Institute of Physics and the Physical Society. The Conference was divided into four sections dealing with the following subjects: "Ionospheric Constitution and Ionizing Radiations"; "Geomagnetism and the Ionosphere"; "Irregularities and Drifts in the Ionosphere"; and "The Mathematics of Wave Propagation through the Ionosphere". The fifth, and concluding, section is entitled: "Preliminary Results from the First Anglo-American Satellite UK1, Ariel". This contains

three papers in which authors working on part of the programme of the British National Committee for Space Research describe some preliminary results obtained from the above satellite, which is variously referred to as *UK1* or *S51* as well as Ariel. The initial orbit parameters of this satellite were perigee 389 km and apogee 1,210 km: and the three short papers outline the preliminary results obtained in X-ray spectrometer measurements, in plasma probe experiments, and some preliminary electron (not election as printed) density measurements above the *F2* region of the ionosphere.

Reverting to the main sections of the volume, each of these opens with a review paper by an invited speaker at the Conference, who at the end of the section also contributes a summary of the papers presented and the resulting discussion thereon. It is clear from the list of authors and the titles of their individual papers that the Conference was successful in bringing together a large number of research scientists from various parts of the world; not only from Europe and North America as might be expected, but also from Africa, Australia, India, Japan and New Zealand, where there is clearly an active interest in the general characteristics of the ionosphere, and their association with radio-wave propagation.

A possible criticism of the publication is that no less than seven of the contributions are merely abstracts. No reference is given as to whether the relevant full papers exist and, if so, where they will be published. With the exception of one which is from the United States, these abstracts are from authors in the United Kingdom and five of them are from a well-known research laboratory. This is perhaps a little unfortunate, as it might give an impression of favoured treatment against overseas authors, all of whom, with the exception mentioned, seem to have produced their full papers in time for the Conference, or at least for the publication of its proceedings.

Apart from this comment, the production of the book and its clear text and diagrams, the arrangement of the contents and the author index are excellent in every way. The organizing committee and in particular the Editor of the Institute of Physics and Physical Society are to be congratulated on the production of this volume and, together with the publishers, for its issue within ten months of the holding of the Conference.

These two volumes taken together comprise an exhaustive and up-to-date work of reference to the physics of the ionosphere, which can now be explored by direct measurements carried out in satellite vehicles.

R. L. Smith-Rose

SPACE AND METEOROLOGY

Proceedings of the First International Symposium on Rocket and Satellite Meteorology

Washington, D.C., April 23–25, 1962. Edited by H. Wexler and J. E. Caskey, Jun. (Sponsored by Committee on Space Research (COSPAR); World Meteorological Organization (WMO); and International Union of Geodesy and Geophysics (IUGG). Pp. ix + 440. (Amsterdam: North-Holland Publishing Company, 1963.) 100*s*.

RESEARCH of any kind in which rockets or satellites are used is now referred to by almost everybody as 'space research'; yet in fact much of this work is concerned not with 'space' as ordinarily understood but with the Earth's atmosphere. At the 1961 Symposium on Space Research organized by the Committee on Space Research (COSPAR), thirty-five per cent of the papers presented were directly concerned with some aspect or other of the atmosphere. Not all these papers were 'meteorological' in the now accepted and restricted meaning of this term. Many were concerned with studies of the electrical properties of the atmosphere (the ionosphere) and others were concerned with the chemical and photochemical processes which occur in the rarified air at very high levels (aeronomy); but a substantial number were concerned with atmospheric densities, temperatures, winds, turbulence and cloud systems and with the radiative processes ultimately responsible for all these things. So when, in the following year, the COSPAR Symposium on Space Research was held in Washington, the late Dr. Harry Wexler proposed that a separate symposium on rocket and satellite meteorology be held immediately before the COSPAR meetings. This volume, now dedicated to Dr. Wexler, is a report of that symposium.

The book has four sections: meteorological rockets; radiation measurements from satellites; cloud photography from satellites; other geophysical observations from satellites. Rocket soundings using grenades and vapour releases are reported on by five countries: the United States (who reports on ten grenade soundings in 1961); Great Britain (with ten grenade soundings over the period 1957–61); and smaller numbers by Japan, Italy and France. The grenade technique gives temperature and winds up to a height of about 85 km. Most of the vapour release techniques are designed to give winds from this level up to heights of 180 km or so, but Prof. Blamont describes an experiment in which temperatures at very high levels are determined from precise spectroscopic measurements on the glow from a sodium release and he concludes that the temperature at 370 km rose by 500 ± 150° K between December 1960 and September 1961. Soundings using rocket-sondes carried by 'small meteorological rockets' are reported by the United States and by the U.S.S.R. The U.S.S.R. measurements were all made during the International Geophysical Year. They extend only to a little beyond 40 km and there is no discussion, or even statement, about the accuracy of the measurements. Two 'cross-sections' are presented, but since these are based on observations from a single ship spread over a period of several weeks, in a region where day-to-day variations are considerable, these cross-sections carry no great conviction. The reports of the American studies do contain a statement (perhaps an optimistic estimate) about the accuracy of the measurements; and charts are presented of results from synoptic soundings from about ten stations over the North American Continent, at levels up to 55 km.

The second section contains three papers on the evaluation of the *Tiros* infra-red radiation data. Two of these are preliminary studies concerned with the interpretation of the data; the third is presentation and discussion of radiation measurements made during a pass over a hurricane. This section also contains one paper on the measurement of solar ultra-violet radiation and some theoretical papers, following familiar lines, on radiation balance problems. The three papers on the *Tiros* radiation data all originate from within the United States; indeed, although the data are readily available to meteorologists of all countries, no studies of these measurements have yet been published anywhere by meteorologists outside the United States. This is almost certainly because instrumental and other difficulties are so great that the present experiments can scarcely do more than indicate the possibilities and emphasize the difficulties. The *Tiros* cloud photographs, on the other hand, being of excellent quality, can be studied by anyone, without resource to an electronic computer, and there are, in Section 3, papers on this topic written by meteorologists of five countries. The novelty of clouds photographed from satellites has largely worn off and all these papers reflect the hard grind which now faces meteorologists who have to learn how to recognize what is significant and, when recognized, how to interpret and use it.

The short final section contains two factual papers describing the determination of snow and ice cover from *Tiros* photographs; and a paper presenting the results of the determination of atmospheric densities at high levels

from an examination of the changes of satellite orbits (leading to what may turn out to be a rather optimistic claim that "the general features of the air density distribution between about 180 and 700 km have now become well established").

In all, the volume contains a comprehensive account of observations and studies made during 1961 (except that only a little Russian work is included and that refers to the International Geophysical Year). Almost all the papers are in English with a Russian summary. Since practically everything has been published elsewhere it is particularly to be regretted that nothing is reported of the discussion which followed many of the papers.

R. Frith

THE ART OF PAPER ELECTROPHORESIS

Hochspannungselektrophorese ihre Anwendungsmoglichkeiten für Biochemische und Klinisch-chemische Trennprobleme

By Dr. Med. Roman Clotten, Dr. Med. Annemarie Clotten. Pp. xv+556. (Stuttgart: Georg Thieme Verlag, 1962.) DM.98

ELECTROPHORESIS on rigid substrates such as moistened filter paper has become one of the more important methods for the identification and separation of compounds, rivalling chromatographic methods. This is not because the technique in itself is more versatile, there only being a limited number of *p*H values which can be used profitably, even in the presence of complexing agents, such as borate or sulphite, but because it is rapid and because it permits a fairly ready interpretation to be made of the nature of the compounds which are being investigated, or, alternatively, a confident prediction of the possible behaviour of unknown compounds.

Rapidity, of course, means the utilization of high voltages, or more properly high-voltage gradients, and this in turn involves the real danger of electrocution, which is not stressed in this text-book, and also the problems of the dissipation of heat and of the expense of the apparatus, both of which increase out of proportion to the voltage. The dissipation of heat is indeed a major problem, and of the two current solutions the authors prefer contact cooling by solids to cooling by immiscible liquids, a choice which is probably to be commended both because of the danger of fire and of the toxicity of some of the fluids used for cooling, but which has to be weighed against other factors such as compactness and general convenience.

An increase in voltage is not only associated with a speed of separation of small molecules which is actually somewhat greater than the voltage increase, largely because of diffusion effects, but it has also become associated with laboratory prestige, possibly because of the aura of danger accompanying high voltages, and this seems to have become a motivating influence with some workers.

Clotten and Clotten have written their excellent text-book with what is undoubtedly a background of considerable experience, largely of the clinical type, and they have endeavoured to make a comprehensive gift of their technical skill to the reader, together with all the background information which they consider he should need. Indeed, they tabulate buffer mixtures, spray techniques, sizes and properties of filter papers, including, incidentally, useful tables of the residual ion-exchange behaviour of commonly used papers and other factors, some relevant, some not. They also describe hydrolysis, concentration and desalting techniques though they omit, when discussing the latter, a particularly important caution to remove those ions common both to the specimen and the buffer because of their relative lack of mobility on subsequent electrophoresis, a phenomenon often puzzling to the inexperienced.

Another strange omission, particularly when one considers the thorough descriptions of eccentric elution techniques and other minutiæ, is the authors' failure to give any attention, apart from one specialized instance, to the problems of the power supplies needed and their construction. These constitute perhaps the most expensive items to purchase but are not at all difficult to build in the laboratory at reasonable prices, particularly now that silicon rectifiers are available.

The greater part of the text is given up to the analytical procedures to be adopted for the investigation of groups of compounds such as sugars, amines, etc., both from a qualitative and from a quantitative point of view, and these are excellently documented, and are illustrated by photographs, both in black-and-white and in colour, most of which are quite excellent, and if one may say so, a welcome change from the dreadful examples which one sees so often in print.

The book is excellently produced on good quality paper with an enormous bibliography and comprehensive index, and can be recommended to anyone interested in these methods who does not mind reading German and has 98 DM. to spend.

Roy Markham

HUMAN GENETICS

Outline of Human Genetics

(Contemporary Science Books). By Prof. L. S. Penrose. Pp. xiv+166. (London: Heinemann Educational Books, Ltd., 1963, second edition). 15*s*.

THIS book is precisely what it purports to be, namely an outline, but it has managed to cover a very wide field because of admirable economy in words. Throughout, Prof. Penrose deals with just those points which are of general interest and particularly topics about which people ignorant of genetics are always asking, for example, Is natural selection still operating in spite of civilization and medical advances?

He starts with a historical survey of the subject—the story of Mr. Scott's colour-blindness in 1777 is most attractive—and then goes on to explain the essential differences between segregating and non-segregating characters. With respect to height (which has undergone such changes) it does, however, seem strange that the inheritors of Galton's laboratory cannot give us any more up-to-date estimate of the correlation coefficient between parent and child than that of 1890.

In "Genes and Populations" good use is made of tables and diagrams, and Penrose clearly realizes the difficulty that a beginner has in understanding the Hardy–Weinberg law—and, arising out of it, such subjects as heterozygous advantage and the results of inbreeding. "Association and Linkage" is for the most part conventional, but the puzzles of the Rh system are interestingly presented and help to introduce readers to the idea of geographical variation in an inherited character.

Not only beginners would benefit from "Environment and Heredity" but also all those (and there are many of them) who accept the magic formula of 'identical twins brought up apart' as the solution to any nature–nurture problem. After reading Penrose's survey they will think again.

Penrose delights in being the bane of the 'planners' in "Eugenics and Dysgenics" and is deliberately provocative with remarks such as the "heterozygous mongrel group can indeed be the genetical backbone in a human population even if the members of this group may theoretically appear to be imperfect specimens".

Practically the only new feature in this second edition is one additional chapter ("Commentary") in which he

discusses some of the advances made since 1959. These could with advantage, though doubtless with a good deal of extra trouble, have been incorporated earlier, where they belong, and what is coming in the final section should surely be referred to in the original chapters. In "Commentary" he explains in more detail how common chromosomal abnormalities, such as those causing mongolism and intersexes, are produced; mentions theories dealing with the possibility of inherited cancer; touches on pharmacogenetics; and outlines the vast amount of genetic variability which is being shown up by the complicated polymorphisms of the blood proteins. Finally, he makes the very good point that while geneticists are continually worrying about the quality of the human race we shall have doubled our numbers in the next 50 years and that birth control is far more important than the fruitless task of planning the superman.

There is no doubt that readers of *Nature* will find the book lucid, wholly understandable and interesting, for it is written (in beautiful English) by one of themselves. The query is whether he will cast a spell on intelligent non-scientists (particularly the young) who have had no previous knowledge of the subject.

C. A. CLARKE

CHANGED APPROACH TO RACE STUDIES

Races of Man

By Sonia Cole. Pp. 131+12 plates. (London: British Museum (Natural History), 1963.) 11*s.* 6*d.*

BY contrast with the transatlantic situation where several race classifications have emerged in the post-war years and where discussion of the number of races to be identified, if any, is as vigorous as ever[1], the present generation of physical anthropologists in Britain has tended to leave severely alone questions of race classification. Taking a more biological view of race than their predecessors, they have thought it less important to spend time and energy over what can only be arbitrary, the number of races and which human groups they include, and more important to enquire more deeply into the characters in which human populations differ, the ways in which they are affected by heredity and influenced by environment during development, the biological significance of race differences[2]. Thus it is that to-day the frontiers of knowledge in race matters are explored, and the most significant advances made, in departments of biology—biochemistry, genetics, serology, physiology, nutrition, growth, etc. Formerly, however, they lay in the museums, to which there was a continual inflow of skulls, photographs, measurements, etc.; material which had to be maintained in some sort of order, so that there was practical reason for concentration on classification.

That the needs of the museums may latterly have been overlooked is suggested by the emergence from the British Museum (Natural History) of this small hand-book *Races of Man*. It was written to complement the exhibit illustrating the races of man, in which the arrangement of the materials was based on Boyd's[3] original six-fold classification by blood group frequencies. The idea—an attempt at classification based on both genetic and morphological criteria—then was good. But its implementation is unfortunate. Lip service is paid to the genetic approach to race, but little besides. Indeed, were it not for the date on the title-page and some half-dozen pages in Chapters 3 and 4, one might think it had been written in the pre-war decades, so discreet is the acknowledgement of post-war thought.

It would be unfair to compare it with the authoritative introductions to physical anthropology that the British Museum has previously published—Oakley's *Man the Toolmaker* and Le Gros Clark's *History of the Primates*. Its treatment and level of presentation are much more elementary, aimed at schoolchildren and the lay public, as indeed they should be in a companion to an exhibit; but that is why especial care should have been taken in order not to give wrong impressions. It is disturbing, for example, to find so little attention to variability, both in the text and in the sketches and diagrams; so much concentration on 'types', with nowhere a discussion of the validity of that concept; so little note of the continuity of the processes of race formation. One can only hope that those who read this book will also read, say, *Human Races* by S. M. Garn, by way of an antidote.

D. F. ROBERTS

[1] *Curr. Anthropol.*, 3, No. 3 (1962); 4, No. 2 (1963).
[2] Roberts, D. F., and Weiner, J. S., "The Scope of Physical Anthropology and its Place in Academic Studies", *Symp. Soc. Study Human Biol.*, 1 (1958).
[3] Boyd, W. C., *Genetics and the Races of Man* (1950).

FISH AS FOOD

Fish as Food

Edited by Georg Borgstrom. Vol. 2: Nutrition, Sanitation, and Utilization. Pp. xvii+777. (New York and London: Academic Press, 1962.) 178*s.* 6*d.*

THE effects of food depend on the amount consumed, the concentration of nutrients in meals, and the physical state of the person eating it. Recent developments lay emphasis on the central role of protein in determining the nutritional value of foods and on the importance of the proportion of the total caloric value of a diet accounted for by protein, Making allowance for biological value, protein concentration must reach certain minimal levels for the maintenance of health and even of life. For the young child protein of ideal biological value must provide about 8 per cent of the total calories of the diet. Diets of lesser protein content will lead to protein-calorie deficiency disease.

It is generally acknowledged that this manifestation of ill-health is the most important form of malnutrition throughout the world and accounts for enormous amounts of sickness and mortality, especially among children. Prevention of such malnutrition lies to some extent in making available larger amounts of food, but especially in raising the concentration of protein of satisfactory biological value in the diet. Much work is being done in nutrition research laboratories to discover special preparations which could be used as food to prevent protein-calorie malnutrition. There are many problems involved not the least of which are in connexion with costs and distribution on a national scale, and satisfactory results of laboratory tests cannot readily be assumed to ensure success in the field. There is a danger that occupation with the elaboration and use of special foods of high protein content may detract from interest in natural foods which people have used throughout many generations. One difficulty associated with the use of these products, however, is in preserving them so as to allow their wide distribution; another is the relatively high physical and monetary costs in obtaining them.

In this context fish is of special value—it can be procured without using up limited land resources, it is widely acceptable as a food, means of preserving it have been known for hundreds of years; but, above all, many kinds of fish have a very high concentration of protein. For example, 95 per cent of the caloric value of the edible part of cod comes from protein, and many fish have protein-calorie concentrations higher than pork, beef, chicken, eggs, and other foods of high nutritional value. Not only does fish contain much protein, but fish proteins are of high biological value; mackerel promotes more growth in rats than does casein. It is clear that addition of sufficient fish to even the poorest of diets would prevent protein-calorie deficiency disease.

In addition, some fish, or some parts of fish, can supply substantial amounts of vitamin A, iron, fluorine, and iodine—nutrients important in the prevention of abnormalities which at present affect considerable numbers of people throughout the world.

But, fish must be harvested and suitably preserved so as to ensure its availability to those who need it. It is well to know that while new methods such as radiation-sterilization may become important in the future, at present, there is no need for other than methods which are well established such as freezing and canning. Application of them "could satisfy all reasonable needs for years to come".

There are some potential dangers in eating fish. This food may carry a number of organisms pathogenic to man, including salmonellæ and *Clostridium botulinum;* there is the curious condition known as Haff disease in which there is excruciating pain in the muscles, probably caused by ingesting fish contaminated with algae; some people develop allergic reactions after eating some kinds of fish; some fish naturally contain materials poisonous to man. Also, those engaged in the fish industry are exposed to a number of hazards to their health.

These and other important topics are discussed in detail in the second volume of *Fish as Food*. The sea, from which so much of our fish is obtained, has no physical national barriers, and it is appropriate that those who have contributed to this book belong to half a dozen different countries. Under the sub-title *Nutrition, Sanitation, Utilization*, Georg Borgstrom has assembled impressive reviews from several authorities. The editor himself has written a considerable amount of the volume.

The book is interesting throughout, and many parts are fascinating. It would be inadequate, however, to leave its assessment there, because it is also an important book and should be studied by everyone having anything to do with human nutrition.

G. R. Wadsworth

WOOD EXTRACTIVES

Wood Extractives and Their Significance to the Pulp and Paper Industries

Edited by W. E. Hillis. Pp. xvii + 513. (New York and London: Academic Press Inc., 1962.) 114*s.* 6*d.*

As Prof. Erdtman remarks in his foreword to this book, the days are long past when wood was regarded as merely "cellulose and lignin", and it is now generally recognized, not only that woods contain a very great variety of extraneous components, but also that these components are responsible for many of the characteristic features of different timbers. The study of the extractives of wood has developed into an important branch of wood chemistry, particularly within the past 10–15 years, and the time is ripe for a survey of the present state of knowledge. This has now been undertaken by Dr. W. E. Hillis of the Division of Forest Products, Commonwealth Scientific and Industrial Research Organization (Australia), with the aid of a number of collaborators, all of whom are well known for their work in this field.

The book opens with an excellent account of the main structural features of wood and of the processes by which a tree grows. This is essential to an understanding of the part played by the minor components and their location in the tree. Particular attention is directed to the process of heartwood formation which is relevant to the question, discussed in a later chapter, of the origin of heartwood extractives. A short account, which might with advantage have been expanded, is given of the influence of extractives on wood properties and utilization.

After this introduction the remainder of the book is divided into four sections, of which three are concerned with the extractives themselves while in the final section some of the ways in which these components influence the utilization of wood for manufacture of pulp and paper are considered. In the treatment of the extractives the main emphasis is placed on the polyphenolic constituents of wood, which form the subject of seven of the nine chapters in this portion of the book. The polyphenols range in complexity from the simple C_9 and C_{15} compounds (flavonoids and hydroxycinnamic acids) to the highly complex tannins and are of such widespread distribution that it is right that they should occupy a prominent place in any discussion of wood extractives.

Dr. Hillis has performed a valuablo service in bringing together the points of view of the organic chemist, the biochemist and the botanist concerning this group of compounds. In his own contribution on the distribution and formation of polyphenols within the tree he discusses the various theories that have been advanced to account for the origin of heartwood polyphenols, and gives reasons for his own view that they are formed *in situ* from carbohydrates. E. C. Bate-Smith's chapter on the simple polyphenolic constituents of plants includes an account of his very interesting studies of the systematic distribution of these compounds—particularly the leucoanthocyanins and trihydroxyphenolic compounds—in the leaves of plants. The analogous work of Erdtman on the heartwood components of conifers, which is more directly relevant to the present book, receives only very brief mention. Other chapters in this section of the book include well-presented accounts of the chemistry of the lignans and the condensed tannins by D. E. Hathway and of the hydrolysable tannins by L. Jurd. Two chapters are devoted to the biosynthesis of polyphenols and reflect the present-day interest in biosynthetic processes in plants, the study of which has been so greatly assistep by the use of radioisotope tracer techniques.

The succeeding section of the book is entitled "Other Extractives in Wood" and consists of two chapters dealing with tropolones and wood resins respectively. J. A. F. Gardner gives a useful survey of the properties and distribution of the relatively small number of tropolones that have been found in wood, while D. B. Mutton describes the resins occurring in softwoods and hardwoods. The discussion of wood resins is confined mainly to North American timbers and no mention is made of the resinous tropical species, for example, the dipterocarps.

Finally, an account is given of a variety of problems arising in the technical processes of pulp and paper manufacture which are related to the presence of extractives in the wood. These include such diverse phenomena as the inhibition of the pulping reaction by pinosylvins, corrosion of digesters, discoloration of pulp, deposition of scale in digesters and piping, colour changes during storage of newsprint, and pitch problems in pulping. The ways in which these problems are tackled provide a good illustration of the practical value of a knowledge of the chemistry of the extractives concerned.

This book is the first major work on wood extractives that has been compiled and is an important addition to the literature of wood chemistry. Much of its value lies in the varied outlooks of the several authors, who approach their subjects from chemical, botanical, biochemical and industrial angles and yet contrive to blend their contributions into a unified whole. Its greatest weakness lies in its bias in favour of polyphenolic compounds, so that other important classes of extractives, including the terpenes (other than the resin acids) and their glycosides, carbohydrates, and nitrogenous compounds, including alkaloids, are neglected or inadequately dealt with. The production is excellent and the book is particularly well provided with literature references and indexes. It is primarily a book for research workers in this field and will give them much food for thought, but it may also be read with profit by a much wider circle.

R. H. Farmer

Annual Review of Microbiology

Vol. 16. Edited by Charles E. Clifton in association with Sidney Raffel and Mortimer P. Starr. Pp. vii+344. (Palo Alto, Calif.: Annual Reviews, Inc., 1962.) 7 dollars.

IN the introduction to the first volume of *Annual Reviews of Microbiology* the editors expressed the hope that subsequent editions would serve the need of microbiologists of diverse interests by providing summaries of work in many different fields. That the present volume deals only with a limited number of topics is not entirely the fault of the editors, who had planned a more varied list of contents, and it does not necessarily detract from its value to the average microbiologist. The specialization of subject-matter which characterizes this year's *Annual Review* has the advantage that it provides a comprehensive account of the selected topics. Thus four reviews are concerned with aspects of microbial genetics. This very important subject is discussed from various points of view, ranging from descriptions of the sex life of bacteria and *Aspergillus* to an account of the biochemical mechanisms of gene action.

Immunological topics are also given generous treatment in this book. A fascinating contribution on the elaboration of antibodies by single cells gives rise to the hope that those genetical and biochemical methods which have been so successful in studying microbial biosynthesis may eventually be applied to such systems. Such an investigation would require more knowledge than we possess at present of the specialized structure of antibody proteins; much recent progress has been made in this field and this is described in an article on the nature of antibodies and antigens. The effects of specific antibodies on tissue cells are discussed in a review of that title and also in an article on cultural characterization of tissue cells where the use of these specific reactions for the practical purposes of identifying cells is described.

A few reviews on more classical aspects of microbiology help to counterbalance the large number of articles devoted to these two specialized topics. Thus "Endogenous Metabolism of Microorganisms" and "Lipid Requirements of Microorganisms" both provide valuable summaries of information collected from widely distributed sources. A subject of personal importance as well as general interest to microbiologists is described in a survey of the methods for control and purification of water. The difficulties which beset workers with oncogenic viruses are brought out in a very clear account of the complex subject of viral leukæmias of mice.

Although this volume undoubtedly provides reviews of interest to most microbiologists it is to be hoped that next year's edition will show a return to the original editorial policy.

A. NEWTON

Histogenetische Studien an den Blüten Einiger Phanerogamen

Von Dr. Helmut Pankow. (Botanische Studien, Heft 13.) Pp. v+106. (Jena: Veb Gustav Fischer Verlag, 1962.) 14.30 DM.

THIS volume is one of a series, edited by Profs. W. Troll and H. von Guttenberg, which is intended for the publication of investigations requiring more extensive treatment than is usually possible in most botanical periodicals. The present contribution is concerned almost exclusively with histogenetic investigations on the ovary, and in particular on the ovules, in a considerable range of angiosperm species. A few gymnosperms were also examined.

In his introduction to this investigation the author rightly stresses that with regard to the morphological interpretation of the angiosperm flower there have been more speculations than serious attempts to ascertain the available facts. It is also pointed out that although in dealing with a structure so complex as the angiosperm flower no line of evidence should be ignored, there have been relatively few investigations of the development of the ovary or ovule. In his own contribution to this field, the author has now provided us with detailed well-illustrated accounts of the development of the ovules and associated structures in a number of representative species from 23 families of angiosperms.

Although the author's personal investigations constitute the greater part of the work, there is also a concise review of other investigations of gynœcium development. From his own investigations the author concludes that in many families the ovules are clearly stachyosporous, that is, originate from cauline tissue, while in other families they are phyllosporous and originate from the carpels. Too much emphasis is not placed on this distinction, and the author draws an analogy with *Selaginella* in which some species have axillary sporangia produced from stem tissue, while other species bear sporangia on leaf tissue at the base of the sporophyll.

The entire work is a valuable factual contribution to our knowledge of gynœcium development and on that basis is a useful addition to any botanical library.

A ALLSOPP

Life of the Wayside and Woodland

A Seasonal Guide to the Natural History of the British Isles. By Dr. T. R. E. Southwood. A new edition based on the work of T. A. Coward. (The Wayside and Woodland Series.) Pp. xii+290+64 plates. (London and New York: Frederick Warne and Co., Ltd., 1963.) 30*s*. net.

THIS is a new edition of *Life of the Wayside and Woodland*, based on the work of T. A. Coward. Although the greater part of the original text has had to be changed, as a result of vast research over the past forty years, this edition should have as wide an appeal as earlier ones. The book is intended to serve as a general introduction to natural history, as well as to act as a reference book for more advanced scholars, or those training to teach. It should also promote interest in, and act as a guide to, practical field study. The text throughout is straightforward. Scientific names are given where appropriate, as they are international: they do not complicate the simplicity, and should serve as a preparatory aid for more advanced study.

The major proportion of the book is made up by dividing the year into six two-monthly sections, and dealing with whatever 'life' is flourishing at that particular time. A very wide range of life is covered.

Following this major section are four appendixes. Appendix 1 gives some common galls, leaf mines and other types of leaf damage: this is most interesting and could in itself stimulate observation. Appendix 2 outlines the Berlese-Tullgren funnel operation for the extraction of animals from soil, plant debris and dung. Appendix 3 gives the names of natural history societies, while Appendix 4 gives classified lists of books for further study. This is followed by an index.

There are 32 plates in colour and 32 in black-and-white. Also there are more than 300 text figures which are very clear and give ample illustration throughout.

B. J. G. MAITLAND

Lasers

Generation of Light by Stimulated Emission. By Bela A. Lengyel. (Wiley Series in Pure and Applied Optics.) Pp. xi+125. (New York and London: John Wiley and Sons, Inc., 1962.) 55*s*.

THIS appears to be the first book specifically devoted to masers working in the optical domain. It was written during a period of great activity in the field and the author realizes that it "will necessarily have a limited useful lifetime". At the time of writing there were very few sources other than the original publications to which

a newcomer to the field might turn, although this is no longer quite the case to-day.

After an elementary introduction to some basic physics, there is a short discussion of the principles governing the operation of a laser. This is followed by separate chapters on solid-state and gas lasers describing most of the pioneering work done in the field.

The book is introductory and evidently addressed to those meeting the subject for the first time. It is unlikely to be of great help to anyone actively engaged on research, who is more likely to turn to some of the recent review articles published in *Applied Optics* and *Proceedings of the Institute of Electrical and Electronics Engineers* for his information. The author has, however, succeeded in producing a readable introduction. The book is clearly written and well produced and as such is a welcome contribution to a field in which clear presentations are not exactly abundant. L. Mandel

Light

By Prof. R. W. Ditchburn. Second edition. (The Student's Physics.) Pp. xxvi+833. (London and Glasgow: Blackie and Son, Ltd., 1963.) 75*s*. net.

Ditchburn's *Light* has established itself as one of the best English physics text-books of the post-war period. The first edition was published in 1952 and it has now been extended by chapters on geometrical optics and the inclusion of such topics as coherence and stimulated emission. The result is a scholarly treatise which will be of great value to both students and teachers at honours-degree level.

The book, containing as it does an immense amount of material, is necessarily rather condensed in spite of its 800 pages, and many students will find it rather formidable; for example, the vector analysis used in the book is outlined in 1½ pages—a useful summary for those already familiar with it, but rather difficult for the new student. Other topics are also introduced as though the reader were familiar with them; for example, St. Venant's principle is not clearly expressed (and is, incidentally, incorrectly indexed).

Many of the additions have been made by adding appendixes in smaller print at the ends of the chapters. Since some of the sections are also in this smaller type, and there are also numerous questions for students to try out, and footnotes and references interspersed through the pages, the book has rather an untidy appearance. It is to be hoped that in any future edition the author will try to cast the book in a more consistent mould.

Mistakes seem to be few. But Fig. 6. 3, as in the first edition, shows fringes only in the central maximum of the diffraction pattern of two slits; the correct pattern is shown in Fig. 9. 11. In Chapter 8, some of the Figures, for example, 8. 7, 8. 9*a* and 8. 9*b*, show rays passing through lenses in impossible paths. Moreover, on Plate 3 Figs. *c*, *d* and *e* are rather deceptive in that they show Fresnel diffraction patterns that must have been taken with different magnifications.

But then no book is perfect and the author and publishers must be congratulated in this very considerable contribution to physics teaching.

Fundamentals of Semiconductor and Tube Electronics

By Prof. H. Alex Romanowitz. Pp. xii+620. (New York and London: John Wiley and Sons, Inc., 1962.) 62*s*.

This book represents an attempt to present to the student the fundamental principles of operation of valves and semiconductor devices in a unified way, and relate these to circuitry problems.

It is doubtful whether this aim is a worthwhile one, since the principles of operation of valves and transistors are fundamentally different.

In addition to this, the relative importance of valves and semiconductors in many applications is, in my own opinion, often misrepresented. For example, in the chapter on rectifiers and power supplies, the silicon rectifier, which is fast becoming the most commonly accepted device for rectification, is not discussed at all. Most of the 36 pages of the chapter are taken up in discussion of valves, selenium and copper oxide rectifiers.

Also much of the information in the book is confusing to the student, and mistakes are far too common. On p. 101, in describing the formation of a point contact rectifier, the author speaks of "atoms of an acceptor impurity, probably phosphorus", diffusing into an *N* type crystal to form a *p–n* junction. In the chapter on "Microelectronics", the author states that the field "has advanced to the point where whole systems such as a multivibrator are grown in the laboratory from a pool of semiconductor material". In my opinion this statement is misleading to the student, leaving him with a false picture of the processes for microcircuit fabrication.

A. A. Shepherd

Progress in Inorganic Chemistry

Vol. 4. Edited by F. Albert Cotton. Pp. 575. (New York and London: Interscience Publishers, a division of John Wiley and Sons, 1962.) 115*s*.

Volume 4 of *Progress in Inorganic Chemistry* appears to be of less general interest than its immediate predecessor. The fact that all the articles are in English will be welcomed by most buyers and it looks as if the article in German in Volume 1 by Becke-Goehring will be the exception of this series. The review by Pearson and Basolo on the *trans* effect will be of wide interest and contains interesting discussions of recent kinetic studies, such as those on isotope exchange in $[Pt(NH_3)Cl_3]^-$. The nephelauxetic series by Jorgensen should again interest a broad spectrum of readers, but is not written in an easy style. Phosphonitrilic polymers have been extensively reviewed and one questions the need for the article by Schmulbach even though it is a good one. More specialized but still interesting reviews are by Vannerberg on peroxides, superoxides and ozonides and on isopolytungstates by Kepert. The last-mentioned review of a difficult subject would have benefited from a more critical approach, but the section on kinetics and the summary are useful. Polymorphic modifications of arsenic trioxide is a very specialized topic. This is reflected in the fact that half the references here are to work by the authors of the review. The general standard of presentation is high and there are few misprints. I. R. Beattie

An Introduction to Genetics

By Prof. A. H. Sturtevant and Prof. G. W. Beadle. (Dover Publication No. S306.) Pp. 391. (New York: Dover Publications Inc.; London: Constable and Company, Ltd., 1962.) 16*s*.; 2.00 dollars.

This issue is a corrected republication. When the book was first published, it was given very good reviews. Using mainly *Drosophila* and maize, with mention of other organisms to illustrate specific points, the authors gave an accurate and comprehensive picture of the behaviour of Mendelian genes and chromosomes. But this was in 1939, nearly twenty-five years ago. Genetics, like time, has not stood still.

In the preface to this inexpensive Dover edition, the authors acknowledge that there have been far-reaching changes in the subject but write: "In spite of these developments, however, we feel that the book gives a fair and balanced account of most of the field covered, i.e., of the chromosome mechanics of higher organisms. And this is an important, basic part of genetics that is often neglected or inadequately covered in recent books"; a statement which does not deny, however, that a few do adequately describe both foundations and later construction.

K. S. Dodds

HISTORY IN THE SCIENTIFIC AGE *

By Sir JOHN NEALE, F.B.A. *

Professor Emeritus in the University of London, and
Honorary Research Associate in University College,
London

AMONG the minor pleasures of growing old is the discovery—which still surprises me—that the familiar setting of one's youth may be news to a younger generation. Consequently, when the Provost of University College, London, asked me to address the Assembly of Faculties, I thought that I might agreeably discharge the obligation by exploring my memory of changes that have occurred in the study of history in the past forty years or so.

When I began my career in 1919, academic history was in what might be described as its scientific phase. The older tradition of Great Britain was in disrepute—a tradition stretching back to Clarendon in the seventeenth century and more recently displayed in Macaulay, Carlyle, Green, Lecky and Froude, which regarded history as a branch of literature. Quite naturally and rightly scholars had succumbed to the professionalizing of their subject by the great nineteenth-century German historians and by the establishment of the École des Chartes in France. In a famous phrase of the German scholar Ranke, they believed that their duty was to reconstruct the past "as it actually was"; and this involved a new critical discrimination in the choice and use of historical sources and the application of severe technical disciplines in evaluating and interpreting them. The supreme virtue of the true historian, they believed, was objectivity; scientific method was the basis of his profession.

In 1903, J. B. Bury, the newly appointed Regius professor of history in the University of Cambridge, who as a student of ancient history was peculiarly sensitive to the achievement of German historians, preached the new gospel in his inaugural lecture, entitled, "The Science of History". "History", he declared in the most frequently quoted sentence of the lecture, "is a science, no less and no more." "It is . . . of supreme moment that the history which is taught should be true; and that can only be attained through the discovery, collection, classification and interpretation of facts—through scientific research." "History is not a branch of literature." "So long as history was regarded as an art, the sanctions of truth and accuracy could not be severe."

Bury's pronouncement was immediately challenged by a young scholar, not as yet an academic historian, though he was later to occupy the famous chair then held by Bury. He was G. M. Trevelyan, a great nephew of Macaulay, whose traditions and own views led him into instant denunciation of Bury. His challenge, entitled *Clio a Muse*, was published in December 1903 and set the issue between history as an art and history as a science—as indeed did the felicitous style in which it was written, contrasted with the humdrum prose of Bury.

When I began my career, these two rival gospels confronted one another; and on the whole academic historians followed Bury and spurned Trevelyan, whom they regarded as an outsider, a graceful amateur who did not make their grade. This is not surprising. Their search for facts and objective truth had led them to work more and more in the Public Record Office and had revealed the utter inadequacy of their literary predecessors, both in critical method and choice of evidence. Basic history was being completely refashioned by professional scholars, and the new discipline was justifying itself.

But that unfortunate assertion of Bury's that "history is a science, no less and no more" encouraged academic historians to concentrate their talents on the process of research and give scant time and care to the literary problem of presenting the results to their public. History, after all, has to be communicated to someone; and if we scrutinize the writings of some of our most respected professors in the Bury epoch, we may think that had they displayed half the ability in prose they revealed in research, their readers would have received much more pleasure and no less enlightenment. Some of the more austere scholars even regarded good writing as the enemy of scholarship and were apt to think that readable history was *ipso facto* bad history. Trevelyan in his *Clio* essay declared that "the public has ceased to watch with any interest the appearance of historical works, good or bad". As late as 1930, when I was writing my biography of Queen Elizabeth, I was conscious that the title of professor was calculated to frighten people off a book; and it was this lack of interest in academic writing that led me to suppress all my footnotes—an act of self-denial which, in the climate of to-day, I would neither need, nor wish, to repeat. An anecdote will give point to my remarks. Shortly after the publication of my *Queen Elizabeth*, Eileen Power—a historian who wrote with style and charm—was dining at one of the women's colleges at Oxford. My book came under discussion, particularly the lack of footnotes, and a history don remarked: "Neale has sold the pass". "I don't know about selling the pass", retorted Eileen Power, "but he has sold twenty thousand copies".

Trevelyan's *Clio* was not a faultless argument. Being a narrative historian of the older school, he believed that "the art of narrative" was "the principal craft of the historian". Few or no academic historians to-day would subscribe to that statement—not even the most widely read among them. With even more certainty we can say that few or none would subscribe to Bury's description of history as a science: they might talk of 'scientific' method, though they would probably agree that 'scholarly' is a less misleading word.

In fact, the old controversy is dead; and if proof were needed of this, it could be found in some of the royalty statements sent by publishers to their academic authors. Professional historians may still write in the *English Historical Review* for a few experts, or in monographs on themes too technical for an extensive audience; they may edit texts published, and only publishable, by university presses; but, generally speaking, they are aware that when their research is finished, there remains the task of communication, that this is a literary problem of supreme difficulty, and that there is a reading public waiting to be wooed, which is commensurate with their skill in writing and the interest of their subject. The academic historian to-day is combining art and scholarship. When successful, he is so vastly superior to the literateur who has art without scholarship that publishers nowadays, instead of regarding professors as a costly and doubtful investment in mere prestige, court them as potential best-sellers, or at worst normal risks: and surely the crowning irony, when compared with the situation forty years ago, is that we now find the literateur adding footnotes to his narrative to create the impression—which, alas, is too often an illusion—of scholarship.

* Substance of an address delivered before the Assembly of Faculties, University College, London, on June 20.

It is worth asking how this change came about. In the nature of things the new 'scientific' gospel was bound to produce a certain asceticism, or lack of humour, in the first generation or so of converts. Equally, we might have expected a younger generation to be less enamoured of ivory towers and come down among ordinary mortals. In fact, this natural process was speeded up by a number of external happenings.

Here, as elsewhere, the First World War was the great divide. Until then—and with a time-lag afterwards—conventional history was mainly concerned with the State and the Church; with kings, statesmen, bishops, domestic and foreign policy, wars. Its hallowed divisions were political, constitutional and ecclesiastical history; and for academic purposes modern history was deemed to end in 1815, 1870 or—where minds were tinged with radicalism—1900. The history of our own times was a subject for the journalist or publicist, since *ex hypothese* the historian could only function where objectivity was attainable.

After all, the historian writes the sort of history prompted by the questions he asks, and his questions are ultimately determined by the spirit and interests of the age in which he lives. In the relatively stable society before 1914 political, constitutional and ecclesiastical history might easily satisfy historical curiosity. Economic history was arriving, and no one, after the appearance in 1919 of Keynes's disturbing best-seller, *The Economic Consequences of the Peace*, could be blind to its importance. But it found its way into the London history curriculum only in the 1920's, and then not without misgivings. The *Times Literary Supplement*'s review in 1924 of Tawney and Power's *Tudor Economic Documents*—a work produced for the London history school—commented: "Theirs is the newer history, the offspring of a democracy growing not merely self-conscious but more curious and intelligent". As for social history, that had to wait a long time to discover itself and become respectable. In 1920 or thereabouts it had no academic standing and was regarded as a subject on which ladies, with a love of the picturesque and the romantic, and no digestion for harder fare, might misguidedly write.

In his *Clio* essay G. M. Trevelyan had declared that the "presentation of ideals and heroes from other ages is perhaps the most important among the educative functions of history". It was a view that Carlyle in the nineteenth century shared with Elizabethans in the sixteenth century—a long stretch of time, indicative of the relative stability of society as well as the conservatism of history. But the years between the two World Wars, when social revolution was at work and communist Russia was influencing thoughtful as well as thoughtless people, could scarcely remain a time for heroes and hero-worship—in any event in Britain. Indeed, in the 1920's Lytton Strachey and his followers were busy debunking all the 'heroes' on whom they could lay their biographical hands.

The age of the common man was arriving; and so was a new academic subject, sociology, which in my early days was struggling to define and establish itself. In depth, sociological studies are historical, needing the historian's sense of time and circumstance for their elucidation; and the questions sociologists ask are often questions the historian can profitably borrow. In this new climate, social history has emerged as an academic study worthy of the finest historical ability, and in so doing has brought local history out of its old, antiquarian limitations and has spread into economic and even constitutional history, humanizing and enlightening these other branches of history to an astonishing degree; and yet I remember clearly how often we bristled at the term 'social history' on our Board of Studies in History, afraid that it might mean the old pretty-pretty, descriptive stuff, void of intellectual guts.

Since Bury and Trevelyan wrote their blast and counter-blast, history has broadened beyond description—certainly beyond description in a half-hour's talk. I have, for example, said nothing of psychology, which, when my career started, was an academic Cinderella in Britain grubbing in the kitchen of philosophy. Lytton Strachey applied psychology to biography and inaugurated what was called "the new biography". It was not an unmixed blessing, as I can testify, who have watched Queen Elizabeth I psycho-analysed by practitioners whose historical competence charity must veil. However, no reputable historian nowadays writes of human beings without being aware that they are creatures of flesh and blood and nerves, and maybe of neuroses: human beings are the stuff of history.

Of late, the world has been too much with us. It has set us asking all manner of questions. Mussolini and Hitler, Stalin and the ideological struggle between East and West have opened the eyes of historians, so that they see the past with a new comprehension. In politics, for example, we have become aware of a historical phenomenon which we now describe as 'ruler-cult'. With other features of our contemporary scene, it has furnished the key with which to open the world of Elizabethan England. In constitutional history, now that democracy and other political terms are used by East and West with utterly different meanings, we can no longer work and write within the framework of Aristotle's *Politics*. Instead, we are probing into the coarser realities of political power, consciously or unconsciously influenced by the Marxist atmosphere in which we live, and borrowing demographic methods from sociology and geography. The results are so striking as to make some of the constitutional and political history of yesterday seem extremely antiquated.

Alongside this broadening of historical studies has come an equally remarkable *rapprochement* with the reading public: and here also a number of casual factors have hastened a natural process. Lytton Strachey and his school of new biography in the 1920's, and the publishers who ran pell-mell after the fashion, were the first mighty influence. Then came broadcasting, with its Third Programme, where—to the great admiration of my American friends—scholars were let loose on those who chose to listen. It had a triple effect. For the more malleable performers—and let it be confessed, some proved unmalleable, and the B.B.C. commendably tolerant—it was excellent training in the art of communication, teaching them the simple but neglected truth that an author must shape his writing for his audience. So far as the audience was concerned, it removed some of the austerity from the title of professor, and wheedled listeners into reading their favourites' books. Finally, scholars were actually paid for their talks. "Bliss was it in that dawn to be alive"; to find one's talks repeated over and over again, with a supplementary fee each time, was very heaven! Scholars made the pleasing discovery that authorship could be remunerative—a wonderful stimulus to the art of writing. The day, alas, came when the number of these heavenly mansions in the Third Programme was drastically reduced; but already television had appeared: and now the public finally lost its fear of professors: indeed, some of them have rivalled film-stars in popularity. As this space-age unfolds and science impinges increasingly on the general interest, we see our science colleagues joining the historians: and the cry is still 'More professors!'.

I must devote a short time to change in the status of historical studies in our universities and our national life. When I began my career, academic historians, in comparison with to-day, were few in number, their students relatively few, and their subject, in scholarly estimation, a very poor cousin to the classics. If my recollection is correct, a majority of the professors had come to history via Greats at Oxford, and none had emerged direct from the 'Redbrick' universities. Moreover, the classics and not history, the old and not the newer universities, formed the main recruiting ground for our rulers in the higher division of the Civil Service.

All this has changed. Numbers, both of staff and students, have increased and are increasing remarkably and history has emancipated itself from subordination to the classics. On the whole, I think it is an advantage that virtually all our professional historians to-day have received their full training in honours schools of history. They think instinctively in historical terms and are more readily inclined to refashion their subject. This refashioning of the subject is a matter of great consequence. I remember being told—sometime in the 1930's—of an experiment at Harvard, intended to demonstrate the broad new sweep of history by assembling a team of scholars, each lecturing on a different aspect of the same subject. When they had assembled their team, they discovered that no one was scheduled to tell the basic narrative story of the period, and they had to conscript someone for what they whimsically called 'straight history'—and spelt, I hope, 'strait'.

'Straight history' and chronology are, of course, fundamental, but where the emphasis in historical teaching is on this sort of history, there is something in the gibe that history is merely a burden to the memory. While the world was stable and the content of history simple, there were some people who thought that laws could be discovered, governing the evolution of human society. I recollect reading a presidential address to the American Historical Association on the laws of history. A beneficent Providence hovered in the background; the idea of progress and Tennyson's endearing picture of freedom broadening down from precedent to precedent were treated as axioms. I read the lecture at the height of Hitler's career, years after it was delivered, and it seemed to me like a liberal manifesto of late Victorian days. Its laws were spurious.

The world is no longer stable and history no longer simple. Far, far more conscious of the infinite variety and detail of the human story, and of the part played by the individual, we have become acutely aware of the casual, the accidental in history. We cannot conceive of laws in any sense that our scientific colleagues would acknowledge. We may hope that good will prevail; but that is a matter of faith or optimism.

On the other hand, to a degree never equalled before, history is a source of wisdom. As one historian has expressed it: "The crowning attainment of historical study is a historical sense—an intuitive understanding of how things do not happen". As the new history probes realistically into more and more of the past, it prepares the mind for coping more successfully with the present. History does not repeat itself; but it does offer parallels with contemporary problems and can warn us of the unexpected. It makes pragmatists of us, and, except when distorted, does not encourage the doctrinaire, whom I regard as the menace of our age. For these reasons I rejoice in the dominant place that it has won for itself in our universities, and in the fact that historically trained men now readily enter the higher flights of the Civil Service. We shall make fewer mistakes as a nation because of the way their minds have been trained: and even in the executive ranks of industry, the instinct to look at questions in their historical setting and "the intuitive understanding of how things do not happen" can be, and are, of immense value.

Moreover, though history is not a science, nor quite what Trevelyan envisaged in his *Clio*, I think that both Bury and Trevelyan could be happy about its state to-day.

LITERACY OF SCIENTISTS AND TEACHERS OF ENGLISH

By A. E. DARBYSHIRE
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TWO recent articles in *Nature*[1] raise again the problem of standards of the English of sixth-form and undergraduate scientists and technologists and the responsibilities of those who have to teach science and English to these young men and women. In the first article, Mr. A. J. Kirkman asserts "that scientists and technologists themselves must take most of the responsibility for the low standards of self-expression in their professions, and that a major change of outlook on their part is the only thing that can bring a substantial improvement in the situation". I believe this statement shows a failure, typical of many teachers of English, to understand the point of the scientists' and technologists' use of language, and expresses an attitude of mind that teachers of English should long since have abandoned. In the second article, Prof. R. S. Scorer mildly rebukes: "The damage is done in classes called 'English' "; and he gives illustrations from his own experience (all too representative, I suspect) which show how easy it is to do the damage.

As a teacher of English myself in a large technical college, may I say how refreshingly right I think Prof. Scorer is, and how out of touch with what he calls the "situation" Mr. Kirkman seems to be? May I further suggest that if there is to be any "major change of outlook" it should come through the teachers and examiners of English setting their own house in order, and that the scientists and technologists be left alone to get on with the jobs they seem to be doing pretty well?

There is nothing outrageous, I should say, in the contention that responsibility for standards of English should rest squarely on the shoulders of those who teach English and organize examinations in it. There are many, indeed, who are prepared to accept this responsibility, and many more, I think, who would like to know precisely what it is, for there is no real agreement among teachers and examiners of English about what they are actually supposed to be doing, about what the "subject" of English really ought to be, or about the function of the teacher of English in modern industrial society.

The problem is easy to state: it is not to get the scientists and technologists to write good English according to the nowadays old-fashioned standards of most teachers of English, but to re-organize English as a "subject" so as to bring it up to date and adapt it to modern social needs. Four aspects of this problem seem to require discussion. They are: (1) the attitude of teachers of English to science and technology; (2) grammar, rhetoric and democracy; (3) English in English language examinations; (4) the wider dissemination of the results of contemporary linguistic analysis and developments in semantics.

(1) Prof. Scorer takes Mr. Kirkman (and by implication other teachers of English) to task for blaming "the bad grammar rather than his own lack of knowledge of the subject-matter for the failure of communication". This, surely, is the point. For the past four hundred years scientists and technologists have been making the kind of world in which we all now live, and in shaping modern industrial and technological society they have had an enormous effect on the English language and on our methods of communication. But in the twentieth century the teachers of English have remained aloof. The teachers of English—and the examiners who are drawn from their ranks—are normally arts graduates who by predisposition

and training are likely to be unsympathetic to science and technology, and who do not often seem to understand how the minds of scientists work. If the teachers of English have passed through an honours school at a university, they will have developed their ideas of what "good writing" is from literature, and especially from poetic literature. Now English poetry is very good indeed—its reputation abroad is much higher than it is at home—but the language of poetry is not scientific language. Many teachers of English will, consciously or unconsciously, accept the famous dictum of Coleridge that "the proper antithesis of poetry is not prose, but science", and in accepting it will tend to believe that in some mysterious way poetry is "superior" to science. There are, of course, no rational grounds for such a belief, but the intuitive methods of literature in arriving at "truths" about man and Nature are likely to seem to teachers of English to be more reliable (and easier) than scientific method or the rigours of mathematics.

Furthermore, the vast range and variety of the subject-matter of English literature tend to convince teachers of English that literary forms of expression use all the resources of language and that there is nothing that cannot be said in the words found in the *Oxford English Dictionary*. Some teachers of English are apt to be surprised, shocked, and perhaps a little hurt, to find that physicists and engineers, for example, are quite content with a mathematical expression of their concepts and see no necessity for expressing them otherwise, or that for technologists drawings, diagrams, graphs, tables and statistics can communicate just as well as words in sentences. It seems never to occur to some teachers of English that in advancing new knowledge scientists and technologists have also advanced new techniques of expressing it.

Obviously teachers of English cannot turn themselves into scientists; but they certainly can, and should, make an effort to understand what the scientists are doing, how scientists think, and above all how scientists use language and why they want to use it in that way. A student, writing a report of an experiment or, say, of a test of some technological equipment, is not producing a work of literature. He is reporting objectively on a factual state of affairs, and the "objective correlate" (to use T. S. Eliot's expression) of the form of his piece of writing is not emotionally apprehended experience which, in the writing, becomes complicated by overtones of feeling and attitude presented to the reader in verbal subtleties of style, but the facts themselves. Admittedly, the report will be a good one only if the facts are properly understood, and a great deal of bad writing comes, not from ignorance of common usage, but from the writers' inefficient understanding of the subject-matter. The job of the teachers of English is not, of course, to teach the scientific facts, but to increase in his students an awareness of what is happening when language is being used, and he cannot hope to do that very well with students of science and technology unless he knows what such students want to use language for.

(2) The standards of "correct" English, and the modifications of ancient classical notions of rhetoric to accommodate them to ideas of "good literary style" as literacy gradually became more widespread in Britain after the introduction of printing, were developed finally in the eighteenth and nineteenth centuries. They still appear, slightly diluted and vulgarized, in text-books of grammar and composition still used in schools, and, what is worse, they are still demanded in English language examinations. They are the standards of a literary aristocracy inspired by the humanist traditions of the Renaissance; they are as out of date as phlogiston; and the terminology used to talk about them is as antiquated as Julius Caesar.

Up till about the middle of the nineteenth century, large numbers of English-speaking people learnt to write English by translating from and into Latin and Greek: and that is probably the best way of learning to write English that has ever been invented; for Latin and Greek are languages sufficiently different from English to enforce concentration on matters of sentence structure and vocabulary to get the translation right, to give constant practice in adapting content to form, and the texts chosen for study gave plenty of good models of clear thinking and cultivated taste. But as a way of learning to write English nowadays it is obviously impractical, for it was an eclectic method suitable for a leisured class of aristocrats in an agricultural society where universal literacy was not important.

However, nothing of comparable efficiency has been worked out to meet the needs of our own industrial, technological and democratic society. When, in the middle of the nineteenth century, English began to emerge as a "subject" in response to a growing need for more and more literate technicians to help cope with industrial advancement, it took over the formal paraphernalia of the Latin and Greek tradition in matters of "correct" grammar and the rhetoric of "good style" and adapted it to its own uses to fix an arbitrary standard. The result was linguistic disaster, a conflict, on one hand, of the natural and spontaneous development of the language of the people on its own lines and, on the other hand, of the imposition by school-teachers of antiquated "rules", fatuous stylistic nonsense (such as not ending a sentence with a preposition), and ill-understood mimicry by the "lower orders" of the idiom of their "betters".

The point is that English as a "subject" has emphasized the formal aspects of composition and emphasized them, as it were, in a vacuum. Form without content is the great aim of English as a "subject" during the past hundred years. Here, I think, is to be found the crux of the difficulties that young scientists and technologists encounter when they have to write in the course of their work. They have been so indoctrinated with ideas of form that they are constipated with them, and nervous about putting pen to paper when they have to pay so much attention, as they rightly should, to the content of their written work. My own view is that there is too much writing in the world, and that people should write only when they have something worth saying to say. But that, it seems, is not the usual view of teachers or examiners of English, who apparently want young people to write formal, abstract, disembodied, unmotivated pieces of writing, all "correct" grammar and all "good style", but who do not care very much whether what is written has any important content or not. No wonder Prof. Scorer says that the damage is done in classes called "English".

(3) It is clear, however, that the damage is done not entirely with the approval of all teachers of English. Examiners who organize English language examinations must take at least some of the blame. In every English language examination conducted in Great Britain that I know of—and I think I know them all—essay writing has a dominant role; it normally carries more marks than any other part of the examination, and often it is separated from the main body to become a little examination on its own. What can possibly be the purpose of this futile and time-wasting exercise? The other parts of the typical English language examination paper can perhaps be justified: obviously young people can rightly be expected to be able to read with understanding, to show some knowledge of how passages of prose are constructed, to know the elements of grammar, and to be able to understand the normal usages of civilized communication. But in preparing pupils to pass English language examinations teachers have to train them to write essays merely for the sake of writing essays, and to write a stipulated number of words or for a stipulated length of time, regardless of whether what the candidates have to say needs to be said at such a length, and regardless, too, of

whether the candidates have anything worth saying to say on the kinds of topics usually chosen, or whether indeed anything should be said on them at all. The process can only encourage the production of padding and verbal hot air, the "leisurely periphrasis" which Prof. Scorer so rightly objects to.

The assessment of such unmotivated writing as English language examination essays can only be formal, and the artificiality of such a procedure at once condemns it, for the formality derives, as I have said, from an old tradition which is inappropriate to the times in which we live. After all, the number of people who want to write essays must mercifully be very small, and those few who feel that they do really want to write them no doubt will do so; but for the rest of us there could well be the blessings of silence or of other more worth-while activities, and we shall write only when we have something to say which we think (deceiving ourselves, perhaps) is worthy of being said.

The skills, if that is what they are, which such examinations as the General Certificate of Education English Language Ordinary Level are supposed to test are those of writers who are separated from their subject-matter. But if one accepts, as scientists and technologists, I imagine, must accept, that pieces of writing exist to communicate their meaning and not just the author's acquaintance with sentence and paragraph structure, grammar and "style", then one must also accept that subject-matter influences form. The examples of so-called errors which Mr. Kirkman gives exhibit slack thinking about subject-matter more than they exhibit anything else. A statement like "The dimensions and weight of the vibrating part of the rod was measured" shows that the author had no clear idea in his mind of what was measured; and the "English" of "The diagram was set up as shown . . ." is as good as one might find anywhere—it is simply that what it says is presumably not true. These examples and many hundreds of others which I have met in my experience with students in further education indicate that our traditional school methods of teaching English fail to make clear the relationship between words and things or to demonstrate to students the importance of meaning.

The great writers of the past were great writers, whether in "pure" literature or science, whether men like Shakespeare or Dickens, or Bacon or Darwin, because they had something significant to say, and what they had to say decided the form in which they said it. Although the object of teaching English in schools is not to produce great writers but only competent ones, the same principles will apply, for it is only the great writers of English who can tell us what good English is.

(4) It ought to be possible to assimilate into English classes in the upper forms of secondary schools and in further education some of the knowledge that has been acquired in this century of linguistic analysis and semantics. There are three fundamental questions which can be asked about linguistic communication and which in our time have been pretty thoroughly answered at the post-graduate level for most practical purposes of training young people to think clearly about the problems of self-expression in writing. They are: (1) What are the uses of language ? (2) What is the meaning of meaning ? (3) How does, or does not, any particular use of language express the meaning its author intended to express ? If one leaves aside highly technical questions of modern linguistic philosophy, cybernetics, neurology and mathematical logic, there is nowadays a sufficient basis of fact about linguistic communication to form a popular account of how language works that could be taught in the upper forms of schools and in further education. A syllabus could easily be devised, and need be no harder in its content, at Ordinary and Advanced Levels of the General Certificate of Education examination, than the mathematics at present taught in schools.

The language itself of many English classes and English language examinations is full of what telecommunication engineers call "noise". A little less "noise" and a little more "information" would be an advantage to everyone concerned.

[1] Kirkman, A. J., *Nature*, **196**, 807 (1962). Scorer, R. S., *ibid.*, **197**, 1049 (1963).

RAY PATHS IN A ROTATING SYSTEM

By Dr. R. C. JENNISON

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THE transverse Doppler effects associated with the rotation of a source and an absorber have been examined using the Mossbauer effect[1] and the principles are well understood[2]. The entire situation may be expressed in terms of an inertial frame where the light paths are straight, and the light paths through the rotating system have received little or no attention. It will be shown that a very beautiful situation is exhibited by the light path within the system and that this leads to some interesting interpretations of the phenomenon of rotation.

There is always difficulty in defining a rotating system or disk, and for this reason I shall use as my context a hypothetical synchrotron machine in which self-luminous particles are orbiting at velocities closely approaching c in an outer ring and at progressively slower speeds in a series of smaller concentric rings. The system may be very large and in the centre we may place a synchronized turn-table equipped with servo controls and sighting tubes through which an observer may confirm that the orbiting particles are maintained in synchronous rotation with a period $1/2\pi\omega$. The observer will have a variety of means for confirming that the instantaneous speed of a particle at distance r is given in his system by the product ωr.

I shall now use this rotating system as a frame of reference and in complex notation may define the position of one of the rotating particles by the elementary transforms:

$$Z = Z'e^{-i\omega t},\ Z' = Ze^{i\omega t}$$

The central observer would then associate the points sighted in synchronous rotation with the condition that Z' for each particle is independent of t.

The history of a light ray through the centre in system Z is simply:

$$Z = cte^{ia}$$

and in system Z':

$$Z' = ct\ e^{i(\omega t+a)}$$

This path is an Archimedean spiral and would have opposite curvature if either the rotation were reversed or the light were propagated in the opposite direction. The result is precisely that which would be obtained in Newtonian kinematics for a projectile moving with

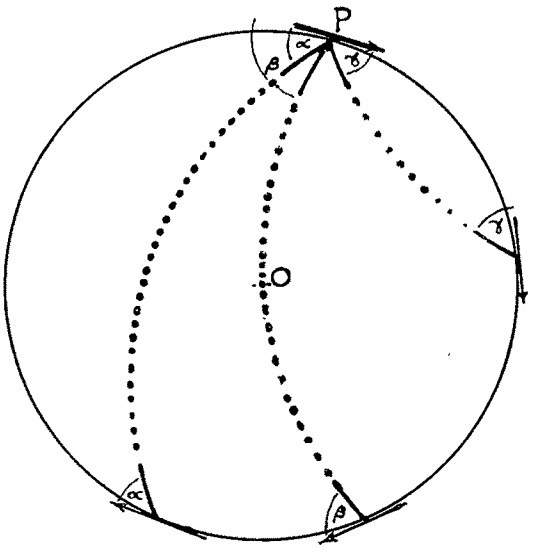


Fig. 1. The symmetry of ray angles for transmission from point P to various receiving points on the same ring. The angles may be obtained by Euclidean projection either directly or through an intermediate inertial frame

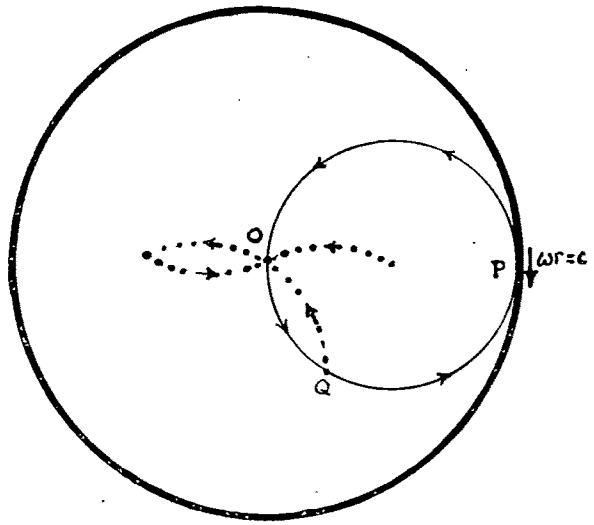


Fig. 2. Light pulse paths to and from the centre for the point P at $r = c/\omega$ and for Q at $r = c/2\omega$. The drawing also shows, dotted, a number of other paths to and from points in the ring at $r = c/2\omega$

velocity c. The Coriolis acceleration gives the Archimedean curve.

One may now query whether or not this really expresses the path within the disk for had I computed the situation in terms of t' where t' is the time applicable to an observer at the source I would have obtained an alternative result in his system. Though the two paths would be simple transformations neither could claim to apply generally and directly within the disk as a whole.

Consider now the more general situation where the path throughout is defined in terms of the various local systems Σ_0, Σ_1, Σ_2 . . . Σ_r through which it passes. In each of the annular systems we have associated local time-scales t_0, t_1, t_2 . . . t_r applicable to all experiences within the regions.

Consider the emission of light from a point in the system Σ_r. Let this light be seen through a sighting tube in the central region Σ_r. The time-scale t_r results in a reddening which one may compute from the Doppler shift:

$$\nu = \nu_r \frac{1 + \frac{\omega r}{c} \cos\theta_r}{\sqrt{1 - \frac{\omega^2 r^2}{c^2}}} \qquad (1)$$

and a corresponding aberration of the ray angles at the source in terms of the systems Σ_0 and Σ_r:

$$\cos\theta = \frac{\cos\theta_r + \frac{\omega r}{c}}{1 + \frac{\omega r}{c}\cos\theta_r} \qquad (2)$$

Neither of these expressions is affected by subsequent change in speed or direction of motion of the source during the transit of the light signal to the observer.

Simple geometrical considerations would suggest that only the transverse Doppler component is applicable and hence equation (1) reduces to:

$$\nu = \nu_r \sqrt{1 - \frac{\omega^2 r^2}{c^2}} \qquad (3)$$

and in particular in equation (2):

$$\theta = \frac{\pi}{2} \text{ and } \cos\theta_r = -\frac{\omega r}{c} \qquad (4)$$

These results are, of course, entirely consistent with the relative dilation in the two systems, and for source and observer in the same ring we find:

$$\nu_{r1} = \nu_{r2} \qquad (5)$$

and:

$$\theta_{r1} = \theta_{r2} \qquad (6)$$

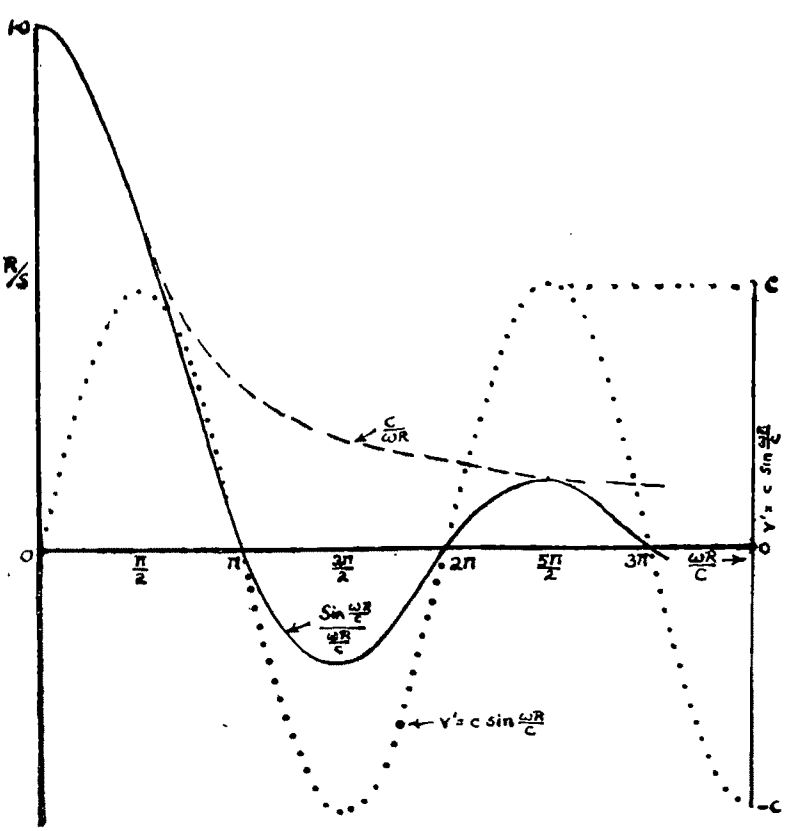


Fig. 3. The distance, relative to the optical path distances, from the centre to the apparent edge of a rotating system ($c/\omega R$), and to a particular ring at radius R, $\left(\frac{\sin \omega R/c}{\omega R/c}\right)$. The function $v' = c \sin \omega R/c$ is also plotted

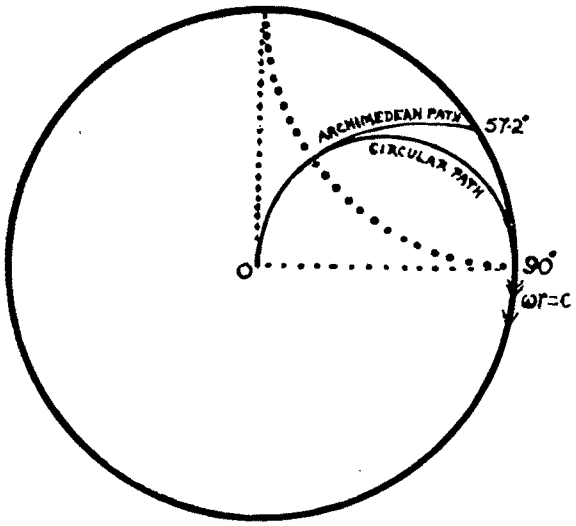


Fig. 4. The light paths to the centre corresponding to a circular arc and an Archimedean spiral; the curves are almost coalescent for $\omega r < 0{\cdot}4c$. The dotted lines are geodesics projected into a system at rest relative to O (after Møller, ref. 3)

The relationship (5) which is commensurate with the equal pseudogravitational potentials is now well known, whereas the associated condition (6), which may be used to fix the ray angles at the two points, has received little, if any, attention. The symmetry is illustrated in Fig. 1.

Equation (4) may be used to determine the complete path of the light ray within the system. It may be used to give precise instructions for the local excavations between the particles for a tunnel to be provided to allow the passage of light through the system. It is assumed for this purpose that the ray path of any given pulse of light is independent of the motion of the source. Equation (4) therefore gives a series of elementary rays which must join together to form a continuous light path, for the light from the outermost point may be seen through a single sighting tube in continuous conjunction with the light from other individual points in the systems Σ_0, Σ_1, Σ_2 . . . Σ_r as applicable. Hence we have for the ray: $\cos\theta_r = \frac{-r\omega}{c}$ which defines a circular arc for Euclidean projection.

The continuous ray constructed in the manner described has a constant curvature within the system and is in this sense a circular arc of radius $\rho = c/2\omega$ (Fig. 2). The curvature is therefore a function only of the angular velocity of the system. The ray path becomes a complete semi-circle for $\omega r = c$.

Some results of the analysis may be summarized as follows:

(1) The curvature of the ray is a function only of the spin or angular velocity ω.

(2) Different paths are taken by the light to and from any pair of points in the rotating system. Holes from a point on the equator to the centre of the Earth would deviate by 30 ft.

(3) It should be possible to determine absolute rotation by measuring the divergence of the paths in opposing directions.

(4) The time-independent or direct radius differs from the action or ray distance. The two are related as the chord is to the arc of a circle:

$$r = S\,\frac{\sin\frac{\omega r}{c}}{\frac{\omega r}{c}} \qquad (7)$$

This function may continue beyond the obviously meaningful region where $\omega r \leqslant c$ and then yields a quantized series of vanishing states (Fig. 3).

(5) The regular anisotropy could be expressed in terms of a spin dependent virtual refractive index within the system. The wave-fronts are not normal to the rays.

(6) Lorentz contraction of the individual segments in Σ_1, Σ_2 . . . Σ_r transforms the circular arc to an Archimedean spiral if expressed in a single frame. Both paths are shown on a hybrid diagram in Fig. 4.

I thank Profs. Bondi, W. H. McCrea, G. C. McVittie and J. L. Synge, and Mr. Howard Smith for criticisms of drafts of this article.

[1] Champeney, D. C., and Moon, P. B., *Proc. Phys. Soc.*, **77**, 350 (1961).
[2] Synge, J. L., *Nature*, **198**, 679 (1963).
[3] Møller, C., *Theory of Relativity*, 242.

EFFECTS OF RADIATION ON TRANSISTORS IN THE FIRST *TELSTAR* SATELLITE

By D. S. PECK and E. R. SCHMID
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THE *Telstar* communications satellite was planned as an experiment to investigate operating features of communications satellite systems, to obtain results of radiation effects in space and to examine, if necessary, the failure mode of the satellite. During the several months of successful operation it served effectively to carry out the communications experiments for which it had been designed. Following the initial failure of the satellite's command circuitry, it has performed additionally as an orbiting laboratory to supplement ground-based studies on the effects of ionizing radiation on transistors and semiconductor diodes. The response of the *Telstar* satellite to changing radiation conditions in its real orbiting environment and to changing circuit conditions in response to command has suggested further experiments to improve the understanding of the effect of ionizing radiation on transistor junction surfaces. Although these investigations have not fully clarified the details of the mechanism by which ionizing radiation causes degradation, they have at least provided a practical understanding of the effects of various pertinent factors.

Ionizing Radiation Effect

Considerable attention has been directed in the past to the effects of radiation on the bulk properties of semiconductor materials. These effects consist of: (*a*) hole-electron pair formation resulting from the collisions of charged particles with bound electrons in the material, resulting in the excitation of electrons to the conduction band and the production of holes in the valence band; (*b*) the generation of defects in the lattice structure resulting from collisions of energetic particles with nuclei of the lattice. The latter effect is that responsible for the degradation of solar cells.

In addition to investigations of these effects, recent work has been done on the sensitivity of transistor surfaces to ionizing radiation[1]. Degradation of surface-dependent characteristics appears to result from the combination of a fringing field at the semiconductor surface

(such as that provided by a reverse-biased collector unction) and the presence of ions and electrons produced by ionizing radiation in the gas of the encapsulation. Surface charges held by the fringing field tend to produce inversion layers, resulting in degradation of surface-dependent transistor characteristics. Fig. 1 shows the essential ingredients of this model of the mechanism for an *npn* diffused transistor structure. Since many semiconductor devices are encapsulated with a gas filling this phenomenon may affect the operation of some types of semiconductor devices in a radiation environment. Evacuated transistors, or those with oxide-protected surfaces, may also be affected, possibly due to ionization directly at the junction surface, but the effect is generally much reduced.

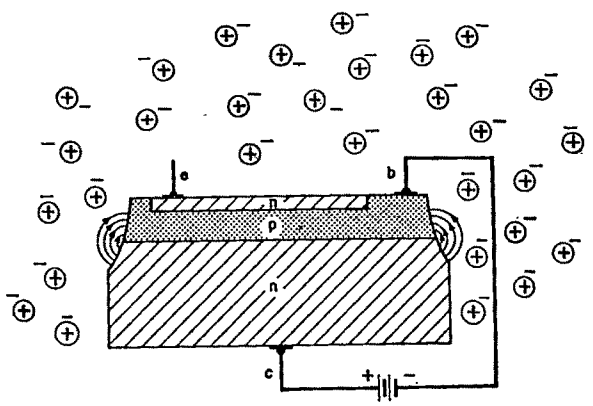


Fig. 1. Model of a reverse-biased transistor in a gas atmosphere ionized by radiation

This surface effect in transistors was recognized as a distinct phenomenon during the period of preparation of semiconductor devices for use in the *Telstar* satellite. The extent of the effect was examined for the various types involved by observing the reverse collector current and current gain characteristics during simultaneous exposure to ionizing radiation and electrical bias. (Depending on the dose rate, an ionization current will flow between leads, and measurement of very low currents may not be possible while in radiation. In this case, the characteristics are measured immediately after removal from radiation.) Some gas-filled silicon mesa transistors were found to have degraded seriously at a total dose of the order of 10^4 rads, corresponding to about 2,000 h in the 5 rads/h dose rate expected in the *Telstar* satellite orbit. Some diffused germanium transistors also showed similar effects but the degradation was less severe and more uniform within each transistor type. Transistors with relatively wide base regions, such as most alloy transistors, are subject to effects of bulk damage at a much lower dose than are diffused transistors and while the surface effect may be present, its seriousness may be masked by that of the bulk damage. Diffused silicon diodes show a reverse-current response similar to that of diffused silicon transistors, although they generally degrade at a higher dose.

Ionization in a transistor in orbit would be caused by high-energy protons and electrons which are not sufficiently shielded by the satellite structure or the transistor encapsulation. For laboratory tests, however, γ-rays may be used to simulate the ionization effect of space radiation. Tests using γ-rays from a cobalt-60 source have disclosed many interesting facets of the radiation effect. Three factors have particular pertinence to the impact of the radiation effect on the *Telstar* satellite circuits.

Effect of Collector Bias

Since the surface effects depend on the presence of both radiation and collector reverse bias we might expect to find that changing the bias will alter the effect. Studies have indeed shown that the degradation of collector reverse current with radiation is strongly dependent on collector bias. Fig. 2 shows the median collector current of a number of transistors, of one sensitive silicon type, exposed at each of several collector voltages.

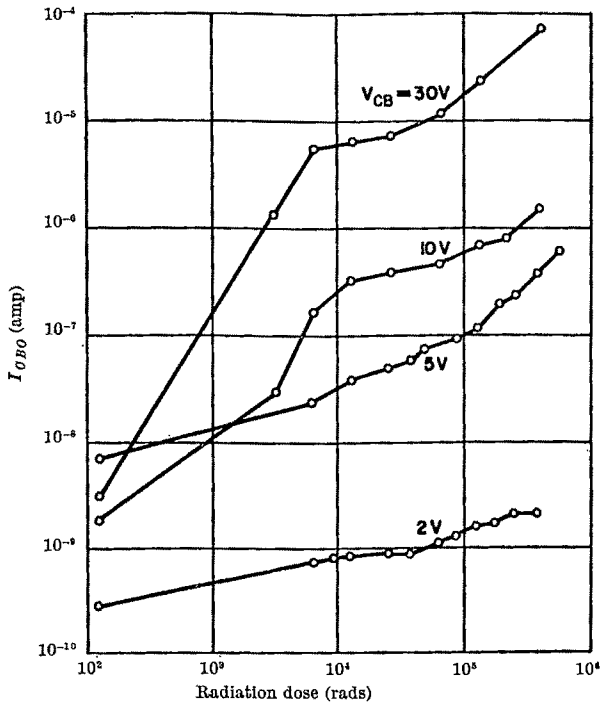


Fig. 2. Dependence of degradation in I_{CBO} on collector bias

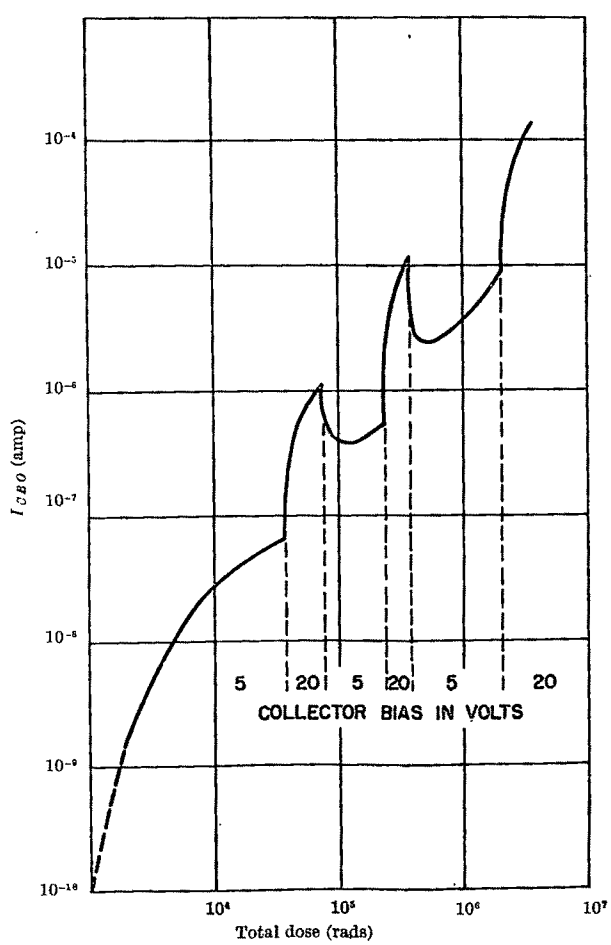


Fig. 3. Influence of collector bias cycling on I_{CBO} degradation

Fig. 3 shows, in addition, the effect on an individual transistor of cycling between two voltage-levels and indicates the manner in which the reverse current degradation is dependent on the bias-level. After cycling from an initial collector voltage to a different voltage and back, the collector current returns quickly to the pattern which might be expected had it remained at the original voltage throughout. This suggests that a surface condition is being developed at a rate relatively unaffected by the voltage value (at least over the range of 5–20 V for this transistor) but that the voltage does affect the resulting surface characteristics.

Effect of Dose and Dose Rate

Investigations of the dependence of collector junction characteristics on dose rate have shown varying results. In general, the degradation appears to be dependent on total dose rather than on dose rate; however, a change of a factor of 10^5 in dose rate can result in an immediate change in collector current by a factor of 10^2–10^3.

The complexity of the phenomenon is demonstrated by the results on devices which were exposed to a 30-sec dose of $8{\cdot}5 \times 10^5$ rads/h as a screening procedure for selecting the most stable devices for use in the *Telstar* satellite. They returned essentially to their initial characteristics when afterwards life-tested in a 5-rad/h dose rate. At a total life dose the same as that of the screening dose, however, approximately the same degradation had developed as in the screening dose. The radiation mechanism apparently allows the ionized surface states to recover to a reduced growth level consistent with the lower dose rate, yet causes eventual degradation corresponding to a specific total dose.

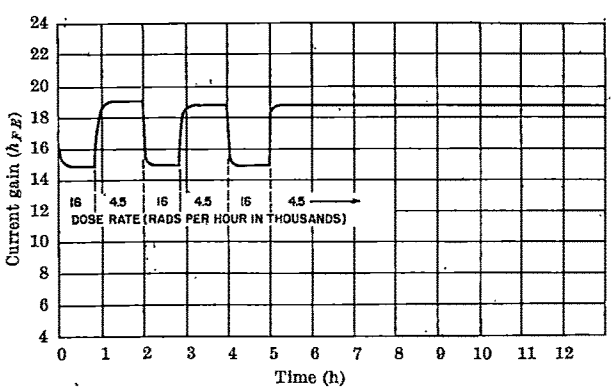


Fig. 4. Influence of radiation dose rate cycling on transistor current gain

More recent experiments by H. K. Gummel at Bell Telephone Laboratories have now shown a repeatable pattern of change in gain resulting from changes in the radiation intensity. Fig. 4 shows typical results of cycling a transistor between two dose rates, indicating a gain level corresponding to each radiation intensity. Over a longer period of time, however, either gain level shows the typical monotonic degradation with total dose.

It would appear that, in addition to the long-term dependence on total dose, a dependence on intensity can be expected in a radiation-sensitive transistor.

Recovery Effects

The surface changes resulting from ionizing radiation are not permanent and the characteristics will essentially recover within a few days at room temperature after removal from the radiation environment. Recovery out of radiation will be somewhat more rapid with the collector voltage removed, since this will allow the surface charges to move more rapidly than if they were held by the reverse-bias field. As might also be expected, recovery will be even more rapid without bias if the devices are left in the radiation environment so that the ionization of the gas can contribute to more rapid movement of the surface-charges. Present results are insufficient to indicate to what extent the recovery rate may be dependent on radiation intensity during recovery, extent of previous degradation or other such factors.

When a recovered transistor is again irradiated it returns to its previous degraded condition during a dose which may be much less than that of the previous exposure. The tests by H. K. Gummel show further that the rate of return to the degraded condition, as well as the degree of degradation, may vary as the cycle is repeated, even for an individual transistor. Predictions of these effects on a degraded transistor are therefore undependable.

Effect of Radiation on *Telstar* Performance

Although an appreciable percentage of the radiations sensitive transistor types in the *Telstar* satellite circuit had been screened by γ-ray exposure in order to select the most stable devices, the malfunctioning (November 19–23, 1962) of the command system showed a pattern of degradation, occasional recovery and recurring degradation which indicated a remaining sensitivity to radiation[2]. The decoders are situated at the outside edge of the electronics canister, receiving the least shielding from other components; they also contain some of the more critical circuitry with respect to allowance for device changes, and many of the decoder transistors are of a type most sensitive to radiation. At about the time of failure it also became clear from other results that the satellite had been exposed to a radiation intensity approximately two hundred times that which had been expected[3]. The total exposure at that time was apparently in the order of 3×10^6 rads, whereas the devices had been screened at about $1{\cdot}4 \times 10^4$ rads; the screening results could not have been extrapolated with any confidence to this unexpected exposure.

The decoder circuits are always under bias from the solar cells and, except for the operation of a specific command, also from the battery. It was recognized that this condition would accelerate degradation of transistor characteristics and that some recovery might be hoped for if voltage could be removed from these circuits for an appreciable length of time. Furthermore, many of the circuits include flip-flops which are left in one state or another depending on the last digit of the latest command signal. The degradation of the transistor left in the 'off' state, with full collector voltage of about 15 V, could be considerably greater than that of the transistor left in the 'on' state, with a resulting collector-base voltage of less than 1 V. Any commands which succeed in changing the states of transistors in the decoder circuits might, therefore, be expected to alter the degradation and recovery rates, hopefully in such fashion as to improve the marginal transistor or transistors. Variations in the response of the decoder circuits during the period of intermittent operation prior to total failure on November 23, 1962, were not correlatable, however, with the final command signal at the end of each pass.

Subsequent effort then concentrated on the development of modified commands which might be accepted by the decoder, taking into account the circuit effects of the possible degradation of various individual transistors. Such modified commands were eventually successful on December 20, 1962, following which the circuits appeared to improve further after periods of complete removal of circuit voltage by disconnection of the batteries and removal of solar cell voltage during eclipse periods. Normal performance was restored by January 2, 1963. During February 4–19, however, one decoder exhibited intermittent response to command, indicating no improvement in response to removal of circuit voltage during eclipse. Although additional modifications of the command signals resulted in improved operation for a short period, by February 21 these commands were no longer effective and the satellite would not respond further.

By that time it was becoming increasingly clear that the periods of loss of response of the command system corresponded to times at which the average radiation intensity was approaching its maximum value because of the changing satellite orbit. Precession of the orbit changes the percentage of time which the satellite spends in regions of accumulation of high-energy electrons in the Earth's magnetic field, resulting in cyclical changes in the average radiation intensity to which the satellite components are exposed. With the development of data indicating the location and intensity of such concentrations, estimates could be made of the average orbital exposure of the satellite, showing that the two failures occurred at periods approaching the maximum intensity, and the January 2, 1963, recovery occurred as the intensity approached the minimum of the cycle. The indication was thus that the transistor response was partly dependent on the dose rate. The confirmation of this dose-rate dependence in the laboratory, as indicated in Fig. 4, makes plausible such an explanation of the response of the satellite circuits.

The response of the satellite to its environment and to command experiments has, therefore, indicated the characteristic ionizing radiation effects on transistors, the variation of degradation with collector voltage, the recovery with removal of voltage, the sensitivity to dose rate and the eventual effect of total dose. Some of these effects were known sufficiently early to guide the efforts aimed at recovering usefulness of the satellite circuitry and others were deduced from the satellite response, to be confirmed on the ground. Toward this end the ability to compare results in the laboratory with those under actual orbiting conditions has been a significant factor in indicating the appropriate testing techniques and exposure-levels to be used in further studies.

The laboratory tests, however, indicate that, with transistor types of appreciable sensitivity to this phenomenon, the repeatability and predictability of response of individual units is certainly open to question and it becomes obvious that the emphasis in development of radiation-resistant circuits must be in the direction of reducing or eliminating the radiation sensitivity of transistor types.

Some of the early tests indicated the insensitivity of most evacuated types of silicon transistors and further work has confirmed that these types show relatively little degradation even beyond a dose of 10^7 rads. Similarly, many planar types, even gas-filled, suffer only limited degradation in the region of 10^6–10^7 rads dose. Hence the availability has been demonstrated of known production techniques for supplying transistors suitable for long life in satellites exposed to ionizing radiation and use was made of these techniques in supplying transistors for the critical decoder circuits for the second *Telstar* satellite. The different response from samples of various types, even of evacuated or planar processing, warns of the need for evaluation of each type for such environment and, perhaps, of each production lot or of each individual device. At least the recognition of the problem has led also to a recognition of solutions which are at hand.

[1] Peck, D. S., Blair, R. R., Brown, W. L., and Smits, F. M., *Bell Syst. Tech. J.*, 42, No. 1, 95 (1963).
[2] Mayo, J. S., Mann, H., Witt, F. J., Peck, D. S., Gummel, H. K., and Brown, W. L., *Bell Syst. Tech. J.*, 42, No. 4, 1631 (1963).
[3] Brown, W. L., Gabbe, J. D., and Rosenzweig, W., *Bell Syst. Tech. J.*, 42, No. 4, 1505 (1963).

NEWS and VIEWS

Physics at Sheffield : Prof. W. Sucksmith, F.R.S.

Prof. Sucksmith has now retired from the headship of the Physics Department at the University of Sheffield, after holding this post since 1940. In that year, he arrived from the University of Bristol, having been a reader in magnetism there, to find a small staff, depleted by National Service calls, and very modest experimental facilities. As a measure, albeit a very inadequate one, of his achievement in building up the Department, there are now more than twenty staff members, plus a post-graduate research school of twice this number, and the Department has recently moved into the new Hicks Building, with splendid facilities for research over a wide field.

Without question a brilliant experimentalist, Prof. Sucksmith has built up a research school in experimental magnetism at Sheffield with an international reputation. In the year of his appointment to the chair in Sheffield, he was elected a Fellow of the Royal Society, after successfully completing outstandingly novel and difficult experiments which led to the measurement of the gyromagnetic ratio for paramagnetic substances, and it is a tribute to the precision of these investigations that it is only now that physicists are requiring improved accuracy. His unique achievements in this field led to his visiting the United States in 1961 as a consultant on a project to measure the gyromagnetic ratio for a wide range of paramagnetic materials. His later research work centred primarily around the physical properties of ferromagnetics. In this work, the Sucksmith ring balance, invented initially to measure the susceptibilities of paramagnetic oxides, was developed as a suitable method for measuring the saturation magnetization of ferromagnetic metals and alloys at different temperatures. His outstanding contributions to experimental methods in the field of magnetism were suitably acknowledged in 1953, when he was chosen as Duddell Medallist of the Physical Society. His wider services to the University of Sheffield were recognized by the Council very recently by the conferment on him of the title of emeritus professor. His many friends in Sheffield are delighted that he and Mrs. Sucksmith will remain among them, and will all join in wishing them a long and happy retirement.

The University has decided to experiment with a system in which henceforth the headship of the Department of Physics will be held in turn for a period of three years by each of the two professors concerned. On the retirement of Prof. Sucksmith, Prof. G. E. Bacon becomes head of the Department for the first period (see *Nature*, 196, 1268; 1962).

Inorganic Chemistry at Bristol : Dr. F. G. A. Stone

Dr. F. G. A. Stone, reader in inorganic chemistry at Queen Mary College in the University of London, has been appointed to the new chair of inorganic chemistry in the University of Bristol. He will be in charge of a Department of Inorganic Chemistry formed by the division of the Department of Physical and Inorganic Chemistry for which Prof. D. H. Everett has hitherto been responsible. With Prof. Everett retaining charge of physical chemistry, the School of Chemistry will consist of three Departments, that is, Organic Chemistry (Prof. Wilson Baker), Physical Chemistry, and Inorganic Chemistry. This reorganization is part of a programme of expansion made possible by new buildings now under construction. A Cambridge graduate, whose interest in inorganic chemistry was stimulated by studies on the boron hydrides with Prof. H. J. Emeléus, Dr. Stone has

held fellowships at the University of Southern California with Prof. A. B. Burg and at Harvard University with Prof. E. G. Rochow. He then remained at Harvard, first as instructor and then as assistant professor, until he returned to England in 1962. He is widely known for his work on the synthesis of organometallic compounds, particularly in the field of the carbonyl complexes and fluorocarbon derivatives of the transition metals, and for his use of modern physical methods in the determination of molecular structure. He has published a monograph on "Hydrogen Compounds of the Group IV Elements", more than eighty research papers and numerous review articles, and has recently become co-editor, with Prof. R. West of Wisconsin, of *Advances in Organometallic Chemistry*, to be published annually by the Academic Press.

Theoretical Physics at Durham: Prof. B. H. Bransden

FOLLOWING the establishment of a chair of theoretical physics at the Durham Division of the University of Durham, Dr. Bransden, of the University of Glasgow, has been appointed the first holder as from October 1. Dr. Bransden graduated in 1945 from University College, London, with a first-class honours degree in mathematics and physics. After a short period in industry he entered the Department of Applied Mathematics of University College as a research student and obtained his Ph.D. in 1950. From 1951 until 1955 he was a lecturer in theoretical physics at Queen's University, Belfast, and since 1955 has been successively lecturer and then senior lecturer in natural philosophy at Glasgow. He was a research associate at CERN in the session 1960–61. Dr. Bransden's research interests have included light nuclear scattering, and, more recently, pion-nucleon, and pion-pion scattering, and the K^* resonance. He has published many scientific papers on these subjects and is the leader of an active research group.

British Scientific Instrument Research Association: Mr. S. S. Carlisle

MR. S. S. CARLISLE has been appointed director of the British Scientific Instrument Research Association (SIRA). The oldest of the co-operative research associations, SIRA has grown rapidly in recent times, having doubled its staff and income over the past seven years.

Mr. Carlisle, who takes up his appointment on November 1, has been with the British Iron and Steel Research Association since 1946. He became head of its South Wales Laboratories in 1954, returning to the London Laboratories four years later as head of the Physics Department. He became an assistant director of research in 1960. Mr. Carlisle's chief concern in recent years has been the development of the Physics Department's long-range research and development activities in such fields as strip handling by new techniques of fluid cushioning which resulted in development of the Hover Pulley, in-line product inspection technology for modern fast steel manufacturing processes, development of new composite materials such as fibre-reinforced steels and composite coatings. He has also been responsible for co-ordination of work on automation and its application in industry. Mr. Carlisle is a vice-president of the Society of Instrument Technology and has been recently elected to the Council of the Institution of Electrical Engineers.

U.S. Funds for Research and Development

A FINAL report on a survey of research and development funds and scientists and engineers in 1960, issued by the National Science Foundation as *NSF* 63–7 in the *Survey of Science Resources Series*, puts expenditure by industrial firms on research and development at 10,500 million dollars out of a total of 14,000 million dollars, an increase of 10 per cent on 1959, and nearly three times the total for 1953 (*Research and Development in Industry, 1960—Final Report on a Survey of R and D Funds and R and D Scientists and Engineers*. Pp. xii+118. Washington, D.C.: Government Printing Office, 1963. 65 cents). Of this 10,546 million dollars, 385 million dollars (4 per cent) was spent on basic research, 2,062 million dollars (20 per cent) on applied research, and 8,100 million dollars (76 per cent) on development; 4,359 million dollars derived from company sources and 6,117 million dollars from the Federal Government. More than 9,000 million dollars was expended by companies with 5,000 or more employees, and almost another 900 million dollars by firms of 1,000–4,999 employees. Federal funds for such research were 8 per cent higher than in 1959 and more than four times the comparable total in 1953. Company-financed research and development was 11 per cent higher than in 1959 and double that in 1953—chemicals and allied products (848 million dollars), electrical equipment and communication (816 million dollars), motor vehicles and other transport equipment (660 million dollars), and machinery (584 million dollars), accounting for 64 per cent of the 1960 total. The survey indicated that the number of companies with research and development programmes did not appreciably change between 1959 and 1960. The 300 firms with the largest programmes in terms of dollars accounted for 92 per cent of the funds for industrial research and development. About three-fifths of the basic research was in the physical and mathematical sciences, one-fifth in the engineering sciences and the remainder in the biological and other sciences. Guided missiles, communication equipment and electronic components and aircraft and parts accounted for 5,500 million dollars out of the 10,200 million dollars utilized for applied research and development. The full-time equivalent of 307,300 scientists and engineers employed in industrial firms on research and development in January 1961 was nearly two-fifths of the total employed in the industrial sector; 85,500 were employed on aircraft and missiles, 72,200 in electrical equipment and communications, 34,400 on chemicals and allied products, and 31,700 on machinery. Of the total, 79 per cent were employed by firms with 5,000 or more employees, and 10 per cent in companies with less than 1,000 employees. The average expenditure per scientist or engineer in research and development in 1960 was 35,000 dollars compared with 34,900 dollars in 1959, the average figure varying with the size of the firm, from 38,900 dollars in firms with 5,000 or more employees to 19,100 dollars in firms employing less than 1,000.

U.S. Federal Funds for Science

IN the note on *Federal Funds for Science* on p. 1144 of the June 22 issue of *Nature*, the figure of 12,300 million dollars represents the anticipated expenditure on research and development and on plant for that purpose (and not for plant only as stated), the 14,400 million dollars being the amount obligated for these purposes. The figure of 368 million dollars is the increase authorized by Congress over the amount originally in the budget for 1963 and not, as stated, the increase in the amount for the Department of Defence, which was 1,010 million dollars, the largest increase over 1962 reported being 2,000 million dollars for the space programme administered by the National Aeronautics and Space Administration.

Primary and Secondary Education in Britain

IN a written answer in the House of Commons on August 1, the Parliamentary Secretary to the Ministry of Education, Mr. C. Chataway, stated that, during the past year, the Curriculum Study Group had been assisting the Secondary School Examinations Council to establish the new Certificate of Secondary Education examinations. The Group was also collaborating with the Nuffield Foundation in their projects for improving science and mathematics teaching and helping to organize the pilot scheme for teaching foreign languages in the primary

schools, for which the Foundation is sponsoring the preparation of teaching materials, as well as planning an enquiry into the content of the secondary school curriculum. It is also concerned with the proposal now under discussion to form an advisory body concerned with schools' curriculum and examinations, and would be providing professional assistance to the reconstituted Central Advisory Council in their study of primary education under the chairmanship of Lady Plowden.

The Colonial Development Corporation

The annual report of the Colonial Development Corporation for the year ended December 31, 1962, records 110 approved projects at the end of the year, of which eight, totalling an investment of £1·63 million, were new (Pp. vi+95. London: H.M.S.O., 1963. 6*s*. 6*d*. net). These were: in the Far East, the Sarawak Electricity Supply Corporation and Fiji Agricultural Trials; in East Africa, the Chinja and the Metaara Tea Supply Co., Ltd., the Mauritius Housing Corporation, and the Nairobi City Water Supply; and in the High Commission Territories, the Basutoland Electricity Authority and Vuvulane Irrigated Farms. Additional investment of £1,882,000 in existing projects has been approved. Of the committed capital at the end of the year, £15·8 million was in the Caribbean, £20·6 million in the Far East, £23·6 million in East Africa, £22·6 million in Central Africa, £21·3 million in the High Commission Territories, and £13·3 million in West Africa. Of this total, 53·7 per cent was for basic development, 34·5 per cent for primary production and processing, and 11·8 per cent for commerce and industry; for 1961 the corresponding figures are 54·8 per cent, 34·4 per cent and 10·8 per cent, respectively.

The Macaulay Institute for Soil Research

As has been the case with many research institutes, the Macaulay Institute for Soil Research was first accommodated in a country house at Craigiebuckler on the outskirts of Aberdeen. As its activities increased, temporary buildings were erected in the grounds and eventually a completely new building was planned and erected. This was formally opened in September 1962 by Mr. D. L. Macaulay, a son of the founder, and is an excellent example of a modern research establishment, finished in a style compatible with the nature of the work being carried out. Every effort has been made to overcome troubles from chemical corrosion, noise and vibration, and air-conditioning is installed in certain sections.

The report for 1961–62 gives a brief description of the great variety of studies in progress (Pp. 55+1 plate. Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1963). It is sufficient to mention that there are departments of pedology, soil survey, spectrochemistry, biochemistry, plant physiology, microbiology, soil fertility and statistics. But the research programme involves considerable collaboration between departments. For example, investigations on crop performance embrace field, pot and laboratory studies, major and micronutrients and statistical evaluation of the results. A few of the topics dealt with in recent publications concern principles of soil classification, direct-reading spectrochemical methods, natural chelating substances, cation-exchange of plant tissues, phosphate-dissolving micro-organisms, fractionation of organic phosphate, minerals in clays, weathering of basic igneous rock, the genetic soil groups of Scotland, response to fertilizers—all indicative of the rapid development of soil science in the past thirty years.

Disarmament and Arms Control

Disarmament and Arms Control, a new international quarterly journal, has been launched to provide a forum for the discussion of disarmament, arms control and related topics in the belief that well-informed discussion of such issues can reduce the present manifest dangers of mankind (1, No. 1; Summer 1963. Pp. v+1–110. Subscription rates: '*A*', £10 (30 dollars) applicable to libraries, Government departments and industrial establishments; '*B*', £2 (6 dollars) for individual subscribers, who certify to the publishers that the journal is for their personal use. London and New York: Pergamon Press, 1963). Its aim is to encourage the exchange of ideas and experience between workers in different countries, and it is hoped to include a regular section of comments by readers; the periodical is dedicated to the improvement of communication and understanding by seeking to formulate and clarify the new concepts required. For the present it is published in English only, with abstracts of each article in Russian, French and German, but a full translation of any article can be obtained on application to the publishers. The present issue is being distributed by the American Academy of Arts and Sciences to its Fellows and Foreign Members. The first number is admirably printed and produced and its quality is illustrated sufficiently by the titles and authors of its five main articles: "First Step—Sealed Records Caches ?", by J. C. Polanyi; "The Role of the Neutrals in the Geneva Negotiations", by M. Samir Ahmed; "Violations of Disarmament Agreements", by R. J. Barnet; "The International Control of Fissile Material Production"—being a shortened version of a paper contributed by the Atomic Energy Authority to the Disarmament Conference at Geneva in August 1962; "A Proposal for an African and Near-Eastern Zone Free from Certain Weapons", by D. H. Frisch. Besides book reviews, there is a section containing short abstracts of current literature and research. The first issue seems well designed for its purpose and should strengthen the efforts in this field which scientists are already making through the Pugwash movement and in other ways.

Atom

The June 1963 issue of *Atom* completes W. F. Wood's review of the reactors at Harwell commenced in the May issue. In the July issue is printed a lecture by J. F. Putman on economic aspects of the technical application of radioisotopes, which gives some impressive savings. Annual savings in the United Kingdom through the use of radioisotopes are estimated at £3·5 million, but potential savings at £14·5 million, including £2·75 million in the paper and board industries, £1·9 million in chemical industry, £1·05 million in iron and steel, and £1·775 million in electrical engineering. The number of British firms using radioisotopes has increased from 658 in 1957 to about 1,200.

Apartheid and the South African Library Association

The *Library Association Record* for June 1963 includes a note from more than thirty members of the staff of the Johannesburg Public Library giving the text of a resolution passed at a general meeting of the Southern Transvaal Branch of the South African Library Association deploring the decision taken by the South African Library Association Conference in resolving to enforce separate racial associations on the library profession (see *Nature*, 196, 1242; 1962). The resolution sets forth reasoned objections. It maintains that common professional ideals, aims, interests and problems form a bond which transcends differences of age, sex, religion, politics, language, race or colour, and therefore strongly objects to any of these fortuitous differences being utilized to build artificial barriers between colleagues. The Association is numerically small and fragmentation can only weaken the Association and produce separate and even impractically tiny bodies. Since the Government intends to enforce apartheid on profesional associations, it is urged that the action of the Conference was untimely and unnecessary.

Had it waited for the separation to be imposed from outside, no wedge need have been driven between its members, many of whom feel strongly and bitterly on the subject; the library profession would not have inflicted a gratuitous insult and slight on its non-white members and through them on their peoples; professional bodies such as the Library Association and the American Library Association, who would doubtless have offered support and sympathy when Government action enforces the establishment of separate associations, would not have been alienated and antagonized; librarians would not have set themselves apart from professional bodies in South Africa, several of whom have already expressed concern at the inevitable consequences of the Government's proposed legislation; and finally, the Association would not have played into the hands of South Africa's critics at the United Nations and elsewhere.

Documentation of Science Organization

An interesting paper by G. Rózsa on "Documentation of Science Organization as an Emerging New Branch of Scientific Information" has been issued as No. 32 in the *Bibliographical Publications of the Scientific Academy of Hungary* (Pp. 15. Budapest: Magyar Tudományos Akadémia Könyvtára, 1962). Directing attention to the prolific growth of scientific organizations, in size and number as well as in scope, Dr. Rózsa suggests that documentation of scientific organization involves covering the following fields: history, theory, philosophy and systematization of science; scientific policy and government administration of scientific work; international collaboration in scientific work; planning management and organization of scientific work; levels of research; methodology of research work; economic problems of scientific work; management of scientific manpower; scientific personnel; scientific agencies and organizations; and scientific information. The first nine of these divisions he regards as containing the concept, themes and systematization of the necessary documentation, but this, he maintains, does not fit into any of the developed and usual schemes of science systematization, or even library or documentary systematization; however, it is probable that not all his readers are likely to accept the contention that science organization, in consequence, appears as a new branch of science. It is quite clear, however, that Dr. Rózsa uses the term 'discipline' in a sense which differs considerably from that in which it is used in academic circles in Britain or in the United States. He is concerned to see that efficient means are developed of handling the problem of communication or of documentation of scientific organization, whether on a general basis or within a particular field of science.

Magnetic Recordings

A revised bibliography on *Magnetic Recordings*, compiled and edited by R. E. Hadady and published by Kinelogic Corporation, 29 South Pasadena Avenue, Pasadena, California, covers the years 1954–61 inclusive, and lists 775 papers and articles from some 120 technical journals. An author index is included and both the names and addresses of the journals cited are given. The numbers of items for the respective years 1954–61 are 95, 62, 67, 70, 55, 63, 159 and 204, and they show how the interest in the subject of magnetic recording has varied, with a considerable revival and growth in the more recent years. The bibliography will be of interest to those engaged in data recording, data storage and retrieval, recording design, automation, and sound and television recording.

Southern Variable Stars

Astronomy has for long suffered from the lack of observations of objects in the southern hemisphere, and the increased *tempo* of results which has followed the installation of the 74-in. telescopes at Pretoria and Canberra is most welcome. The latest issue of *Monthly Notices of the Royal Astronomical Society* (125, No. 5/6; 1963) contains two contributions on southern variable stars. M. W. Feast (pp. 367–415) describes very extensive observations of long-period variable stars made at the Radcliffe Observatory, Pretoria, during the years 1952–60, when 281 spectra of 114 stars were obtained. Radial velocities were obtained from this material; for only a very few of these stars had similar observations been made previously. The velocities were obtained from both absorption and emission lines. Combination with data for northern stars yielded a very homogeneous set of velocities for 405 long-period variables. These data were subjected to statistical analysis for systematic motions, velocity ellipsoids and galactic rotations. The variables cover a wide range of population types. The shortest period variables have anomalous motions and may perhaps be pulsating in the first overtone. A. W. Rodgers and R. A. Bell (pp. 487–527) report on an analysis made at the Mount Stromlo Observatory, Canberra, of high-dispersion spectra of the southern cepheid variable κ Pavonis. This is a relatively bright star and is probably a member of the disk population somewhat similar to W Virginis cepheids. Variations in the parameters describing the atmosphere of the star were obtained at various phases of the cycle, and the density fluctuations studied. The abundances of the chemical elements in the star were also obtained.

The Zone of Fracture, and the Wave Spectrum of Earthquakes

Dr. Hans Berckhemer has examined this question and written about it in a paper entitled "Die Ausdehnung der Bruchfläche im Erdbebenherd und ihr Einflub auf das Seismische Wellenspektrum" (*Gerl. Beitr. Z. Geophys.*, Bd. 71/1). The amplitude-period spectrum of a seismogram is determined by what happens in the focal zone as well as by the conditions of wave propagation and the characteristics of the seismograph. Dr. Berckhemer describes a method which admits the separation of the influence of the focal process from the others. His investigation covers 20 earthquakes of magnitude from 4·5 to 8. It can be seen immediately that long-period waves are favourably excited by strong earthquakes. The comparison of isoseismal maps suggests that differences in the wave spectrum are caused by the different sizes of the fractured zones at the hypocentre. A simplified theory for a focal process with an extended focus is given in order to explain the observations. The assumptions made concerning the rupturing velocity are based on the results of laboratory experiments. Dr. Berckhemer has found that the relationship $\log F = 0{\cdot}45 + 1{\cdot}7\ M$ between the earthquake magnitude, M, and the size of the fractured area in square centimetres, holds for values of M between 5·5 and 8. A corresponding expression in terms of earthquake energy instead of magnitude is $\log F = 0{\cdot}85 \log E - 5{\cdot}7$. By comparing the last formula with an expression for the elastostatic energy of the fractured area given by Keylis–Borok, it is possible to obtain information about the shape of the seismic fault and to determine the shearing strength of the crust in the focal area (2–5·10^7 dynes/cm^2). Deviations from laboratory measurements may be explained by stress concentration at geological scars within the Earth's crust.

Powder Technology

A course of instruction in "Powder Technology" will be held in the Faculty of Engineering, King's College, University of London, for one year commencing in October. The course is designed for students with a first degree in engineering, physics, chemistry or certain other approved branches of applied science, and its successful completion will lead to the award of a College postgraduate

diploma. Further information can be obtained from the Registrar, King's College, Strand, London, W.C.2. The fee for the course will be £87.

Solid-State Electronics

A POSTGRADUATE course in "Solid-state Electronics" will be held in the Electrical Engineering Department of the Manchester College of Science and Technology commencing in October. The course is available to those with a good honours degree in physics, electrical engineering or related subjects, and will lead to the degree of M.Sc.Tech. by examination and dissertation. The course will last for four terms, the first three being devoted to lectures, tutorials and laboratory work, and the fourth entirely to work on a research project. Further information can be obtained from the Registrar, Manchester College of Science and Technology, Manchester 1.

Methods of Chemical Analysis

A FULL-TIME residential course on "Modern Methods of Chemical Analysis", with specialization in either spectroscopic methods or electroanalytical methods, will be held at Loughborough College of Technology for one year, beginning on October 1. The course will consist of general studies and practical work, and satisfactory completion can lead to the award of the Associateship of Loughborough College. The course is intended as an immediate postgraduate course for those obtaining a degree or equivalent qualification.

The following symposia and courses in modern chemical analysis will be held at Loughborough College of Technology during 1963–64. *One-day lecture symposia:* automatic analysis and control (October 8); modern gas chromatography (October 15); modern infra-red spectroscopy (November 19); modern polarography (February 4); nuclear magnetic resonance and electron spin resonance (March 17). *One-week residential courses:* gas chromatography (October 14–21); infra-red spectroscopy (November 18–25); polarography (February 3–10); atomic spectroscopy (March 9–15); X-ray methods of analysis (April 26–May 2).

Further information can be obtained from the Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire.

University News: London

PROF. R. S. NYHOLM has been appointed to the headship of the Department of Chemistry, University College, in succession to the late Prof. E. D. Hughes.

Sheffield

DR. JOHN STEPHEN FLORRY has been appointed "Glass Manufacturers' Federation" Senior Research Fellow in the Department of Glass Technology at the University of Sheffield as from October 1. Dr. Florry graduated with honours in metallurgy at the University of Sheffield in 1957 and then undertook postgraduate research in the Refractories Technology Department. From October 1960 until the present time he has been a metallurgist at the Material Research Laboratory of the Mullard Radio Valve Co. He was awarded a Ph.D. in December 1962.

Announcements

PROF. C. L. OAKLEY, professor of bacteriology in the University of Leeds, has been appointed a member of the Agricultural Research Council in succession to Prof. J. W. Howie, who retires on September 30.

A MEETING of the German Society of Polar Research will be held in Karlsruhe during October 6–9. The subjects under discussion will be "The Antarctic" and "High Alpine Glaciers". Further information can be obtained from Deutsche Gesellschaft für Polarforschung, 23 Kiel, Eckernforder Strasse 1.

A THREE-DAY postgraduate course on "Electron Probe X-Ray Microanalysis" will be held at the Bristol College of Science and Technology during October 3–5. The fee for the course will be six guineas. Further information can be obtained from K. Humphreys, Bristol College of Science and Technology, Ashley Down, Bristol 7.

A SYMPOSIUM ON "Insect Reproduction", arranged by the Royal Entomological Society of London, will be held at the Imperial College of Science and Technology, University of London, during September 19–20. Further information can be obtained from the Registrar, Royal Entomological Society of London, 41 Queen's Gate, London, S.W.7.

A CONFERENCE on "Generation, Detection and Properties of Coherent Radiation of Wave-length Less than One Millimetre", arranged by the British Radio Spectroscopy Group, will be held in Bournemouth during September 24–25. Further information can be obtained from G. Phillips, Research Division, Signals Research and Development Establishment, Christchurch, Hants.

THE annual general meeting of the Photobiology Group will be held in the University of Cambridge during September 26–27. The programme will include a short symposium on "Woodland Light Climates", together with a number of papers on various other photobiological topics. Further information can be obtained from Dr. L. R. Fisher, National Institute for Research in Dairying, Shinfield, Reading.

A CONFERENCE on "The Physics of Optical Glass", arranged by the Optical Group of the Institute of Physics and the Physical Society, will be held at the Research Laboratories of Pilkington Bros., Ltd., Lathom, Lancashire, during October 3–4. Topics under discussion will include: special glasses; measurements of optical properties; working and testing of surfaces; physical studies of glass; instrumental requirements. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A ONE-YEAR graduate course in "Solid State Electronics Engineering" will be held in the University of Birmingham, commencing October 1. The aim of the course will be to provide a broad and fundamental treatment of this sector of electronic engineering, and it will include groups of lectures on: quantum mechanics; structure of matter and physical properties of materials; theory and applications of solid-state and quantum devices; computers and digital systems; selected topics in engineering mathematics, as well as practical work and a three-month study or research project. Successful completion of the course will lead to the degree of M.Sc. (for degree students) or to the Diploma of Graduate Studies (for non-graduate students). Further information can be obtained from the Registrar, University of Birmingham, Birmingham 15.

THE following short evening courses will be held at Hendon College of Technology: digital computers and their industrial applications (twelve lectures, commencing September 23); a design course for electronic engineers (twelve meetings, commencing October 1); transistors and transistor circuit design (sixteen lectures, commencing October 16); an introduction to microwaves (twelve lectures, commencing September 26); global communications (nine lectures, commencing October 10); high-fidelity sound reproduction (nine lectures, commencing October 9); statistics (two consecutive courses, each of six lectures, commencing September 26 and November 7); electronics for engineers (sixteen meetings, commencing October 29); relativity and space (seven lectures, commencing October 9); introduction to plastics technology (six lectures, commencing October 8). Further information can be obtained from Hendon College of Technology, The Burroughs, Hendon, London, N.W.4.

PHYSICS IN AFRICA

PROBLEMS and opportunities facing physics departments in African universities were discussed at the Leverhulme Inter-Universities Conference held at Salisbury, Southern Rhodesia, during June 10–20. The delegates, drawn mainly from the region between the Sahara Desert and the Limpopo River (with two advisers from the United Kingdom) were of many nationalities—Indian, Norwegian, French and Belgian, as well as British, Nigerian and South African. The institutions which they represented included small and recent foundations (such as Roma, Basutoland, with 173 students) along with others of greater size and seniority.

Many African universities, having grown out of institutions established in Colonial days, retain close links with the mother countries. French influence is still strong at Dakar (Senegal) and Abidjan (Ivory Coast); the University of Lovanium, in Léopoldville, is run on characteristic ally Belgian lines. Several colleges in former British territories were originally in special relation with the University of London; only one (the University College of Rhodesia and Nyasaland) retains this status, the others having all taken steps to secure autonomy. New foundations in Ghana, Nigeria, Tanganyika and elsewhere will, it is expected, grant their own degrees from the outset.

The universities of mid-Africa present a rich diversity of constitution, tradition and academic outlook, but they have many problems in common. The formation of new universities—now proceeding at a rate unequalled even in the United Kingdom—is part of the movement towards independence. It would be an over-simplification to regard the universities in developing countries merely as symbols of national pride, but it must be accepted that the advancement of learning in many African territories is closely linked with the contemporary political ferment.

The first problem discussed at the conference was the role of the universities in an emerging country. The definition offered recently by an African politician ("factories for graduates") expresses one view of the situation. The repatriation of Colonial administrators and experts of all kinds creates serious problems in newly independent countries, where facilities for higher education have often been very limited. It is, however, not certain that university expansion should have first priority in the effort to build up the local supply of professionally trained people. Several universities are short of students, particularly in science, because of deficiencies in the provision of secondary education for African children. Many universities follow the present English practice by requiring candidates for admission to have at least two advanced-level passes in the General Certificate of Education. This is a higher standard than is now required in a number of advanced countries, including South Africa, India, Scotland and the United States. Its attainment in African conditions is extraordinarily difficult. In Southern Rhodesia, for example, 129,000 African children began their primary education in 1962 but fewer than 6,000 were enrolled in secondary schools and only 81 remained at school after taking the Ordinary-level examination for the General Certificate of Education. About half these students may eventually graduate and not all of them are likely to become teachers.

The Conference agreed that expansion of secondary education will be necessary before the universities can play their full part in the communities that they serve. Meanwhile, the universities are under pressure to organize (as some have already done) a preliminary year to bring students of limited academic attainment up to the standard required for entry to a degree course.

The shortage of African students is both the cause and the consequence of the shortage of teachers. Since few countries in the world have enough well-qualified teachers, it is becoming more difficult for African schools to recruit staff from abroad. An imaginative scheme sponsored by the United Nations, at a cost of more than £300,000, will attempt to deal with this situation by bringing several hundred recent graduates, from all over the English-speaking world, to Salisbury. They will study at the University College of Rhodesia and Nyasaland for the postgraduate certificate in education of the University of London and will then teach in various African countries.

The inter-dependence of school and university was discussed further when the conference passed to its second main topic, the teaching of physics to undergraduates. The African child, reared in a culture based on folk-lore and witchcraft (which the white man has disturbed without adequately replacing) does not take readily to the study of science. Having had no contact with modern technology, he finds difficulty in scientific and mechanical tasks which his contemporaries in Europe or North America would consider trivial (one delegate suggested the distribution of 'Meccano' in primary schools as a useful preparation for later scientific studies). The African teenager may even be suspicious of experimental science as an activity more akin to manual labour than to intellectual exercise.

Against this background it is unreasonable to plan for a large output of highly qualified honours graduates—particularly since the abler students can often obtain scholarships to support their undergraduate studies in foreign universities. It is judged that a considerably increased supply of physicists at the level of the general honours or the pass degree would best serve the short-term needs of most African countries.

Although the universities are not yet able to produce great numbers of physicists, there is much to be done in the teaching of physics as a subsidiary subject at undergraduate-level. Reference was also made to the physicist's role in the advancement of technical education. Training courses for technicians are not popular, even where the industries which need the products are already established. The educated African hopes to obtain a degree, which confers much improved status and earnings. Short of a degree, he will often prefer clerical employment to technical work, which is not yet esteemed as the foundation of a 'respectable' career.

The conduct of research was the third major theme of the conference. A physics department in Africa has, as elsewhere, three main tasks: (*a*) the teaching of students; (*b*) the advancement of knowledge; (*c*) the application of scientific ideas and methods to practical problems arising in industry, agriculture, defence and other public activities. An active research effort is, the Conference urged, essential for the fulfilment of all three of these responsibilities. The maintenance of such an effort is difficult. The most serious handicap reported by many delegates was the lack of research students. The output of honours graduates suited for further training is small, and most of them prefer to go abroad for their research experience. On the other hand, expatriate staff are not generally recruited below the lecturer-level. In these circumstances, many African universities are not able to offer the Ph.D. degree. It was agreed that a successful physics department must have some measure of continuity, through the postgraduate training and eventual absorption (as staff members) of its own students. Free movement of scientists is important, but every department should be able to recruit a proportion of its staff from among its own graduates.

Some of these problems will solve themselves as African universities grow in stature and resources. Meanwhile, the Conference recommended, two steps should be taken. First, the institution of an intermediate research degree,

at the M.Sc. level, would be a useful measure towards the growth of participation by African students in the research programmes of physics departments. Secondly, the help of the Department of Scientific and Industrial Research and of other research-sponsoring organizations should be sought in the recruitment of research students from overseas.

In the absence of research students, much of the experimental work in a project has to be done by the lecturers, or by the small number of full-time research fellows. Progress is further retarded by the shortage of technicians. The supply of assistance at this level depends on the provision of secondary education (up to Ordinary-level) in mathematics and physics and also on the development of full-time or part-time technical education to the level of the Ordinary National Certificate or the City and Guilds final examination in appropriate subjects.

Educational facilities of this kind are essential to the development of a healthy industrial economy and are, therefore, of great importance in many African countries. Their provision will be a slow process, and physics departments will have to rely on expatriate technicians—who can do valuable service in the training of indigenous staff.

Physics departments in Africa are isolated in the geographical sense, but they also face the danger of intellectual isolation. Many active areas of modern physics are closed to them, through lack of funds for equipment and staff. Contacts with other physicists are also very limited. Chemists and biologists can be found in most countries, going about their normal work in industry, agriculture or Government service. Physicists are needed mainly in industrialized communities; consequently teachers in African universities can seldom sharpen their wits by contact with practising physicists in the outside world. African universities in general make reasonable provision for overseas leave; the Conference emphasized the value of attendance at scientific conferences and of frequent discussion with visiting research workers.

Despite many obstacles and discouragements, a lively spirit of enquiry is evident in many physics departments. Good use is being made of the excellent local opportunities for research in geophysics (including seismic studies and palæomagnetism), meteorology, cosmic radiation and ionospheric investigation. The cost of this research is largely defrayed by European or American sources or by international organizations.

Physics departments in Africa face some of the difficulties which were known in English provincial universities forty years ago. Student numbers are small (one African university has 186 first-year students in history, but only 15 in physics), because physics is not yet seen to have any immediate relevance to the needs of the community. The teaching load is nevertheless heavy. Funds for equipment are meagre and research is a constant struggle. British physics advanced rapidly as a consequence of the Second World War; the political upheaval which is now transforming the African scene must, for its full consummation, be accompanied by an upsurge of science and technology. Physicists now working in African countries are fully alive to their responsibilities at this exciting time.

J. M. A. Lenihan

FREE RADICALS

The sixth International Symposium on Free Radicals was held in the Department of Physical Chemistry in the University of Cambridge during July 2–5. The meeting had an extensive scientific programme; but pre-printing of the papers made it possible to avoid parallel sessions. The delegates were accommodated in Trinity Hall, Sidney Sussex and Downing Colleges, and the social functions, which included a reception in the Guildhall and a guest night dinner held in St. John's and Downing Colleges, provided plenty of opportunity for discussions. The three hundred delegates included Dr. W. A. Noyes, jun. (president of the International Union of Pure and Applied Chemistry), Academician V. N. Kondratiev, Prof. S. Claesson, Prof. P. A. Giguère, Dr. G. Herzberg, Academician W. Kemula, Dr. Bernard Lewis, Dr. F. O. Rice and Prof. G. Semerano.

The inaugural lecture was given by Prof. R. G. W. Norrish, who discussed the many ways in which kinetic spectroscopy and flash photolysis have contributed to the understanding of the properties of free radicals.

As with previous symposia, the emphasis of the plenary lectures and contributed papers was primarily on free radicals in the liquid and solid states, although H. Schüler and G. Arnold (Göttingen) discussed the dissociation of *cyclo*hexane and *n*-butane into different radicals and diradicals by slow electrons in the positive column of a glow discharge. Electron bombardment in the gas phase was also used by R. Marx, S. Leach and M. Horani (Orsay, Paris) to produce free radicals from water. The radicals which could be condensed from an electric discharge through water vapour were also discussed by P. A. Giguère (Laval, Quebec). The closely related problem of using electron spin resonance to identify the radicals produced in the radiolysis of ice and hydrogen peroxide–water systems at low temperatures was discussed in a plenary lecture by R. Livingston (Oak Ridge, Tennessee) and in papers by D. Schulte-Frohlinde and K. Eiben (Karlsruhe), I. Safarik, B. C. Green and J. W. T. Spinks (Saskatoon) and S. J. Wyard and R. C. Smith (Guy's Hospital, London). The existence of hydrogen superoxide (H_2O_4) is not unequivocally established in these systems, but there is clear evidence for the trapping of OH and HO_2. The nature of the active species in the radiolysis of aqueous solutions was also discussed in a plenary lecture by J. Weiss (King's College, Newcastle upon Tyne).

Several papers were concerned with the identification and study of the electron spin resonance spectra of trapped radicals produced by irradiation of both organic and inorganic solids. It was significant that a high proportion of the work reported was carried out on single crystals. These papers were preceded by a plenary lecture on the interpretation of hyperfine coupling by D. H. Whiffen (National Physical Laboratory, Teddington), and the parent compounds studied included persulphates by P. W. Atkins, J. A. Brivati, A. Horsfield, M. C. R. Symons and P. A. Trevalion (Leicester); nitrates by J. Cunningham (Chicago) and R. M. Golding and M. Henchman (Cambridge); fluorinated organic compounds by J. K. Brown and D. S. Stiles (Birmingham); succinimide by N. M. Atherton (Sheffield); olefines and alkyl halides by P. B. Ayscough and H. E. Evans (Leeds); organic acids by R. J. Cook, J. R. Rowlands and D. H. Whiffen (National Physical Laboratory, Teddington); and acetylene by E. L. Cochran, V. A. Bowers and F. J. Adrian (Applied Physics Laboratory, Johns Hopkins).

Three contributions described the use of electron spin resonance to examine reactions of radicals produced by irradiating both hydrocarbon polymers (H. Fischer, K. H. Hellwege and P. Neudörfl (Darmstadt) and A. Charlesby, C. Graves, P. G. Garratt and P. M. Kopp (Royal Military College of Science, Shrivenham)) and polymethylsiloxane (H. Ormerod (Royal Military College of Science, Shrivenham)). Electron spin resonance was one of the many techniques mentioned by C. H. Bamford (Liverpool) in his plenary lecture on radical polymerization in the solid-state. The unusual reactivity and exceptional surface area of fluffs obtained by freeze-

drying polymer solutions were reported by R. B. Ingalls (North American Aviation) and L. A. Wall (National Bureau of Standards, Washington). Surface properties were also considered by V. B. Kazanskii and G. B. Pariiskii (Moscow), who were able to obtain electron spin resonance spectra of free radicals adsorbed on surfaces of insulators but not of semiconductors.

The electron spin resonance spectra of organic diradicals such as substituted methylenes were reported by W. A. Yager, K. W. Murray, G. Smolinsky, A. M. Trozzolo and E. Wasserman (Bell Telephone Laboratories).

Chemical reactions studied in the solid-state using electron spin resonance spectroscopy included the benzene photosensitized decomposition of hydrocarbons (N. V. Fock, B. N. Shelimov and V. V. Veovodskii (Moscow)), the reaction of ethylene or oxygen using an ingenious rotating cryostat (J. E. Bennett and A. Thomas (Shell, Thornton)), and the photolysis of methyl iodide at $\lambda = 2537$ Å, where the methyl radicals rapidly extract hydrogen from the methyl*cyclo*hexane cage (J. M. Flournoy, H. S. Judeikis (Aerojet, California)).

Chemical analysis was used by R. H. Duffett and G. J. Minkoff (British Petroleum, Sunbury) to examine the reaction of hydrogen and deuterium atoms with solid olefines. Fluorescence spectra of benzyl radicals in *cyclo*hexane glasses were studied by L. Grajcar and S. Leach (Orsay, Paris) to obtain information about the structure of the solid-state. For their studies oxygen in liquid and solid argon, A. M. Bass and H. P. Broida (National Bureau of Standards, Washington) used ultra-violet spectroscopy. The formation and decomposition of purple sulphur at low temperatures was illustrated with a colour film by F. O. Rice (Notre Dame, Indiana).

It is more difficult to maintain significant concentrations of reactive species in the liquid state than in the solid phase. Three of the papers, however, reported electron spin resonance investigations of short-lived radicals under liquid conditions. The use of a fast-flow system in which the reactants were mixed close to the sampling cell was reported by P. L. Kolker, T. J. Stone and W. A. Waters (Oxford), who produced aromatic radicals by oxidation of phenols, and by W. T. Dixon and R. O. C. Norman (Oxford), who formed various aliphatic radicals using titanous salt–peroxide systems. L. H. Piette (Varian, California) showed that both rapid response and good sensitivity would be obtained with an electron spin resonance spectrometer on a statistical basis. Electron spin resonance studies of radicals produced by the photolysis of electron-donor–acceptor system were reported by C. Lagercrantz (Göteborg) and the spectra of terphenyl anions by K. H. Hausser, L. Mongini and R. van Steenwinkel (Euratom, Ispra). The production and properties of ketyl radicals formed by polarographic reduction were described by Z. R. Grabowski and M. K. Kalinowski (Warsaw).

The importance of diffusion of radicals in solution was discussed in papers by J. E. Guillet and J. C. Gilmer (Tennessee Eastman) and A. D. Osborne and G. Porter (Sheffield) respectively. The former showed that solvent cages are important in the decomposition of diacyl peroxides and that the activation energies for radical escape from a cage and for diffusion are identical. The latter workers showed, by studying the decay of active species in solvents of different viscosity, that the Debye equation is valid when the reacting species do not differ greatly in size from the solvent molecules.

This stimulating meeting was assisted by the Royal Society, by many British industrial firms, and by the provision of transport for many North American Delegates by the United States Air Force. It is planned to hold the seventh Symposium in 1965 in Italy, probably in Venice.

B. A. Thrush

INSPECTION OF ALKALI AND CEMENT WORKS

THE ninety-ninth annual report on alkali, etc., works in England and Wales covering the year 1962 by the Chief Inspector * notes the extension by the responsibilities of the inspectorate by the Alkali, etc., Works Order, 1961, to cover control of emissions from calcination of black liquors from paper manufacture and from operations involving uranium, beryllium, selenium and their compounds. At the end of 1962, 2,077 works were registered under the Act, involving the operation of 3,247 separate processes, and 10,768 visits and inspections were made during the year, including 140 special visits by the Chief and Deputy Chief Inspectors. During the inspections, 4,331 quantitative analyses were made of gases evolved from processes, compared with 6,992 in 1961, but the latter total included 3,065 tests taken during a special investigation into emissions from the salt glazing of earthenware.

In addition, 647 special samples were taken and submitted for analysis to the Government Chemist. Specific complaints investigated during the year, largely at the request of local authorities, concerned 297 works, compared with 283 in 1961, 292 in 1960 and 354 in 1959, and of these 297 works 233 were registered under the Act, and the complaints were most numerous against gas and coke works (47), ceramic works (41), electricity works (33), iron and steel works (27), aluminium works (14) and cement works (13). The degree of justification for complaint varied, and once again was most bitter against some of the smaller works. There was also some general complaint against overall emission in particular areas rather than against any specified works, and of the 16 examples include the blue-brick making area of Staffordshire and The Thames and Medway cement-making areas. Listed infractions, numbering 10, were again confined to the pre-1958 processes and remedies were applied on formal notification by the inspector without institution of proceedings. The root cause of the trouble in the Thames cement-making area was the approaching exhaustion of available Eocene clay supplies in North Kent and the consequent use of ever-increasing proportions of estuarine clay from the Cliffe marshes, and the resulting increased dust burden in the kiln gases has in turn led to overloading and erratic operation, or even failure, of electrical precipitates. Planning consent was sought to win sufficient Eocene clay from Essex to replace all the Cliffe clay in use and at the end of the year only one of the four works on the south bank of the Thames was on Cliffe clay. The report includes an interesting historical review of the evaporation of lime in open pans, a process for common salt now practically out of use and there is also a brief discussion of the problem of emissions from the Trophas steel converter. Although the Inspectorate is still much preoccupied with grit, etc., emissions from the intermediate-sized coal-fired power stations, considerable progress can be recorded in modernization and replacement of grit and dust collection plant, and there are reasonable grounds to believe that such emissions will be reduced to a satisfactory level, but concern is still expressed over the minimization of emissions of acid-soot from oil-fired power stations.

The annual report for 1962 of the Chief Inspector for Scotland, published under the same cover, records a decrease in the number of registered premises from 297

* Ministry of Housing and Local Government; Scottish Development Department. Alkali, etc., Works Regulation Act, 1906, and Alkali, etc., Works Orders 1928–1961. Alkali, etc., Works Regulation (Scotland) Acts, 1906, 1951, and Alkali, etc., Works (Scotland) Orders, 1952 and 1958). Ninety-ninth Annual Report on Alkali, etc., Works by the Chief Inspectors, 1962. Pp. 75. (London: H.M.S.O., 1963.) 5s. net.

to 285, chiefly due to closure of gas works by extension of the grid system of distribution. The 575 visits of inspection included 36 by the Chief Inspector and there were 142 further miscellaneous visits and 64 visits not under the Acts. The dust-testing team from the Department of Scientific and Industrial Research completed during the year 20 months of its three-year contract period and made 28 tests of dust emission from registered premises. The work has shown that circumstances in field work may require substantial modification of procedures laid down in accepted standard methods for dust measurement. Maintenance of operation of plants to prevent discharges of noxious and offensive gases from registered premises was generally satisfactory.

THE BRITISH COKE RESEARCH ASSOCIATION

THE nineteenth annual report of the British Coke Research Association* is noteworthy for the record of continued expansion and progressive research undertaken during the year 1962. One immediate objective has been the direction of attention to the more practical aspects of the results of research. This has been stimulated by the Association's participation in the Special Assistance to Industry Scheme, sponsored by the Department of Scientific and Industrial Research. The use of films, a fully equipped mobile laboratory and an expansion of the technical liaison and advisory services are now additional means of 'putting over' the great amount of 'know-how' which has been learned during past years about the correct methods of sampling, sample-preparation, physical testing and analysis of coke.

Naturally, with the competitive challenge of alternative fuels and of alternative methods of metallurgical practice, the question of the supply and demand of fuels manufactured from coals must constantly be a matter for vigilance and close examination. In recent years there have been changes in the demand for blast-furnace coke, partly as a result of the reduction in the production of steel. The demand has also fallen for the technical reason that blast furnaces now use less coke per ton of pig iron produced, because of the extension and improvement of sinter practice and the successful application to blast-furnace operation of the injection of fuel oil and of powdered coal. Given, however, a reasonable level of expansion of the national economy, the demand should not change much in coming years. Moreover, it has been concluded that the blast furnace will remain the principal producer of iron in the foreseeable future. While the coke-oven process, with improvements, will continue to be used to meet the demand for blast-furnace coke, there is, however, a possibility of a new process being developed, using cheaper coal and giving a product of uniform shape and size, described as a 'formed coke' which might prove to be more economic in use than the conventional product. Depending on capital and running costs, such a process might be economically competitive with the coke-oven process. The development of a formed-coke process, involving the erection of costly prototypes, would undoubtedly prove to be enormously expensive and might possibly be best pursued by international co-operation. The Russians and the Poles, among others, have devoted much time and money in recent years to this question. It is very satisfactory to learn that the British Coke Research Association is well aware both of the possibilities and the difficulties of adopting such a process, which in the event may best be suited to localities of the world remote from supplies of good coking coal.

A formed-coke process should provide means for the supply of a solid fuel tailored to the future requirements of the blast furnace; but the Association in fact is mounting a two-headed attack on this difficult question of new types of fuel for the iron and steel industry. The other possibility which is being investigated is that of influencing the properties and more particularly the size of coke produced in the conventional coke-oven by controlling the fissuring process during carbonization. Undoubtedly news of developments in both these fields will be awaited with great interest.

Passing to the record of the research achievement, painstaking effort, not always rewarded with equivalent positive result and calling for much detailed experiment, has continued in the general study of additions to the coal blend for carbonization. Examples of the additions in question are coal-tar pitches, petroleum residues, breeze and heavy petroleum oil. Nevertheless, results have arisen that are worth all the effort. Thus in the case of the possible substitution of breeze for a Welsh coal it was found that 3 per cent of fine breeze could replace the most costly component of the blend while providing coke of similar characteristics. Again, in respect of the use of sodium carbonate, the addition of which is known to improve the reactivity of coke for domestic purposes, with a 0·75 per cent addition of this salt before coking not only was the reactivity improved but also the size of the coke and the resistance to impact increased, though the resistance to abrasion fell considerably. Other features of economic importance have been revealed, for example, the determination of the end-point of carbonization, at which the 'soaking' of the charge has reached its optimum in terms of the useful properties developed in the coke.

The provision of means for carrying out coking trials at various ranges of capacity, from a full scale involving a 10 tons charge to as small a quantity as 15 lb., has apparently paid off handsomely, for one reads of trials that are necessary in the large oven and others of equal importance being run in the small ones. Further trials of new blends may be run in the large oven, when it has been established in the small ovens that an improvement is likely and that there is no danger of coking pressures large enough to damage the oven.

Since the earliest use of coke for iron production, the demand for metallurgical coke of low sulphur content has persisted and with the progressive exhaustion of the best coking coals the problem of sulphur impurity has remained of growing importance. More particularly does the difficulty arise in respect of the behaviour of the organic sulphur constituents. The objective is, of course, to discover whether these reactions may ultimately be capable of being controlled. In a very complete examination of the sulphur–carbon complexes to be found in coke, involving the most modern techniques, electron-spin resonance measurements among others, it has been concluded tentatively that the surface sulphides make an important contribution to the organic sulphur content of coke and that dehydrogenation by sulphur, and the interaction of sulphur with free radicals present in the coke, contribute to the formation of these complexes during the coking process. Their preservation in the coke seems to depend on their being of stability greater than that of their carbon–oxygen counterparts.

The Applied Studies Laboratory at Chesterfield has been concerned particularly with problems bearing on the thermal behaviour and fissuring of coke, methods of analysis and measurement and the use of oven coke in domestic appliances. The housewife requires a coke of ready combustibility, that is to say, she wants a fuel that

* Nineteenth Annual Report of the British Coke Research Association, 1962. Chesterfield, Derbyshire.

lights easily and does not go out overnight; accordingly, the questions of coke reactivity and size become the pertinent issue and the metallurgical requirement is the direct contrary of the domestic need. There are, however, situations in which hard coke may suffice for the domestic boiler; they may be defined by suitable experiment.

Progress has been made in the Association's contribution to the provision of cheap and effective methods of liquid-effluent treatment and measures to be taken for the reduction of the emission of smoke, dust and grit at coke ovens. With regard to the former, the Association is supporting, both financially and technically, basic research at the University of Leeds into the biological treatment of gas liquors. Considerable strides have been made in the study of the activated sludge process as applied on a coke-oven plant and of the relative merits of percolating filter processes. It is most interesting to know that these microbiological processes can destroy such undesirable constituents of waste liquors as phenols, cyanides and thio-salts, including thiocyanates, substances which by their poisonous activity can persuade even the scientists to describe some of them as 'devil liquor'. Perhaps even more interesting to the layman is the fact that spoil heaps may be brought into service as percolating filters, and where they also have lagoons these may serve as means of aeration. To bring the rejected refuse of the mine back as an active agent in solving surface effluent problems is indeed to raise slumbering devils to serve mankind. The genus *Thiobacillus* would appear to give much scope for study, since preliminary results indicate that the organisms reported in the literature are only a fraction of those that exist in Nature. In the matter of atmospheric pollution much has been done in the mitigation of air pollution as the result of the training of coke-oven staff and operatives.

In the associated work of the Northern Coke Research Laboratory the tradition of the devotion to fundamental studies has been sustained. They involve methods of adsorption and microwave spectroscopy. The objectives include the unravelling of the mechanism of the adsorption on carbon of oxygen, carbon dioxide and nitrogen under controlled conditions. It is a problem which has challenged the scientific world since the earliest physical chemists applied the methods of chemical kinetics to the basic problems of combustion. The latest methods permit rapid determination of surface area, an essential parameter.

Coke science is not like nuclear science, a subject which makes popular news, but progress which is respected by that section of the scientific world able to appreciate the problems involved is just as worthy of recognition. The Council of the Association, its director, Mr. G. W. Lee, his colleagues and staff are therefore indeed to be congratulated on their achievements. R. J. SARJANT

GEOMETRICAL OPTICS

THE Optical Society of America, at its Rochester meeting in October 1962, awarded the Frederic Ives Medal for distinguished work in optics to Dr. M. J. Herzberger, the head of the geometrical optics department and senior research associate, Research Laboratories, Eastman Kodak Co. The Medal was presented on October 4 by D. L. MacAdam, the president of the Optical Society. A list of some 150 of Dr. Herzberger's publications is given in the June issue of the *Journal of the Optical Society of America* (53, 659; 1963) together with the text of his address entitled "Some Recent Ideas in the Field of Geometrical Optics". Dr. Herzberger discussed three particular optical problems on which he had worked—the analysis of optical images, the improvement of achromatic systems, and the algebraic method of designing optical systems. The use of spot diagrams to describe image formation in optical systems is illustrated by application to the analysis of the so-called 'rim-ray curves' and the four-element air-spaced compound triplet, an *f*/5, 300-mm 'Tessar'-type lens, is taken as a numerical example. The method will appeal to those who prefer to think in pictures rather than numerically. In 1959, Dr. Herzberger discovered that it was possible to design lenses, with or without fluorite, which were corrected for colour over a wide range of wave-lengths. The fundamental idea is to consider the refractive index n, and not its square, as a characteristic physical quantity, and to utilize the dispersion formula:

$$n = A + B\lambda + C/(\lambda^2 - \lambda_0^2) + D/(\lambda^2 - \lambda_0^2)^2$$

where λ_0 is a constant which for the wave-length range 365 mμ to 1·01μ and 176 glasses and other transparent substances used in optical design has the value 0·168μ. Four data are therefore required to fix the dispersion of an optical substance in the visible region. The data suggested are discussed, and it is shown that two glasses can be used to design an achromat, and if three lens elements made of different glasses are used a super-achromat can be designed which is corrected for four colours.

The basic mathematical problems associated with the algebraic design of optical systems, that is without ray tracing, have recently been solved by Dr. Herzberger, and the work remaining consists only of the elementary, but tedious, job of the algebraic solution of certain linear equations with algebraic coefficients and the same non-zero determinant for all orders. In his address Dr. Herzberger explained the meaning of the 'characteristic function' or 'eikonal' and posed the two questions, the answers to which would reduce the designing of lenses to pure mathematics. He then outlined the procedure adopted to determine the focal eikonal to any desired order for an image formation given by a single thick lens with two aspheric surfaces. Dr. Herzberger concluded by saying that he felt confident that it would not be long before the brain achieves a decisive advantage over the computer in the design of optical systems.

FLUOROCARBONS

THE origin of the organic fluorochemicals industry lay in the refrigeration industry wherein, following several fatal accidents, there arose an urgent demand for a non-inflammable, non-toxic, low boiling-point refrigerant. The pioneers working for General Motors were Drs. McNary, Midgley and Henne, who carried out research on compounds of carbon, fluorine and chlorine, particularly CCl_2F_2 made by the action of SbF_3 on carbon tetrachloride. Prof. M. Stacey of the University of Birmingham contributes an interesting article on this subject in the Spring 1963 issue of *Catalyst*, the excellently written and illustrated industrial journal of the Shell Chemical Co., London. "Success was immediate and dramatic", says the author, "and, in 1931, in collaboration with Du Pont, General Motors formed the corporation Kinetic Chemicals which began the commercial production of a range of colourless odourless non-toxic and non-inflammable fluorochlorocarbons which were named the Freons".

These compounds derive from the reaction of anhydrous hydrogen fluoride on carbon tetrachloride, chloroform or ethylene. There are five major ones, CCl_2F_2, CCl_3F, $CHClF_2$, $C_2Cl_3F_3$ and $C_2Cl_2F_4$; these together constitute 95 per cent of the fluorochlorocarbons at present being used in industry. To-day they are produced in fourteen different countries and are severally known by titles such as 'Freons', 'Arctons', 'Isecons', 'Genetrons', 'Foranes', 'Eskimons', etc. World annual production is now in the region of 150,000 tons.

Besides refrigerants, however, there are other important applications of and developments from these fluorocarbons. One example is found in certain pressure packaging 'aerosols'. "As aerosol propellants, their production has risen dramatically in ten years from about 40 million units (cans) to 500 million units, and world production is still growing". Another development is a remarkable plastic based on tetrafluoroethylene ($CF_2=CF_2$). In its polymerized form, $(CF_2)_n$, this plastic possesses quite exceptional properties; it is chemically inert to solvents, acids, alkalis, oxidizing agents, steam, lubricants, etc.; at temperatures up to 300° C only fluorine gas and molten alkali metals attack it. As polymerized tetrafluoroethylene is thermally stable up to 300° C and has a constant and low power factor, it is especially valuable for electrical uses also in highly corrosive environments.

Prof. Stacey states: ". . . a number of new oils and plastics are now made from trifluoro chloroethylene and co-polymers. Various fluoro alcohols are being developed as monomers for polymers, and the esters of these alcohols are being used as stable lubricants". On a practical laboratory scale, it is found that the life of ground glass joints can be prolonged by covering them with polymerized tetrafluoroethylene sleeves as a protection against action of corrosive liquids. The article also gives details of the biological developments of these compounds and concludes: "Thus it will be seen that potentialities for synthesis are even greater than in the vast area of coal tar chemistry. There are prospects for new dyestuffs . . . new drugs and medicinals, as well as new materials for the study of reaction mechanisms".

H. B. Milner

REDUCTION OF OXYGEN AT A CARBON ELECTRODE EMERGING FROM AN ELECTROLYTE

By SAM R. FARIS

Socony Mobil Oil Co., Inc., Field Research Laboratory, Dallas, Texas

P. HERSCH[1] has described an experiment in which the corrosion of a metal is facilitated by the presence of a three-phase boundary between metal, electrolyte and the atmosphere. Weber[2] investigated the transport of oxygen to metal cathodes. He found that metal cathodes afforded higher current densities when partially submerged. Will[3] examined the oxidation of hydrogen at platinum electrodes. He found that a large increase in current occurs when the electrode is partially immersed in the electrolyte. The entire electrode was wetted by the electrolyte and no three-phase boundary existed; the oxidation occurred almost exclusively in a narrow region at the upper edge of the electrolyte meniscus and the adjacent film close by.

The purpose of this article is to describe similar experiments in which oxygen reduction at porous carbon cathodes is enhanced by raising the electrodes in the electrolyte.

One of the factors which limits the capacity of a fuel cell is mass transport of oxygen to the cathode. It is generally believed that oxygen first dissolves in the electrolyte and then diffuses to the surface of the cathode for reaction. It is desirable to have an electrode of large surface covered with a thin film of electrolyte in which the dissolved oxygen reaches this surface by diffusion along a short path. Establishment of large extents of this electrolyte film of optimum thickness is one of the difficulties encountered in the preparation of fuel cell electrodes.

A porous carbon electrode partially submerged in an electrolyte which wets it should have on it a film of varying thickness. An electrode under these conditions should show a larger current capacity than when it is completely submerged. This increase in current has been observed for porous carbon cathodes at which oxygen is being reduced in an electrolyte of aqueous potassium hydroxide.

The electrolyses were carried out in an H-shaped glass vessel with separate electrode compartments. The electrode assemblies were held in place by rubber stoppers. The anode was a porous block, 2·5 × 2·5 × 7 cm, made of Stackpole No. 139 carbon. The cathodes were made of porous carbon rods, 6–7 cm long, with circular cross-sections. These cathodes were sealed in U-shaped holders of glass with either epoxy resin cement or Corning '*RTV*-731' silicone rubber. The cathode compartment of the electrolysis cell was provided with a tube for entry of oxygen into the catholyte via a fritted glass gas dispersion tube. Another tube permitted oxygen to enter only the vapour space above the electrolyte. A saturated calomel electrode was used as the reference electrode. The electrolyte was aqueous potassium hydroxide of density 1·302 g/cm³ at 25° C (a nominal 40 wt. per cent solution). The potassium hydroxide solutions were prepared with A.C.S. reagent grade potassium hydroxide pellets using water having a specific resistivity of 2·0 – 4·0 × 10^6 ohm cm. The oxygen was U.S.P. grade twice washed with water before use. Fig. 1 illustrates the principal parts of the electrolysis cell.

After assembly of the cell with the cathode in the submerged position, oxygen, saturated with water vapour, was bubbled through the potassium hydroxide solution for a period of 2–4 h. The oxygen was then arranged

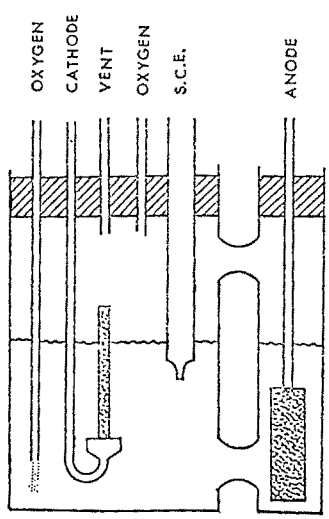


Fig. 1. Electrolysis cell

not to bubble through the electrolyte but to sweep continuously through the vapour space above the electrolyte.

The potential of the cathode was held at − 1·0 V versus the saturated calomel electrode with an electronic potentiostat. The current flowing through the electrolysis cell was continuously measured and recorded by a strip-chart recorder. To raise the cathode, electrolyte was pipetted from the cathode compartment or the glass electrode holder was raised. Both methods were used. The height of cathode raised above the free surface of the electrolyte was measured with an Ealing 11–550 cathetometer. At all times during the experiments the anode remained submerged. The temperature of the electrolysis cell was maintained constant to ± 0·1° C in a water bath.

Two kinds of carbon were used to make cathodes: Stackpole No. 139 with an apparent density of 1·35 g/cm³ and surface area of 0·72 m²/g; and Great Lakes carbon Type *HPL* with an apparent density of 1·73 g/cm³ and surface area of 0·18 m²/g. The surface areas were measured by the Brunauer–Emmett–Teller method using nitrogen as the adsorbate.

In Fig. 2 are shown the current versus height of emergence data for the two carbons used as cathodes in aqueous potassium hydroxide at 30° C. While the cathodes are submerged a small current of the order of 0·1 m.amp flows. As the cathode is raised in the electrolyte the current rises rapidly to a limiting value. The third curve shown in Fig. 2 was obtained for a Great Lakes carbon cathode after it had been made 'wet-proof' by painting it with a solution of paraffin in isooctane. After evaporation of the isooctane, a thin layer of paraffin coated the cathode. On emergence this treated cathode was not wetted by the electrolyte. These electrodes were rod-shaped, 6–7 cm long and with circular cross-sections of 0·62 cm diameter.

A cathode of Stackpole No. 139 carbon, 0·64 cm diameter and 6·0 cm long, was used in the electrolysis cell with air sweeping through the vapour space above the electrolyte. The air had been filtered and saturated with water vapour before it was introduced into the electrolysis cell. The results obtained at 30° C are shown in Fig. 3 and are there compared with the results for the same electrode with oxygen sweeping through the vapour space above the electrolyte.

Current versus height of emergence data were obtained for Stackpole No. 139 carbon cathodes at 10° C intervals over the temperature-range 10°–40° C. The currents increase as the temperature increases. Fig. 4 shows the

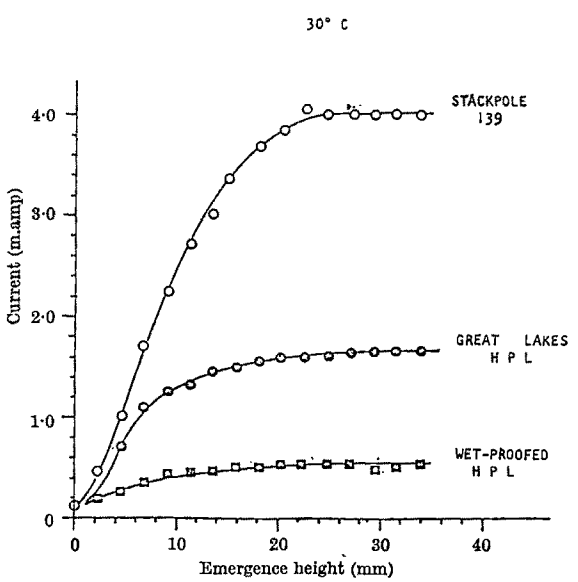


Fig. 2. Current versus emergence height for carbon cathodes

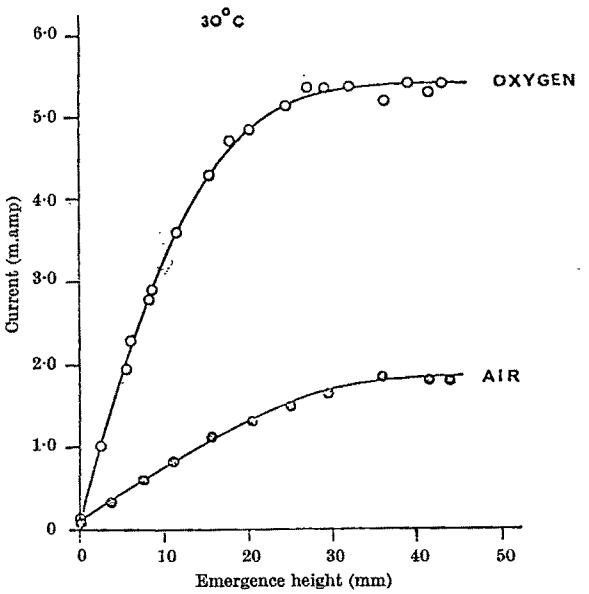


Fig. 3. Use of air in vapour space

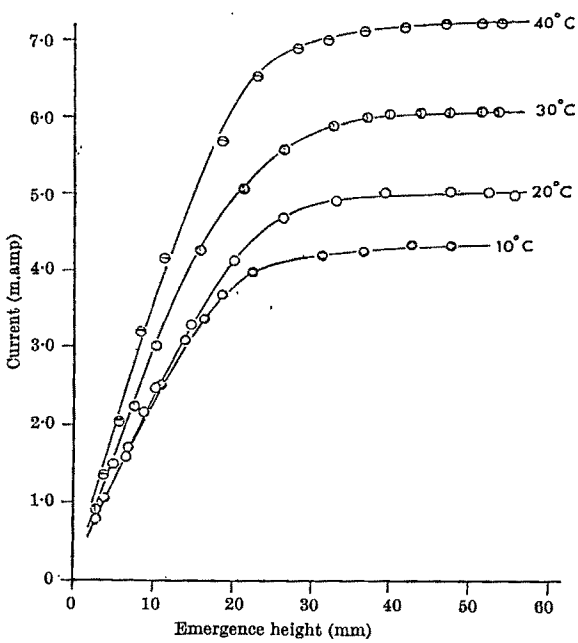


Fig. 4. Variation of current with temperature

variation of current with temperature for a cathode 7·0 cm long and 0·62 cm diameter. Additional results to illustrate the variation of current with temperature are given in Fig. 5, where the logarithm of the limiting current value at a constant value of height of emergence (46·6 mm in this case) are plotted against $1{,}000/T$ °K over a temperature-range of 10°–50° C. In the temperature-range 10°–35° C the variation of the logarithm of the limiting current with $1/T$ is approximately linear.

The slope of the linear portion of the curve in Fig. 5 corresponds to an activation energy of about 3·6 kcal/mol. This value is of the same order of magnitude expected for the energy of activation for diffusion of oxygen in the electrolyte.

Similar data for the variation of current with temperature for electrodes submerged in the electrolyte are difficult to obtain. These currents are erratic and not reproducible. A number of measurements of the current with the cathode submerged were made at 30° C and the current values varied from 0·12 to 0·40 m.amp.

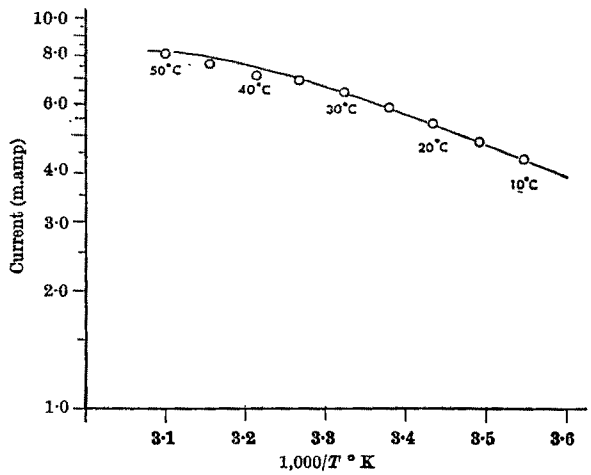


Fig. 5. Variation of current with temperature

The very small values of current for the submerged electrodes are very nearly the maximum to be expected from the diffusion of dissolved oxygen in the bulk electrolyte to the electrode surfaces. On raising the electrode a thin film of electrolyte is created through which oxygen transport by diffusion is considerably increased. As withdrawal continues the current increases to a limiting value. This indicates that the extent of optimum thickness electrolyte film is limited and does not increase with emergence beyond a certain value. Towards the top of the emergent electrode the electrolyte film is so thin that ionic mobility is hindered and the electrical resistance in the film has increased. In Fig. 6 the film of electrolyte above the point *A* on the emerged electrode is too thin to afford adequate ionic conduction. Consequently, this extent of electrolyte film above *A* contributes nothing to the current capacity of the electrode.

The electrolyte film located between points *A* and *B* on the emerged electrode is sufficiently thick to provide adequate ionic conduction but thin enough to allow rapid transport of oxygen by diffusion. When the electrolyte film between points *A* and *B* has been formed by raising the electrode, the limiting value of the current has been reached. Electrolyte situated below point *B* on the electrode is essentially the same as that encountered by the submerged electrode. According to Fig. 4, for example, the 'band' of optimum electrolyte film thickness between points *A* and *B* is formed at a height of emergence of about 3·0 cm for Stackpole No. 139 carbon cathodes. To raise these electrodes further only produces a film of electrolyte too thin to contribute to the current capacity of the electrodes.

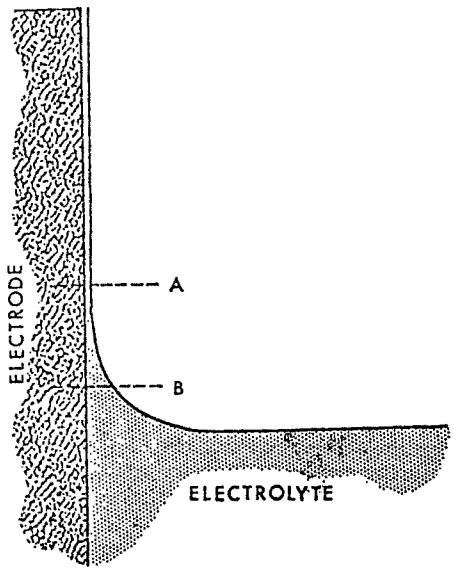


Fig. 6. Diagram of electrolyte meniscus and film

Unless the electrode is wetted by the electrolyte no film (or at best an insufficient film) is formed on emergence. This is clearly shown in Fig. 2 for the 'wet-proofed' cathode made of Great Lakes carbon. For the 'wet-proofed' cathode the limiting current was about 0·5 m.amp compared with a limiting current of about 1·7 m.amp for the untreated cathode. Both cathodes exhibited a current of about 0·1 m.amp when submerged. Even the small limiting current of 0·5 m.amp is probably the result of incompleteness of the 'wet-proofing' procedure. Presumably if the entire surface of the electrode were coated imperviously with paraffin the electrode would be a non-conductor so far as ionic conduction is concerned and an open circuit would result. For the Great Lakes carbon cathodes the *A* to *B* region of electrolyte film is formed at a height of emergence of about 2·0 cm. The Great Lakes carbon is a 'smoother' carbon than Stackpole No. 139 and has only about one-fourth the specific surface area.

The results of these experiments reinforce the belief that oxygen transport by diffusion through the electrolyte is the slow or current limiting step in the reduction of oxygen at porous carbon cathodes.

I thank the Socony Mobil Oil Co., Inc., for permission to publish this article.

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FORMATION AND PROPERTIES OF THE BLACK FORM OF CARBON DISULPHIDE

By E. G. BUTCHER, M. ALSOP*, J. A. WESTON and H. A. GEBBIE

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IN 1941, P. W. Bridgman[1] reported that carbon disulphide slowly changed its character when subjected to a pressure of 45,000 atmospheres and a temperature of 175° C. The new form was a black solid which for practical purposes was stable at room temperature and atmospheric pressure. Later, Whalley[2], in presenting results of infra-red investigations on this black material, gave as the conditions of formation 55,000 atmospheres and 185° C, while in 1962 Dachille and Roy[3] referred to it as a waxy polymer which was produced at 42 kbar at 100°–200° C.

This communication gives some results of further investigations of this material carried out here. Using the tetrahedral anvil apparatus[4] with a modified cell assembly (which will be described elsewhere) comparatively large samples (0·5 cm diam., 1·0 cm long, weighing 0·1 g or more) of the black high-pressure form of carbon disulphide have been produced, and it has been found

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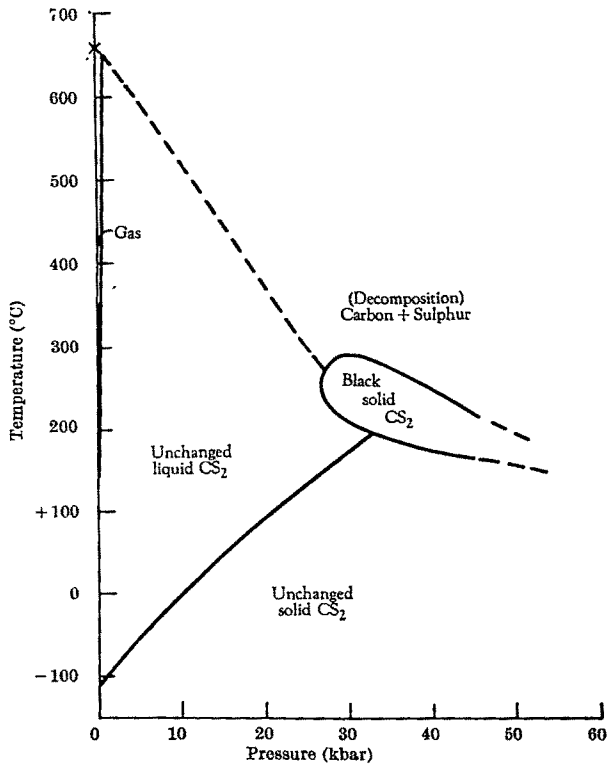


Fig. 1. Formation diagram of black carbon disulphide

that the formation of this black form occurs over a wide range of pressure but over a relatively narrow range of temperature. Fig. 1 shows a diagram of the formation conditions from which it will be seen that the black solid is formed at pressures as low as 27,000 atmospheres at temperatures of 220°–280° C. Above 280° C the material starts to decompose into carbon and sulphur. As the pressure is increased the formation temperature decreases so that at 45,000 atmospheres black carbon disulphide is formed over the temperature range of 165°–235° C, some decomposition into carbon and sulphur occurring at 240° C. The time taken for the change to occur in our apparatus is of the order of 10 min at the higher pressure (45,000 atmospheres) and somewhat longer at the low pressures. Under these conditions the yield approaches 100 per cent.

The black form of carbon disulphide is a solid at ordinary temperatures and pressures. It is stable for an indefinite period and no conditions have been found under which it reverts to the original liquid form. When heated it softens at about 70° C and decomposes into sulphur and carbon at 170° C. It is insoluble in all the normal laboratory solvents. Its density is found to be 1·89 g/ml. and this value compares with that for carbon 2·0–2·2 g/ml. (graphite) and 1·95–2·05 g/ml. for yellow sulphur. Our value does not agree, however, with the density value reported by Bridgman, who stated that the black solid had a density 4 per cent greater than a mechanical mixture of sulphur and carbon.

A determination of the stoichiometry of the black solid shows that the molecular composition is carbon disulphide. Measurements on three samples gave the proportions 82·8 per cent sulphur and 16·33 per cent carbon, compared with 84·22 per cent and 15·77 per cent of sulphur and carbon respectively which would be expected from the atomic weights.

X-ray powder photographs and electron diffraction measurements gave very little information, since in both a broad diffuse pattern was obtained indicating that there is little or no long-range order.

At room temperature the material has a very low electrical conductivity (approximately 10^{-15} ohm cm^{-1}), but this increases rapidly with temperature suggesting that the material is a semiconductor. In Fig. 2 the measured electrical conductivity is plotted against the reciprocal of the temperature and the slope of the resulting straight line indicates an energy gap of 1·15 eV. Some support for this value is obtained from near infra-red measurements. The material is black with high absorption in the visible region, but at a wave-length of approximately 8,000 cm^{-1} (1·2μ) the transmission increases and this locates roughly an optical absorption edge corresponding to an energy of 1 eV. The infra-red absorption spectrum in the frequency-range 2,000–1,000 cm^{-1} has been recorded using powdered samples and the potassium chloride disk technique. We confirm the results of Whalley in finding a strong absorption band at 1,063 cm^{-1}, which he assigns to C=S stretch. In addition, however, we find bands at 1,389 and 1,351 which at present we are unable to assign. Perhaps more revealing are some results of measurements in the far infra-red which are shown in Fig. 3. In this region liquid carbon disulphide and the solid made by freezing it show only weak absorption features, but with the black material there is strong absorption throughout the range 350–120 cm^{-1} with reproducible absorption

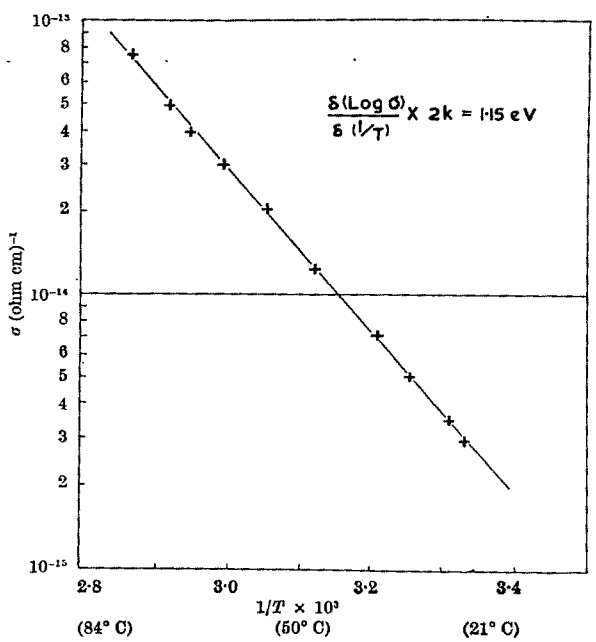


Fig. 2. Temperature dependence of electrical conductivity (σ)

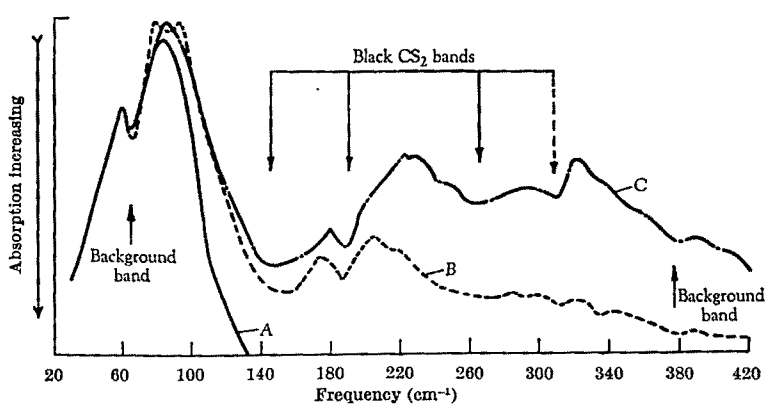


Fig. 3. Far infra-red absorption spectrum of black carbon disulphide with instrumental background spectrum superimposed. *A*, specimen 250μ thick; *B* and *C*, specimen ~100μ thick

maxima at 265 cm^{-1}, 190 cm^{-1} and 150 cm^{-1} and a less-definite feature at 310 cm^{-1}. No quantitative values of absorption coefficient in this region have been obtained so far, but that the absorption is high may be judged from the fact that transmission could be measured only when the sample thickness was reduced to be of the order of 100μ even when using an interferometer method where relatively large energy is available. The strong absorption in the region 350–120 cm^{-1} is in great contrast with the behaviour of liquid carbon disulphide and suggests the presence of polar groups in the black modification.

At frequencies less than 100 cm^{-1} the transmission increases and this has allowed us to make a rough measurement of refractive index for a frequency band at 30–40 cm^{-1} by an interferometric method[5]. A value of approximately 2·0 was found corresponding to a dielectric constant of 4·0. Measurement of dielectric constant at microwave frequencies (10^{10} c/s) and radio frequencies (10^{8} c/s) gave the values 4·2 and 4·0 respectively with negligible dielectric loss at both frequencies. The high values of dielectric constant of the black variety of carbon disulphide may also be contrasted with that of liquid carbon disulphide, which for low frequencies has the value 2·62.

It is evident from the foregoing that Bridgman's black carbon disulphide has a structure very different from ordinary carbon disulphide and that a polymeric structure formed by breaking the molecular double bonds is plausible, but it is also clear that more studies as well as refinement of the present measurements will be necessary before this structure can be described unambiguously.

We thank Mr. G. Mitchelmore for valuable help during the investigation of the formation conditions, Mr. R. Rushton of the Standards Division of this Laboratory for the measurements of dielectric constant, the National Chemical Laboratory for the microanalysis results, and the Warren Springs Laboratory for the density determination.

This work is published with the permission of the Director of the Laboratory.

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A NEW APPROACH TO STUDYING THE PROTEIN-BINDING PROPERTIES OF PENICILLINS

By P. ACRED, D. M. BROWN, T. L. HARDY and K. R. L. MANSFORD
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THE interaction of an antibiotic with serum is generally believed to result in a decrease in antibacterial activity. When the natural penicillins were originally examined, it was found that in the absence of serum, penicillin *K* was more active than penicillin *X*, and penicillin *X* was more active than penicillin *G*, but in the presence of serum, penicillin *G* was the most active. The reversal in activity was attributed to the fact that penicillin *G* was less bound to protein than penicillin *K* or *X*, and hence a greater concentration of free penicillin was available for antibacterial action[1].

The methods used to examine protein binding of penicillins have relied largely on *in vitro* static dialysis, but such methods, however, bear little relationship to the dynamic conditions in the body. We have, therefore, studied the phenomenon of protein binding with the view of determining more precisely its significance to antibacterial activity *in vivo*. Gel-filtration and both static and continuous dialysis methods have been used in this investigation.

The penicillins which were selected were penicillin *G*, 'Ampicillin' and '*BRL* 1071' (triphenylacetylpenicillin).

Static dialysis. To 5 ml. of human serum, 5 mg of the pencillin under examination were added. This was placed in a dialysis bag of 'Visking' tubing 0·75 in. diameter and suspended in 20 ml. normal saline and dialysed at 8° C for 24 h. Several dilutions of retentate and dialysate were prepared in M/20 phosphate buffer *p*H 7·0, and the amount of antibiotic was assayed by the cup-plate technique using *Sarcina lutea* as the test organism. The samples of each antibiotic were assayed against dilutions of known concentration of the same antibiotic.

Continuous dialysis. 5 mg of each antibiotic in 5 ml. serum were placed in 'Visking' tubing as described. The bags were suspended in a beaker through which water flowed continuously. The final concentration of the antibiotic was assayed in the serum at 24 h and the percentage obtained was calculated in terms of the concentration in serum at zero and 24 h.

Gel-filtration. 5 mg of each antibiotic were dissolved in 5 ml. serum and allowed to stand for 18 h at 10° C. 3 ml. of the serum were applied to a column jacketed at 10° C and containing 100 ml. 'Sephadex *G*25' gel equilibrated with water, the flow rate being 20–25 ml./h. Fractions (5 ml.) were collected and protein elution was followed by optical density measurements at 280 mμ on a 'Unicam' *S.P.* 500. The fractions were assayed for antibiotic content. Aqueous solutions containing 1 mg/ml. of the antibiotics were similarly treated.

Protein fractionation. Further examination of the bound fraction from the 'Sephadex *G*25' column was carried out using DEAE-'Sephadex *A*50'. After concentration by ultra-filtration the bound fraction was passed down a jacketed column kept at 10° C and containing 30 ml. of fully swollen DEAE-'Sephadex *A*50' equilibrated with 0·02 M phosphate buffer at *p*H 8·6. Stepwise elution was then carried out with 0·02 M phosphate buffer followed by saline of molarities 0·07, 0·12, 0·18 and 0·3 M, the change-over point being based on the optical densities of the fractions at 280 mμ.

Results

(*a*) *Dialysis.* The results obtained for the degree of protein binding are shown in Table 1. By static methods '*BRL* 1071' is bound 80–90 per cent to the serum proteins; penicillin *G* is much less bound, while 'Ampicillin' is only slightly bound. When examined by continuous dialysis, the final percentages bound were reduced but the reduction in the binding of '*BRL* 1071' was less than with the other penicillins (Table 1).

Table 1

Penicillin	Bound	
	Continuous	Static
Penicillin *G*	14·2 (5)	46·4 (21)
'*BRL* 1071'	41·4 (6)	91·6 (3)
'Ampicillin'	0·9 (3)	10·2 (6)

(*b*) '*Sephadex.*' Typical elution patterns from three penicillins of different binding properties obtained on 'Sephadex' columns are shown in Fig. 1. With the penicillin alone the antibiotic activity is confined to fractions 8–10. With serum alone the protein as determined by optical density at 280 mμ emerged in fractions 4–6. When the incubated penicillin serum mixture was passed down the 'Sephadex' column the protein again emerged in

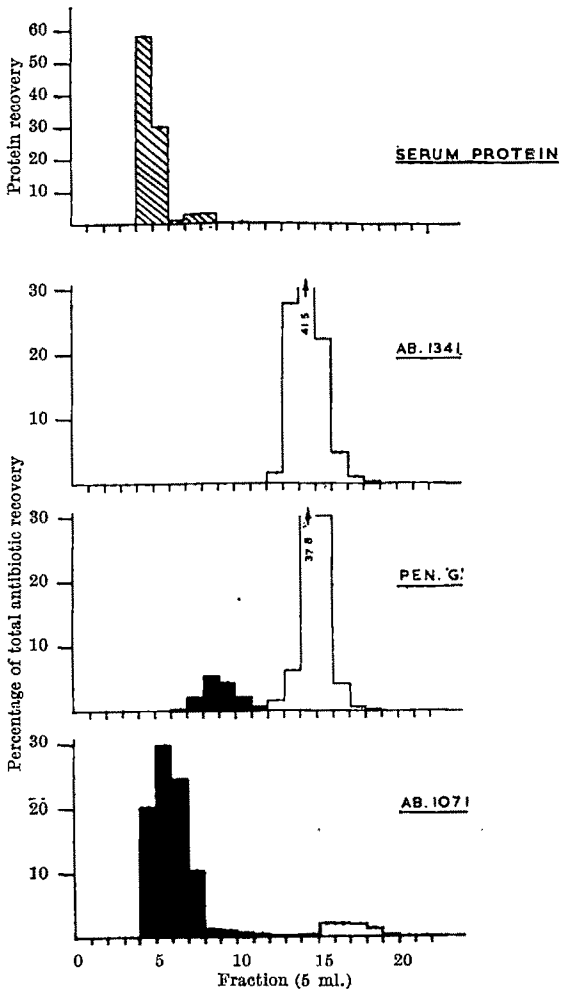


Fig. 1. Elution patterns from 'Sephadex' columns

fractions 4–6, whereas the antibiotic activity showed two peaks, if there was significant binding. One peak was associated with the protein and the other again emerged in the protein-free fractions 8, 9 and 10. The degree of protein binding was then calculated from the antibiotic content of the appropriate fractions. The 'Sephadex' method[2] offers considerable advantages over dialysis methods since the fractionation occurs in less than 2 h, and after a short wash with water the column is ready for further use. The dilution factor of both the bound and unbound material is small, and this aids the sensitivity of the method. The reduction in time needed for the separation of bound and unbound compound is also an advantage when dealing with labile materials.

'Sephadex *G25*' grade has a lower limit for complete exclusion of molecular weights of 3,500–4,500 and is the preferred grade for protein binding studies. With the '*G50*' and '*G75*' grades of different degrees of cross-linkage, the protein peak is flattened and tends to tail into the unbound antibiotic peak.

(*c*) *Protein fractionation.* The bound fraction from the 'Sephadex *G25*' column, on further examination on DEAE-'Sephadex *A50*', gave the bulk of the antibiotic activity in the 0·18 M saline fractions, whereas aqueous penicillin solutions gave a peak of antibiotic activity in the 0·07 M saline fractions with some tailing into the 0·12 M saline fractions. Starch-gel and cellulose acetate electrophoresis was then carried out on the protein fractions. The material eluted with 0·02 M phosphate was found to consist of pure γ-globulin. The other fractions were all multi-component, but the 0·02 M, 0·07 M and 0·12 M fractions contained no albumin and were made up of mixtures of α- and β-globulins. The material eluted with 0·18 M saline consisted almost entirely of albumin whereas the material eluted with 0·03 M saline contained some material of the same mobility as albumin together with a high electrophoretic 'pre'-albumin content. It is evident from this method that the bound penicillin is associated with the albumin fraction of the serum protein.

Discussion

From the results of the three methods described, it is evident that penicillins can differ in the degree and nature of their binding to serum proteins. Static dialysis shows that 'Ampicillin' is slightly bound, penicillin *G* is moderately bound while '*BRL* 1071' is considerably bound.

The nature of the binding of '*BRL* 1071' and penicillin *G* is evident from the results under the different conditions of continuous dialysis and gel-filtration. The binding of penicillin *G* is shown to be of a very loose nature and the binding of '*BRL* 1071' to be very firm. Such differences in binding can affect activity *in vivo*. If a compound is firmly bound to the serum proteins the concentration of free penicillin is reduced and hence its intrinsic activity is reduced. The *in vivo* activity, however, may not be significantly influenced if the binding is of such a loose character that the penicillin is readily available for antibacterial activity. This is the case with penicillin *G* which is bound to the extent of 46 per cent to serum protein yet its activity is apparently uninfluenced by the presence of serum in the same way as a compound, for example, 'Ampicillin' which is only slightly bound. On the other hand, '*BRL* 1071' is very firmly bound and its activity is much reduced in the presence of serum *in vitro* and *in vivo*. Therefore, before conclusions on *in vivo* activity can be drawn from *in vitro* results, information should be available on both the actual degree and nature of the binding to serum proteins.

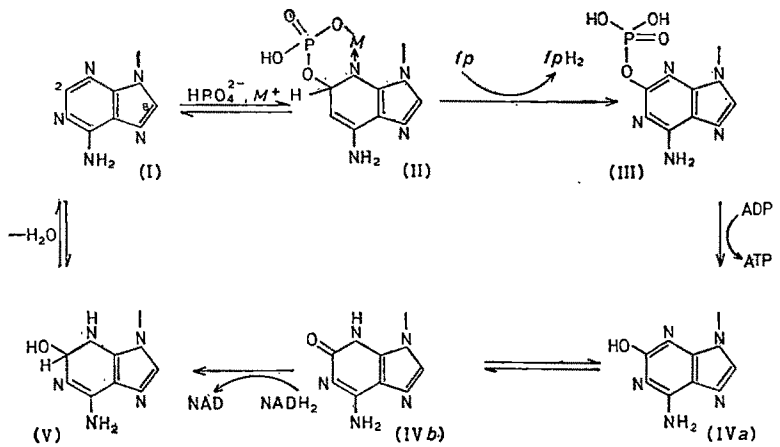
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MECHANISMS FOR OXIDATIVE PHOSPHORYLATION AT THE PYRIDINE NUCLEOTIDE/FLAVOPROTEIN LEVEL

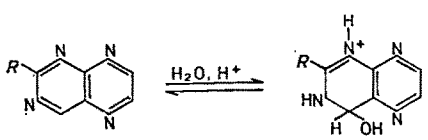
By Dr. J. A. BARLTROP, P. W. GRUBB and B. HESP
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IN respiratory chain oxidative phosphorylation, one molecule of adenosine triphosphate (ATP) is formed during the oxidation of reduced nicotinamide-adenine-dinucleotide ($NADH_2$) by flavoprotein[1]. This article presents chemical evidence for two mechanisms for this process, one involving the adenine ring of $NADH_2$ or of NAD and the other the dihydronicotinamide ring of $NADH_2$. In both mechanisms, it is postulated that part of the NAD ($NADH_2$) of mitochondria is involved in a nucleotide cycle of phosphate activation and transfer and is thus functionally distinct from the rest of the NAD ($NADH_2$) concerned in cellular redox reactions.

For participation of the adenine ring, the following cycle is proposed:

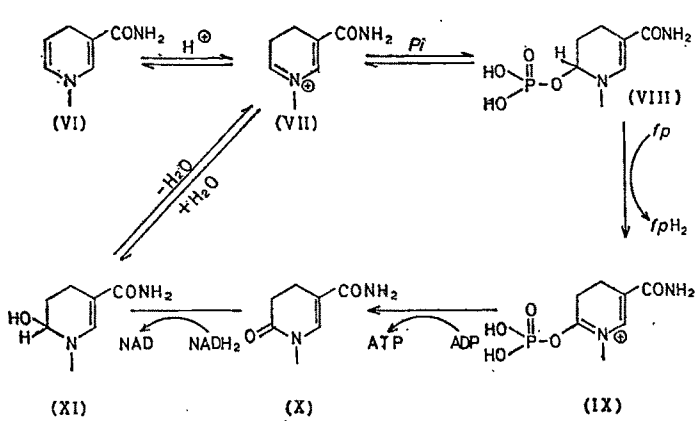


The adenine residue (I) is, in principle, susceptible to nucleophilic attack by phosphate (P_i) in either the 2- or the 8-position. The presence of certain metal ions (M^+) will assist, by chelation, the formation of the 2- or 8-phosphate adduct (for example, II). This step is analogous to the reversible formation of stable hydrates from 2-amino- and 2-hydroxy-pteridine[3,4]:



The oxidation of the phosphate adduct (II) by flavoprotein (*fp*) gives an adenylphosphate (III) which would be expected, by analogy with pyridyl phosphates[5], to be a high-energy phosphate capable of phosphorylating ADP to ATP. Such a reaction would yield the tautomeric 2-hydroxy-6-aminopurine derivative (IV*a*, IV*b*), which might be reduced by $NADH_2$ to the ψ-base (V) and hence to $NADH_2$. The sequence is therefore a cyclic process, one revolution of which generates one molecule of ATP while a pair of electrons is transported from $NADH_2$ to flavoprotein. This mechanism could equally well apply if the pyridinium ring of the molecule were in the oxidized form throughout, in which case (I) would represent NAD instead of $NADH_2$. A similar formulation can also be given for attack of P_i at the 8-position instead of at the 2-position, as shown.

An alternative mechanism, involving the dihydronicotinamide ring of $NADH_2$, is the following:



The acid-catalysed formation of the phosphate adduct (VIII) from $NADH_2$ (VI) via the ion (VII) has analogy in the chemistry of vinylamines. Oxidation of this adduct gives an imidoyl phosphate (IX), and these compounds are known to be high-energy phosphates capable of phosphorylating orthophosphates to pyrophosphates[2]. ATP is formed at this stage. The resulting amide (X) on reduction with $NADH_2$ should give the ψ-base (XI) and hence, by equilibration, (VI), (VII) and (VIII).

The thermodynamic and mechanistic feasibility of the early stages in these mechanisms (up to the phosphorylation step) was investigated using model systems in which compounds containing dihydronicotinamide or adenine rings were allowed to equilibrate with excess P_i and Mg^{2+}, then oxidized and warmed. The isolation of pyrophosphate (PP) was taken as indication that the system was potentially capable of producing the PP link of ATP.

In these experiments, mixtures containing two or more of the components (substrate, Mg^{2+}, and radioactive orthophosphate ($^{32}P_i$) were incubated with KBr_3 solution. Aliquots were applied to Whatman No. 4 paper moistened with 1 N acetic acid saturated with EDTA, then separated by electrophoresis at 20 V/cm for 2–2½ h. The P_i and PP areas were located by the Hanes–Isherwood reagent[6], excised, and the ^{32}P activity in each was counted with a Geiger–Müller tube.

For the first mechanism, adenosine was used as a model compound for NAD.

(i) PP from the Oxidation of Adenosine/P_i Mixtures

Omissions from incubation mixture*	Complete mixture	(−Mg^{2+})	(−Adenosine)	(−KBr_3)
PP/P_i ratio × 10^4	5·84	2·94	0·28	0·24
Yield of PP (per cent)†	2·9	1·5	—	0·12

* Incubation mixture: P_i, 50 μmoles (*p*H 7); Mg^{2+}, 20 μmoles; adenosine, 1 μmole; KBr_3, 50 μmoles. Total volume 320 μl. incubated at 50°–70° for 15 min.
† Calc. on adenosine present.

The experiment indicates that PP is formed in significant yields by the system adenosine/P_i/KBr_3, that omission of magnesium causes a severe drop in yield, and that omission of adenosine or of oxidant brings the yield of PP virtually to zero. The following experiment shows that the yield of PP formed is proportional to the concentration of adenosine present.

(ii) Effect of Adenosine Concentration

Adenosine in incubation mixture*	1 μmole	½ μmole	¼ μmole	0
PP formed in μmoles	0·033	0·017	0·0072	0·00072
Yield of PP (per cent)†	3·3	3·4	2·9	—

* Incubation mixture: P_i, 50 μmoles (*p*H 7); Mg^{2+}, 20 μmoles; KBr_3, 50 μmoles. Total volume 320 μl. Incubated at 65° for 10 min.
† Calc. on adenosine present.

In the experiments so far described, the PP might have been formed not by the mechanism proposed but by some oxidative reaction involving the sugar side-chain. This has been shown not to be the case by the failure to isolate PP when D-ribose is incubated with P_i, Mg^{2+} and KBr_3, and by the demonstration, in the next experiment, that adenine itself gives rise to considerable amounts of PP.

(iii) PP from Adenine

Omissions from incubation mixture*	Complete mixture	(−Mg^{2+})	(−Adenine)	(−KBr_3)
PP/P_i ratio × 10^4	13·4	6·15	0·43	0·009
Yield of PP (per cent)†	6·7	3·1	—	0·0045

* Incubation mixture: P_i, 50 μmoles (*p*H 4·6); Mg^{2+}, 20 μmoles; adenine, 1 μmole; KBr_3, 100 μmoles. Total volume 320 μl. Incubated at 64° for 2½ h.
† Calc. on adenine present.

The following experiment, conducted on 1,4-dihydro-*N*-methylnicotinamide (DHMN) as a model for $NADH_2$, shows that pyrophosphate links are produced by oxidizing the dihydro-compound in the presence of P_i.

(iv) PP FROM OXIDATION OF DHMN/P_i MIXTURES

Omissions from incubation mixture*	Complete mixture	(−Mg^{2+})	(−DHMN)	(−KBr_3)
PP/P_i ratio × 10^4	3·73	3·05	0·54	1·45
Yield of P_i (per cent)†	2·5	2·0	—	0·97

* Incubation mixture: P_i, 50 μmoles (*p*H 4·6); Mg^{2+}, 20 μmoles; KBr_3, 100 μmoles; DHMN, 0·75 μmoles. Total volume, 330 μl. Incubated at 60°–65° for 1 h.
† Calc. on DHMN present.

This experiment shows that significant yields of PP are formed by the complete system, that the yield is reduced slightly by omission of Mg^{2+} and greatly by omission of the other components. The relatively high yield of PP produced in the absence of added oxidant is presumably due to aerial oxidation.

It thus appears that ATP could, in principle, be formed by phosphate addition to, and subsequent oxidation of, the adenine ring of $NADH_2$ or NAD, and also by a similar process involving the dihydronicotinamide ring of $NADH_2$. Both mechanisms are chemically feasible, and that involving the pyridine ring is particularly attractive in view of the work of Griffiths and Chaplain[7], who have isolated from mitochondria a compound which appears to be a phosphorylated derivative of NAD reduced in the pyridine ring and capable of converting ADP to ATP.

Biochemical experiments are in progress to determine which (if either) of these mechanisms is the correct one.

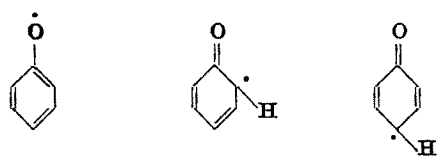
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REACTION OF PHENOXY RADICALS WITH METHYL RADICALS IN THE GASEOUS PHASE

By M. F. R. MULCAHY and D. J. WILLIAMS

C.S.I.R.O., Division of Coal Research, Sydney

REACTION of phenoxy radicals with other free radicals can be expected to occur mainly at the 'free electron positions' of the main canonical type-structures:



I II III

but theory is not yet able to predict the relative reactivities of the three positions. We have investigated the relative reactivities towards methyl radicals experimentally by examining the products of the reaction with methyl radicals in the gaseous phase.

Methyl radicals were generated by thermal decomposition of di*tert*-butyl peroxide:

$$[(CH_3)_3CO]_2 \rightarrow 2(CH_3)_3CO\cdot \rightarrow 2CH_3\cdot + 2(CH_3)_2CO$$

and phenoxy and methylphenoxy radicals by hydrogen abstraction from phenol, *o*-cresol and *p*-cresol:

$$(CH_3)_3CO\cdot + RC_6H_4OH \rightarrow RC_6H_4O\cdot + (CH_3)_3\,COH$$
$$CH_3\cdot + RC_6H_4OH \rightarrow RC_6H_4O\cdot + CH_4$$

The reactions were carried out in the temperature-range 445°–547° K at total pressures of 0·35–3·2 cm mercury in the stirred-flow reactor described elsewhere[1]. The liquid products were analysed by vapour-phase chromatography. About 50 per cent of the peroxide was decomposed and an excess of the unreacted phenol was always recovered.

With phenol itself the main liquid products found (apart from acetone and *tert*-butanol) were *o*- and *p*-cresol together with smaller amounts of anisole and 2,4- and 2,6-dimethyl phenol. No *m*-cresol, or mono- or di-hydroxy diphenyls or other phenolic substances could be detected, the detection limits for *m*-cresol and hydroxy diphenyls being respectively 5 and 0·5 per cent of the total cresols. The chief aromatic product from *p*-cresol was 2,4-dimethyl phenol. *o*-Cresol produced mainly 2,4- and 2,6-dimethyl phenol. Both cresols gave smaller amounts of 2,4,6-trimethyl phenol and of the methyl anisole corresponding to the original cresol. Neither produced a significant amount of any compound with methyl substituted *meta* to the hydroxy group: of the total dimethyl phenols produced in each case, *p*-cresol gave less than 0·2 per cent 3,4-dimethyl phenol.

Direct substitution by methyl radicals in the aromatic ring of the phenol molecule is evidently inappreciable under these conditions. This follows, first from the absence of *meta* substitution considered in the light of the nucleophilic character[2] of methyl radicals, and secondly, from the fact[3] that replacement of the hydroxylic hydrogen of phenol by methyl reduces the rate of reaction of the molecule with methyl radicals by an order of magnitude. We therefore conclude that methyl substitution in the ring occurs by combination of a methyl radical with the phenoxy radical at the *ortho* or *para* position (structure II or III), and that this is followed by hydrogen migration to give the *ortho*- or *para*-substituted phenol. Anisoles are formed directly by combination at the oxygen atom (structure I).

Since cresols react much faster than anisole with methyl radicals the relative rates of efflux of anisole and total cresols from the reactor (n_{an}/n_{cr}) must be corrected for secondary methyl attack to obtain the relative rates of formation. It can be shown that:

$$\frac{n_{an}}{n_{cr}} \simeq \frac{k_{ox}}{(k_o + k_p)}\left(1 + \frac{K[CH_3]}{v}\right)$$

where k_{ox}, k_o and k_p are the bimolecular rate constants for methyl addition to the oxygen atom and the *ortho* and *para* positions of the phenoxy radicals, v is the total volumetric rate of flow from the reactor and K is a con-

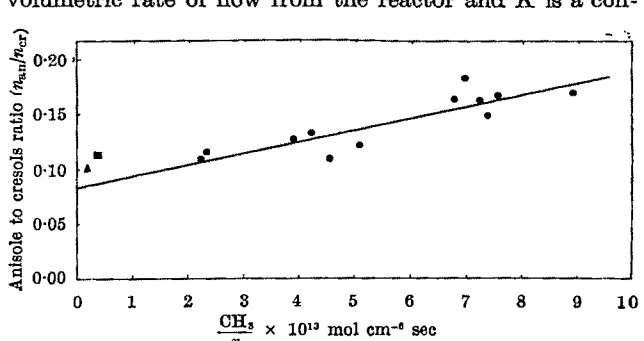


Fig. 1. Variation with $[CH_3]/v$ of relative rates of efflux of anisole (n_{an}) and cresols (n_{cr}) from the reactor. (Line drawn through points at 482° K by method of least squares.) ●, 482° K; ■, 516° K; ▲, 547° K

Table 1. Relative Reactivities towards Methyl Radicals and Relative Free Spin Densities of Reactive Positions of Phenoxy and o-Methyl Phenoxy Radicals

	Phenoxy			o-Methyl phenoxy		
	ortho	*para*	Oxygen	*ortho*	*para*	Oxygen
Relative rate constants	1·1 ± 0·1	1·0	0·17 ± 0·05	0·4 ± 0·1	1·0	0·11 ± 0·09
Relative reactivities of positions	0·55 ± 0·05	1·0	0·17 ± 0·05	0·4 ± 0·1	1·0	0·11 ± 0·09
ESR proton coupling constants (ref. 4) (a_i)	6·6 ± 0·3	10·4 ± 0·5	—	6·0 ± 0·3	11·5 ± 0·3	—
Relative spin densities (Q = 22·5) (ρ_i)	0·63 ± 0·06	1·0	> 0·26	0·52 ± 0·5	1·0	> 0·23

stant. $[CH_3]$ is obtained from the rate of formation of ethane, which is assumed to occur by combination of methyl radicals. Values of n_{an}/n_{cr} obtained from experiments with phenol, in which $[CH_3]$ ranged from 3×10^{-13} to 2×10^{-12} and $[C_6H_5OH]$ ranged from 1×10^{-8} to 6×10^{-7} mole cm^{-3}, are shown as a function of $[CH_3]/v$ in Fig. 1. Extrapolation to $[CH_3]/v = 0$ yields the value 0·08 ± 0·02 for $k_{ox}/(k_o + k_p)$. Values of similar magnitude were obtained from smaller numbers of experiments with the cresols, namely, 0·08 ± 0·06 for the *o*-methyl phenoxy and 0·14 ± 0·045 for the *p*-methyl phenoxy radical.

The relative rates of production of *o*- and *p*-cresol from phenol (n_{oc}/n_{pc}) do not vary significantly with changes in $[CH_3]/v$ (or other conditions). This accords with the relatively small difference in reactivity between *o*- and *p*-cresol[3]. The mean result from twenty-five experiments at four temperatures in the range stated here is:

$$\frac{k_o}{k_p} \simeq \frac{n_{oc}}{n_{pc}} = 1{\cdot}1 \pm 0{\cdot}1$$

The corresponding ratio for the *o*-methyl phenoxy radical obtained from five determinations of the relative rates of production of 2,6- and 2,4-dimethyl phenol from *o*-cresol is, as expected, about half this value, namely, 0·4 ± 0·1.

Taking into account the two *ortho* positions, the relative reactivities of the three distinguishable reactive positions of the phenoxy radical are in the proportion $\frac{1}{2}k_o : k_p : k_{ox}$. The relative values, together with the corresponding (less accurate) result for the *o*-methyl phenoxy radical, are given in Table 1.

It is interesting to compare the reactivities with the free spin densities (ρ_i) at the three positions. Relative values of ρ_i estimated from electron spin resonance measurements made by Stone and Waters[4] are also given in Table 1. The spin densities at the *ortho* and *para* positions were obtained from the relationship $\rho_i = \alpha_i/Q$ where α_i is the proton coupling constant at position i and Q = 22·5 (refs. 5 and 6). The lower limit for the spin density on the oxygen atom was estimated by subtracting the algebraic sum of the densities on the carbon atoms from unity, the values for the *meta* carbon atoms[4] and the (unknown) value for the carbon adjacent to the oxygen atom being taken as negative[7]. There is a clear correlation between reactivity and spin density at the *ortho* and *para* positions. However, it seems that, unless the value chosen for Q is too high, the oxygen position is less reactive than its spin density would indicate. This effect, to which a parallel may be found in the low reactivity of the ('saturated') carbonyl bond towards alkyl radicals, is presumably associated with the electro-negative character of the oxygen atom.

We thank Mr. J. W. Sweeting and Mr. J. Wilmshurst for carrying out the gas-chromatographic analyses.

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STABILITY OF THYMIDINE LABELLED WITH TRITIUM OR CARBON-14

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In view of the considerable interest in our recent paper on the decomposition of organic compounds labelled with tritium[1], we now publish a more detailed account of the stability of the important deoxyribonucleoside thymidine, labelled with tritium or carbon-14. Labelled thymidine is one of the most important tracer compounds at present used by research workers throughout the world. The compound is used for labelling the deoxyribonucleic acid (DNA) of proliferating cells and many papers on its use have been published[2]. A knowledge of the stability of the label in such a compound, under both chemical and biochemical environments, is obviously of great importance.

A detailed examination of the decomposition products of tritium labelled thymidine in aqueous solution by self-irradiation is being undertaken in another laboratory[3]. We have therefore confined ourselves mainly to a study of the gross effects of radiation decomposition of tritiated thymidine labelled generally, in the 6-position (predominantly) and in the 5-methyl group (exclusively), and thymidine labelled in the 2-position with carbon-14. As in our previous paper[1], various storage conditions were examined. For comparison, the effect of γ-radiation on solutions of tritiated and carbon-14 thymidine and on freeze-dried tritiated thymidine were also investigated.

Preparation of labelled thymidine. Thymidine-2-^{14}C, -6-T and -5-methyl-T were prepared by enzymatic coupling of the correspondingly labelled pyrimidine base, namely, thymine-2-^{14}C, -6-T or -5-methyl-T respectively, with 2-deoxy-D-ribose[4]. Generally labelled tritiated thymidine was prepared by an isotope exchange reaction between thymidine and tritiated water or tritiated acetic acid in the presence of a platinum catalyst[5]. Such exchange labelling procedures do not give exclusive tritium labelling in the pyrimidine base portion of nucleosides, as has been claimed in earlier papers[6].

Self-decomposition of labelled thymidine. The various ways by which radio-isotopically labelled compounds can undergo decomposition by self-irradiation are now well described[1,7]. In the case of thymidine-2-^{14}C we can neglect the decomposition due to primary (internal) decay of carbon-14 to nitrogen over the period of time we have investigated, due to the very long half-life of carbon-14 (5,680 years). The actual decomposition due to this primary (internal) decay is less than 0·01 per cent per annum. Decomposition products of thymidine-2-^{14}C will

therefore arise through primary (external), secondary or chemical effects. Decomposition products of tritiated thymidine can arise by all the modes of self-irradiation effects[1].

Observations with thymidine-2-^{14}C. Thymidine labelled with carbon-14 specifically in the 2-position of the pyrimidine ring at a specific activity of 18·3 mc./millimole, had a decomposition rate of only 0·9 per cent per annum. This rate of decomposition, examined over a period of almost three years, was found to be the same whether the compound was stored in aqueous solution or as a freeze-dried solid at +2° C or at −40° C. Identical results were also obtained by storage on paper (cf. ref. 7) under the same temperature conditions. At room temperature stored in aqueous solution the thymidine-2-^{14}C gave 1–2 per cent thymine-2-^{14}C per annum. For comparison we kept a sample of thymidine-2-^{14}C in tap water exposed to air and occasional sunlight for 5 months in the laboratory. At the end of this period analysis showed that the radiochemical purity was still 92 per cent and 5 per cent thymine-2-^{14}C was found. This remarkable stability of the carbon-14 labelled thymidine contrasts markedly with the stability of the tritiated compound. A comparison of the decomposition of thymidine-2-^{14}C and thymidine-T(G) (G = generally labelled with tritium in non-labile positions but the labelling is not necessarily uniform) in the freeze-dried state is shown in Table 1.

Table 1. DECOMPOSITION OF FREEZE-DRIED THYMIDINE-2-^{14}C AND THYMIDINE-T(G)

Compound	Specific activity (mc./m.mole)	Activity in ampoule (mc.)	Wt. of thymidine (mg)	Temp. of storage (°C)	Age months	Radiochemical purity (%)
Thymidine-2-^{14}C	18·3	0·1	2·4	−40	33	98
Thymidine-2-^{14}C	18·3	0·01	0·24	−40	33	98
Thymidine-2-^{14}C	16·0	0·01	0·15	0	25	100
Thymidine-T(G)	98	2	4·9	−40	38	60
Thymidine-T(G)	82	2	5·9	−40	36	50
Thymidine-T(G)	82	2	5·9	−40	36	70
Thymidine-T(G)	82	1	2·95	0	36	70
Thymidine-T(G)	261	2	1·83	0	39	65

Observations with tritiated thymidine. The decomposition of thymidine generally labelled with tritium, in aqueous solution, has been previously studied by Murray and Petersen[8]. They found that after 10 months' storage in aqueous solution at +4° C, thymidine-T(G) at a specific activity of 0·55 curies per millimole gave rise to only two decomposition products which were identified as thymine-T(G) and 2-deoxy-D-ribose-T(G). We have confirmed these results by storing thymidine-T(G) in aqueous solution for 39 months at −40° C. Analysis showed that the radiochemical purity of the thymidine-T(G), having a specific activity of 0·26 curies per millimole, at the end of this period was 35 per cent as thymidine-T(G) with 55 per cent of the remaining activity as thymine-T(G) and approximately 10 per cent 2-deoxy-D-ribose-T(G). These results were different from those we obtained on storing thymidine-6-T or thymidine-5-methyl-T in aqueous solution. These specifically labelled thymidines gave relatively small amounts of thymine-T (less than 5 per cent per annum) and mainly a radiation decomposition product(s) of R_F 0·0 on paper chromatography of the specifically labelled thymidine samples in ethyl acetate saturated with phosphate buffer at *p*H 6·0 (ref. 9) as solvent.

The radiation decomposition product(s) exhibited an ultra-violet light absorption spectrum in aqueous solution at *p*H 7·0 similar to thymidine, having a maximum at 267–268 mμ and a minimum at 242 mμ. This compares with the maximum at 267 mμ and minimum at 235 mμ for thymidine under the same *p*H conditions. The spectrum was unchanged in acid solution, but in alkaline solution the spectrum was entirely different from that of thymidine[20]; both the maximum and minimum disappeared and only an inflexion at 275 mμ was observed. However, re-acidification of the solution caused the re-appearance of the maximum at 267 and the minimum at 242 mμ, demonstrating that the decomposition product(s) is not decomposed in alkaline solution. The decomposition product(s) proved quite stable on keeping in aqueous solution at room temperature and remained unchanged after 7 months storage under these conditions. We did not investigate this product(s) further. The decomposition of thymidine-methyl-T and thymidine-6-T at a number of different specific activities, in aqueous solution, are shown in Tables 2 and 5 respectively. We have repeatedly made the observation that thymidine-T appears to undergo more rapid decomposition in the frozen state (0° C and below) than at +2° C and above[1]. Thymine as well as its nucleoside and nucleotide are reported[10] to be much more sensitive to decomposition under ultra-violet irradiation in the frozen state than in solution.

A similar phenomenon seems to apply to decomposition of thymidine-T by self-irradiation. Insufficient decomposition occurred in the case of the thymidine-2-^{14}C to enable similar conclusions to be drawn. The decomposition of freeze-dried thymidine-6-T at a specific activity of 9·5 curies per millimole is shown in Table 3. We were unable to detect any thymine-T formed as a decomposition product in this case, only the radiation decomposition product(s) having R_F 0·0 on paper chromatography in the ethyl acetate–phosphate solvent described here was detected.

Effect of γ-irradiation on labelled thymidine. The decomposition of thymidine labelled with carbon-14 or tritium in aqueous solution on γ-irradiation (2·5 megarads) was as to be expected. Virtually all the thymidine was destroyed during the 30-min irradiation and paper chromatography of the products in ethyl acetate–phosphate as here, gave mainly the radiation decomposition product(s) of R_F 0·0. This product(s) exhibited the same ultra-violet light absorption characteristics as the product(s) isolated from the self-decomposition of thymidine-T or carbon-14. A good correlation between the products formed from self-decomposition and those formed by the radiolysis of inactive thymidine in aqueous solution by X-rays at 0° C have previously been reported[3,11]. We have found that appreciable quantities of thymidine-T survive the γ-irradiation if a free radical scavenger, in this case benzyl alcohol, is present. Some results are recorded in Table 4.

Chemical stability of the label. Chemically, thymidine is a relatively stable substance, and it can be heated in neutral or alkaline solution up to 120° C for at least 1 h without decomposition[5]. This can be used as a method for sterilizing aqueous solutions of tritiated or carbon-14 labelled thymidine. On keeping for long periods in aqueous solution, hydrolytic cleavage of the *N*-glycoside linkage occurs at a rate which increases with increasing temperature of storage. Thymidine is hydrolysed by mineral acids giving thymine and 2-deoxy-D-ribose[17]. The carbon-14 label at carbon-2 is lost only by complete disruption of the thymidine molecule. In contrast, tritium atoms in organic molecules are sometimes lost by exchange with the hydrogen atoms of the solvent in which the compound may be dissolved[14]. This exchange, of course, occurs without changing the chemical form of the compound. However, the tritium atoms in the 6-position and the 5-methyl group of thymidine are firmly bound to the molecule and are not exchanged for hydrogen when the compound is heated in acid or alkaline solution. Only in the presence of a hydrogen transfer catalyst, for example, platinum metal, is the tritium label likely to be exchanged, and then only at elevated temperatures, to any appreciable extent.

Biological stability of the label. Provided the thymidine is not metabolized, the carbon-14 label at C-2 remains intact. Chemically stable tritium atoms may become labile under biological conditions[14,21]. There is at present, however, no evidence to suggest that the tritium label at C-6 or in the 5-methyl group becomes labile and hence

Table 2. Decomposition of Thymidine-5-methyl-T in Aqueous Solution

Specific activity (mc./m.mole)	Activity in ampoule (mc.)	Volume of solution (ml.)	Wt. of thymidine (mg)	Temp. of storage (°C)	Age months	Radiochemical purity (%)
1060	1	1·4	0·23	0	2	100
1520	1	2	0·16	0	3	95
3200	2	0·53	0·15	−40	14	85
4700	2	0·5	0·1	−40	8	92

Table 3. Decomposition of Freeze-dried Thymidine-6-T

Sample No.*	Age (months)	Wt. of thymidine (mg)	Storage state	Temperature (°C)	Radiochemical purity (%)
1	2	0·053	Vacuum sealed	−40	85
2	2	0·053	Vacuum sealed	−80	93
3	3	0·053	Vacuum sealed	room (20)	95
4	3	0·053	Vacuum sealed	room (20)	92
5	3	0·053	Air sealed	−80	89
6	4	0·053	Vacuum sealed	0	98
7	4	0·053	Air sealed	0	92
8	4	0·053	Air sealed	0	85
9	4	0·053	Vacuum sealed	0	80
10	6	0·053	Vacuum sealed	0	90
11	6	0·053	Vacuum sealed	0	65
12	6	0·053	Vacuum sealed	−40	75
13	6	0·053	Air sealed	−40	60
14	6	0·053	Vacuum sealed	room (20)	90
15	6	0·053	Air sealed	room (20)	90
16	6	0·053	Vacuum sealed	−80	70
17	6	0·053	Air sealed	−80	70

* Each sample contained 2 mc.

Table 4. Effect of γ-Radiation on Thymidine-6-T and -2-^{14}C (Dose: 2·5 × 10^6 rads for 30 min at room temperature)*

Thymidine-6-T Sample No.	Wt. of thymidine (μg)	Activity (mc.)	Vol. of solution (ml.)	Benzyl alcohol (% by vol.)	Radiochemical purity (%)
1 (control)†	92	1	1	1	100
2	92	1	1	1	70
3	192	1	1	nil	<5
4	292	1	1	nil	<5
5	392	1	1	1	85
6	492	1	1	nil	<5
7	592	1	1	1	70
8	692	1	1	nil	<5
9	792	1	1	1	80
10	892	1	1	nil	<5
11	992	1	1	1	70
12 (freeze-dried solid)	53	2	—	—	98
13 (freeze-dried solid)	53	2	—	—	92
Thymidine-2-^{14}C Sample No.					
1	1,323	0·1	0·83	nil	<1
2	132	0·01	0·42	nil	<1

* Carried out by the irradiation service U.K. Atomic Energy Authority, Harwell, Berks.
† Not irradiated.

Table 5. Decomposition of Thymidine-6-T in Aqueous Solution

Sample No.	Specific activity (c./m.mole)	Activity in ampoule (mc.)	Wt. of thymidine (μg)	Vol. of solution (ml.)	Age (months)	Temp. (°C)	Radiochemical purity (%)
1	2·5	2	200	0·29	12	−40	72
2	2·5	2	200	0·29	7	−40	90
3	2·5	2	200	0·29	8	0	90
4	1·14	0·5	90	0·25	10	room (20)	100
5	14·1	2	34	0·5	1·5	−40	90
6	14·1	2	34	0·5	12	−40	55
7	14·1	2	34	0·5	3·5	room (20)	100
8	14·1	1	17	0·5	5	room (20)	97
9	3·6	0·2	134	0·2	10	−40	80
10	1·4	1	170	0·5	10	−40	90
11	12·1	0·5	10	0·5	10	0	65
12	4·8	0·2	10	0·5	10	0	70

lost by exchange in biological systems. In the presence of thymine oxidase the tritium label at carbon-6 is lost[12], due to the formation of methyl barbituric acid, and in systems where demethylation of the 5-position occurs the tritium label in this position is also obviously lost[13]. Some thought is therefore necessary when selecting the correct position of isotopic labelling for specific investigations.

Conclusions. Thymidine-2-^{14}C is stable for a number of years when stored in aqueous solution below +2° C. The rate of decomposition by self-irradiation under these conditions is less than 1 per cent per annum, but there is no evidence that this state of storage is superior to keeping the compound in the freeze-dried state or on paper[7,16,18]. As no thymine-2-^{14}C could be detected at this storage temperature, the small amount of thymine-2-^{14}C which is formed on keeping the solution of thymidine-2-^{14}C at room temperature is probably produced by slow hydrolysis of the thymidine rather than by radiolytic fission of the *N*-glycoside bond. Table 1 shows that thymidine labelled with carbon-14 is much more stable than the tritiated compound of comparable specific activity, allowing for the difference in the energy of the carbon-14 β-radiation (max. 0·155 MeV) and the tritium β-radiation (max. 0·018 MeV).

In the solid state the self-absorbed energy per unit time for the tritiated thymidine will be greater than for the carbon-14 labelled material, due to the low range and penetrating power of the tritium betas. Primary (external) effects would thus be expected to cause more rapid decomposition in the case of the tritiated thymidine, in agreement with the experimental observations. Thymidine-6-T at high specific activity (9·5 curies/m.mole) stored as a freeze-dried solid, kept better than we had expected. The rate of decomposition in this condition, even at room temperature, is only 20–30 per cent per annum. The freeze-dried compound is less stable when stored at low temperatures, for example, −80° C. We are unable to explain this sensitivity to radiation at low temperatures at the present time.

In general, the presence of air in the ampoules did not affect the rate of decomposition.

Even more surprising is the stability of freeze-dried thymidine-6-T to γ-irradiation[15]. Only a few per cent decomposition was recorded, as can be seen from Table 4. No thymine-T or carbon-14 labelled thymine was detected in the decomposition of freeze-dried thymidine-T or carbon-14 respectively, indicating that no appreciable breaking of the *N*-glycoside bond occurs by primary (external) decomposition. Solutions containing thymidine labelled with tritium or carbon-14 cannot be sterilized by γ-irradiation without decomposition of the thymidine, even in the presence of a free radical scavenger. However, in the latter circumstances a high proportion of the compound may survive as is seen from Table 4. The γ-irradiation of thymidine solutions produces at least six different products and the main product(s) has R_F 0·0 in ethyl acetate–phosphate buffer on paper chromatography. This product(s) is similar to the product(s) produced by self-irradiation of labelled thymidine[19]. Weiss and collaborators[19] have shown that pyrimidine ring structures remain intact on irradiation and do not break down into smaller molecules. The ultraviolet light absorption spectrum of our radiation decomposition product(s) would also suggest this although we did not further investigate the product.

The decomposition rates for thymidine-5-methyl-T and thymidine-6-T at comparable specific activities are respectively 0·5–1 per cent per month and 1–2 per cent per month. This rate of decomposition increases when the solutions are stored below 0° C. Thymine-T is formed as a decomposition product even at low temperatures and probably arises by radiolytic fission of the *N*-glycoside bond and not by hydrolytic fission. Analysis of a number of samples of thymidine-6-T kept in aqueous solution by distillation of the solvent showed that less than 5 per cent tritium is 'exchanged' into the aqueous phase on keeping samples for 1 year. This result again supports the view that practically no degradation of the pyrimidine ring occurs forming smaller molecules which may be volatile or 'leak' their tritium into solution by exchange.

Decomposition products will vary to some extent depending on the position of the label within the molecule, which further complicates the interpretation and comparison of such data. The analytical methods used for our radiochemical purity determinations were as previously described[1]. Inactive thymidine was detected on paper

chromatograms with the ultra-violet light from a 'Chromatolite' Engehard Hanovia lamp (ultra-violet-range 250–280 mμ).

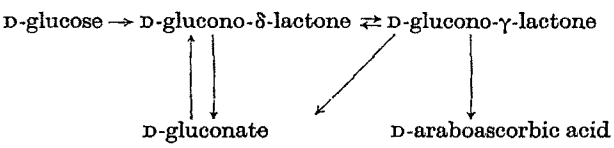
We thank Dr. John R. Catch and Mr. D. A. Lambie for their interest and the U.K. Atomic Energy Authority for permission to publish these results.

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TRANSFORMATION AND HYDROLYSIS OF D-GLUCONO-γ AND δ-LACTONE

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WE[1,2] have proposed the following scheme for the pathway of D-araboascorbic acid synthesis by *Penicillium*:



The theory[6] of the transformation of D-gluconolactones was as follows:

D-glucono-γ-lactone ⇄ D-gluconate ⇄ D-glucone-δ-lactone;

but M. A. Jermyn[3] has reported that lactones transform directly without involving gluconate. We have observed by other methods the direct transformation of these lactones and consider that this reaction would occur during the D-araboascorbic acid synthesis. For the purpose of perfecting this pathway, experiments on transformation and hydrolysis of D-gluconolactones were carried out. As a result, a new scheme is proposed.

D-glucono-δ-lactone (δ-lactone) was given by Fujisawa Pharm. Ind. Co., Ltd. D-Glucono-γ-lactone (γ-lactone) was prepared from δ-lactone by the method of Isbell and Frush[4]. For quantitative analysis of lactones, Hestrin's method[5], slightly modified, was used. The identification of γ- and δ-lactones were carried out by paper chromatography. The following solvent was used: acetonitrile/acetone/water/acetic acid = 80 : 5 : 15 : 1. Hydroxylamine and ferric chloride were used for colour reaction.

(1) *Paper chromatography of lactones.* About 1 mg lactone was added at room temperature in 0·2 ml. of water and N/10 *tris*-maleate buffer solutions of various *p*H. The solution was spotted on the filter paper (Toyo Roshi, Co., No. 51) as soon as δ-lactone was dissolved. The paper chromatograms (Fig. 1) show that γ-lactone (R_F value 0·55) is produced from δ-lactone (R_F 0·35) in *p*H 4·6 or higher and its concentration becomes higher still with *p*H of buffer solutions. In N/10 phthalate–disodium phosphate buffer, which was used for the examination on the pathway of D-araboascorbic acid synthesis by dried cells of *Penicillium*, the same results were obtained. Lactones were not produced from gluconate under the same conditions. Therefore, it is clear that γ-lactone is formed from δ-lactone and not by way of gluconate. This reaction proceeds spontaneously and very rapidly at room temperature. The *p*H of D-araboascorbic acid synthesis from glucose and the lactones coincide roughly with that of this reaction. On the other hand, the formation of δ-lactone from γ-lactone is slight, under the same conditions (Fig. 2). The concentration ratios of both lactones were not seen to change with time.

From these results, the existence of γ-lactone in the broth resulting from gluconic acid fermentation was expected. Fig. 3 shows the paper chromatograms of γ- and δ-lactones in the broth of *Aspergillus niger*, *Penicillium notatum* and *Acetobacter roseum*. The cultural conditions are shown in Table 1. γ-Lactone was not found in the broth of *Pseudomonas fluorescens*. It seems

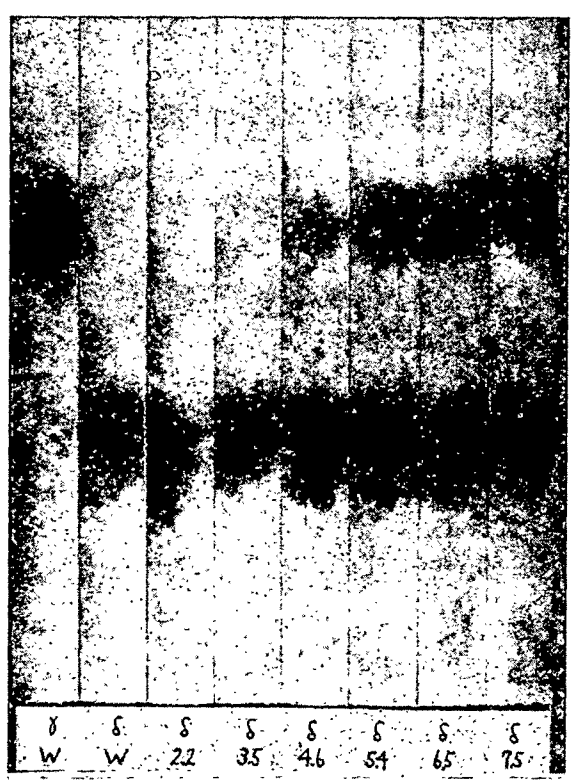


Fig. 1. Paper chromatograms of D-glucono-γ-lactone formation from δ-lactone in various *p*H buffer solutions

γ, D-Glucono-γ-lactone; δ, D-glucono-δ-lactone; *w*, water. Numbers, *p*H of N/10 *tris*-maleate buffer solution

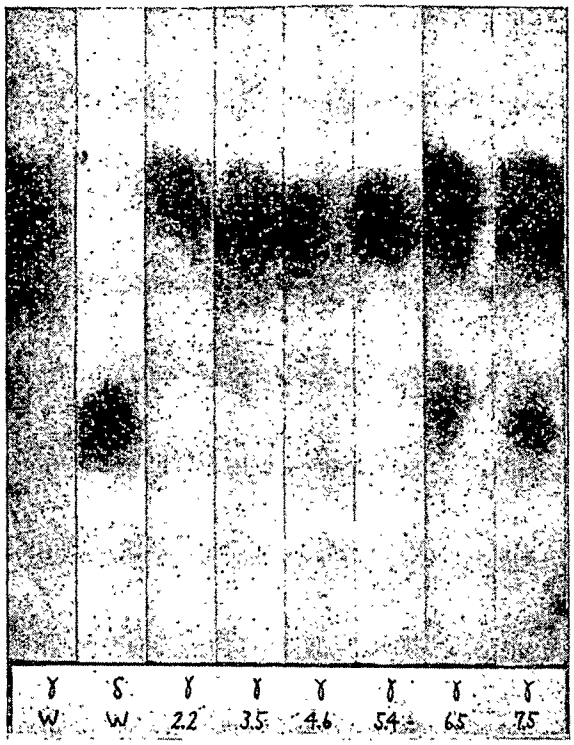


Fig. 2. Paper chromatograms of D-glucono-δ-lactone formation from γ-lactone in various *p*H buffer solutions

γ, D-Glucono-γ-lactone; δ, D-glucono-δ-lactone; *w*, water. Numbers, *p*H of N/10 *tris*-maleate buffer solution

that it was hydrolysed owing to a comparative high end *p*H (*p*H 4·5) of broth.

(2) *Hydrolysis of lactones.* About 10 mg of lactones were dissolved in 10 ml. N/3 *tris*-maleate buffer solution at various *p*H's. All experiments were carried out at 30°. Figs. 4 and 5 are spontaneous hydrolysis curves of γ- and δ-lactones. They show roughly straight lines with slight deviation and no lag period, so both hydrolysis reactions follow a first-order reaction. The deviations do not depend on the accumulation of gluconate or decrease in *p*H of the buffer solution.

Our results coincide with Jermyn's at the point of direct transformation of lactones; but did not at the hydrolysis pathway. Paper chromatograms show that the equilibrium of the transformation between both lactones inclines to γ-lactone and does not to δ-lactone, and that the velocity of transformation is very rapid. During all conditions tested, γ-lactone is more stable than δ-lactone. Therefore, we do not consider that at any *p*H large amounts of δ-lactone accumulate as an intermediate during the hydrolysis of γ-lactone. This opinion is supported by hydrolysis curves.

We propose a new scheme of spontaneous transformation and hydrolysis of D-gluconolactones. The scheme would be applicable in a wide range of *p*H.

Table 1. Cultural Conditions of Micro-organisms for Paper Chromatography of D-Glucono-lactones

	Aspergillus niger *IFO* 4415*	*Penicillium notatum* 1026†	*Acetobacter roseum* *G*2‡
Medium (%)	Glucose 10 $(NH_4)_2SO_4$ 0·1 KH_2PO_4 0·2 $MgSO_4 \cdot 7H_2O$ 0·025	Glucose 10 $NaNO_3$ 0·2 KH_2PO_4 0·25 $MgSO_4 \cdot 7H_2O$ 0·1 $ZnSO_4 \cdot 7H_2O$ trace	Glucose 10 Yeast ex. 0·1 Peptone 3·0 KH_2PO_4 0·3 $MgSO_4 \cdot 7H_2O$ 0·2
$CaCO_3$(%)	1·0	0·2	none
Init. *p*H	5·6	5·6	5·8
End *p*H	2·6	2·8	2·0

Reciprocating culture, 72 h, 25° C

* Gluconic acid producer (Institute for Fermentation, Osaka).

† D-Araboascorbic acid producer isolated by us; but this condition is not suitable for its production.

‡ Gluconic and 5-ketogluconic acid producer isolated by Dr. T. Asai.

New scheme γ-lactone ⇄ δ-lactone
↖↘ ↙↗
gluconate

Jermyn (below *p*H 7) γ-lactone ⇄ δ-lactone ⇄ gluconate
Accepted γ-lactone ⇄ gluconate ⇄ δ-lactone

This is a scheme in which direct hydrolysis of γ-lactone is added to Jermyn's theory; that is to say, a consolidation of the accepted and Jermyn's theory. According to this



Fig. 3. Paper chromatograms of D-glucono-γ- and δ-lactones in cultural broth of micro-organisms

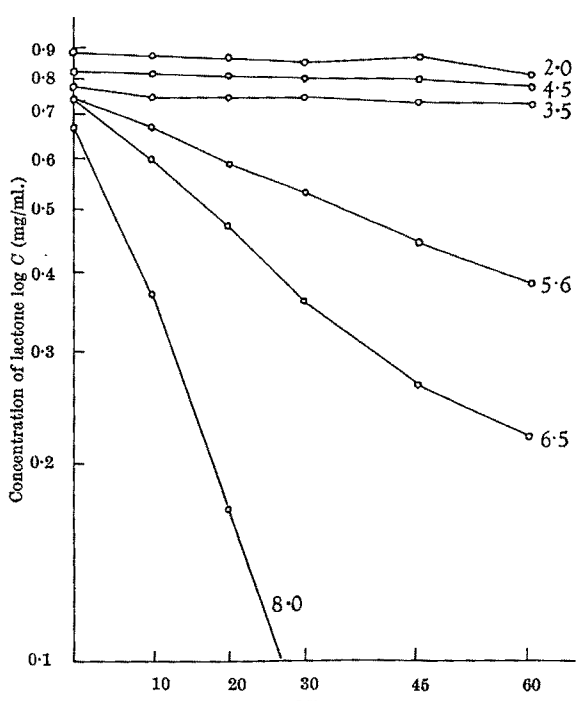


Fig. 4. Hydrolysis curves of D-glucono-γ-lactone in N/3 *tris*-maleate buffer solution of various *p*H, 30° C

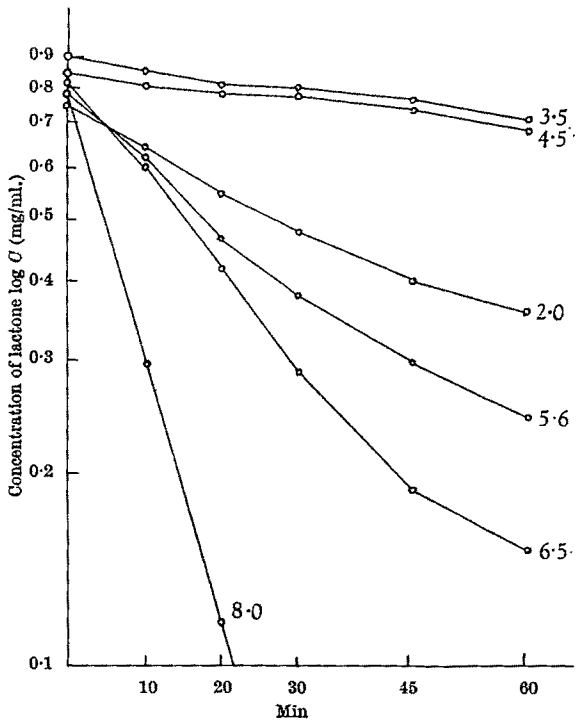


Fig. 5. Hydrolysis curves of D-glucono-δ-lactone in N/3 *tris*-maleate buffer solution of various *p*H, 30° C

scheme, we can reasonably explain the results of paper chromatographs and hydrolysis curves of gluconolactones.

For example, when δ-lactone is added in buffer solution, a part of it transforms to γ-lactone as soon as it is dissolved.

The concentration ratios of γ- and δ-lactone in buffer solution depend on *p*H of solution and initial concentration of δ-lactone. The velocity of transformation is very rapid, so their ratios do not depend on time. Both lactones in solution would be hydrolysed to gluconate, respectively, in first-order reaction. When γ-lactone is added, the formation of δ-lactone is slight at any *p*H. The hydrolysis of gluconolactones, especially δ-lactone in higher *p*H buffer solution, is always accompanied by their transformation (Figs. 1 and 2), and it is difficult to show true hydrolysis curves and their kinetic analysis.

This phenomenon shows that the transformation of lactones in D-araboascorbic acid synthesis is spontaneous and not enzymatic. Of course, we are not able to deny the existence of an enzyme catalysing this transformation. This has yet to be proved.

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EVOLUTION OF ETHYLENE BY SUB-CELLULAR PARTICLES FROM TOMATOES AS INFLUENCED BY COMPONENTS OF THE SYSTEM

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APART from evidence that energy is required for ethylene production, little is conclusively known about the biogenesis of this gas[1]. Recently we demonstrated that subcellular particles from tomatoes, *Penicillium digitatum*, rat liver and rat intestinal mucosa produce ethylene, and that evolution of the gas *in vitro* is related to the structural integrity of these particles[2–4]. In these investigations the particulate fractions were tested for ethylene production in a reaction mixture containing sucrose, potassium dihydrogen phosphate, magnesium sulphate, manganese sulphate, L-malic acid, adenosine triphosphate (ATP), and diphosphopyridine nucleotide (DNP). In this article, we have evaluated the contribution of this reaction mixture, chosen empirically on the basis of known biochemical properties of mitochondria, towards the production of ethylene by particulate fractions. The effect of temperature on ethylene evolution will be discussed in a later report.

Details of our preparative and analytical procedures have already been described[4,5]. The sub-cellular particles (separated by centrifugation at 35,000*g* for 15 min) were suspended in a reaction mixture and sonicated for 4 min in a Raytheon sonic oscillator (10 kc/s) operated at 1·1 amp and 0° C. When ATP and DPN (both obtained from the Sigma Chemical Co.) were used they were added after sonication. The mixture containing the suspended particles was divided equally and two 25-ml. portions prepared, one being used as a control. All reactions were conducted at room temperature.

The evolution of ethylene by sub-cellular particles from tomatoes as a result of free radical formation during sonication was suggested by Meigh[6]. While artefacts are, of course, a possibility, it should be noted that our preparations of sub-cellular particles produce ethylene not only when they are sonicated but also when instead of being sonicated they are aged *in vitro* or are disrupted mechanically by suspension in a 'Servall Omnimixer' or 'Virtis' homogenizer[4]. Treatment with phospholipase *A* or cholic acid, which affects the permeability and structural properties of mitochondria, also activates the ethylene-producing system[7]. Liberation of fatty acids, which have been implicated in the control of ethane production by sub-cellular particles from apples and tomatoes[6,8], could be expected to occur with the latter treatments as well as during ageing.

In addition to our use of a mercuric perchlorate collection system, whereas Meigh analyses the gases directly,

Table 1. COMPARISON OF ETHYLENE PRODUCTION BY TWO HALVES FROM A SINGLE PREPARATION OF SUB-CELLULAR PARTICLES FROM TOMATOES

Run	Halves	Ethylene, mμl./mg N (total mμl. C_2H_4 evolved, in brackets) 0–1	1–2	2–3	3–20 h
1	*A*	18·2 (153)	1·3 (11)	3·2 (28)	9·0 (76)
	B	21·9 (185)	—	—	17·6 (111)
2	*A*	16·7 (44) →		5·3 (14)	5·3 (15)
	B	15·4 (52) →		19·5 (66)	79·8 (269)
3	*A*	22·1 (88) →		*T*	70·5 (249)
	B	23 (71) →		9·2 (33)	28·1 (99)
4	*A*	—	4·3 (45)	4·3 (45)	44·2 (67)
	B	—	7·3 (77)	1·0 (11·3)	14·2 (150)
5	Reaction mixture, sonicated	*N*	*N*	*N*	*N*

Reaction mixture: 0·5 M sucrose, 0·125 M KH_2PO_4 (10^{-3} M), $MnSO_4$ (10^{-3} M), L-malic acid (0·05 M), ATP ($1·98 \times 10^{-3}$ M), *p*H 7. System was swept with a continuous stream of purified air, and the reaction mixture stirred, except where indicated. *N*, not detectable; *T*, trace.

Table 2. Endogenous Production of Ethylene by Sonicated Sub-cellular Particles from Tomatoes

C_2H_4, mμl./mg N (total mμl. in brackets)

Time (h)	Run No. 1 A	1 B	2 A	2 B	3	4	5	6 Reaction mixture sonicated
0–1	274 (214)	281 (219)	66·0 (478)	54·0 (349)	12·0	8·0	17·1	*N*
1–2	82 (64)	82 (64)	16·1 (77)	5·2 (43)	9·0	9·0	10·6	*N*
2–3	*N*	*N*	*N*	1·7 (11)	25·0	23·5	11·9	*N*
3–4	32 (25)	21 (16)	3·8 (28)	4·3 (28)	↓	↓	↓	↓
4–20	21 (16)	—	34·2 (248)	16·1 (105)				

Reaction mixture: 0·5 M sucrose, 0·125 M potassium dihydrogen phosphate, *p*H 7·0. *N*, not detectable.

Table 3. Effect of DPN ($3·3 \times 10^{-4}$ M) on Production of Ethylene by Sonicated Sub-cellular Particles from Tomatoes

Ethylene mμl./mg N (total mμl. in brackets)

Time (h)	Run No. 1 +DPN	1 −DPN	2 +DPN	2 −DPN	3 +DPN	3 −DPN	4 +DPN	4 −DPN
0–2	21·7	77·1	36·6	90·0	24·6	50·4	(38)	(106)
2–20	18·6	97·1	40·6	93·7	5·9	33·4	(82)	(11·1)

	5 +DPN	5 −DPN	6 +DPN	6 −DPN	7 +DPN	7 −DPN
0–2	23·5 (141)	15 (90)	42·5 (281)	59·5 (478)	19·2 (251)	11 (143)
2–4	10·5 (63)	*N*	22·6 (150)	*N*	0·5 (7)	0·1 (1·6)
4–16	↓	↓	—	—	6·5 (85)	—
16–17	*N*	*N*	—	—	0·5 (6·3)	1·3 (17·3)

Reaction mixture: runs 1–3, as in Table 1; run 4, as in Table 2, plus ATP ($1·98 \times 10^{-3}$ M); runs 5, 6, 7, as in Table 2. *N*, not detectable.

there are several other important differences in procedure. Meigh[6] adds ATP and DPN before sonication, rather than after, as we do. He uses a 2-h incubation period, whereas we make several consecutive collections extended over a much longer period of time. Meigh also uses an enclosed system for incubation of the sub-cellular particles, whereas we pass a continuous stream of purified air over the particles.

Meigh[6] detected 'measurable quantities' of ethylene when a solution of sucrose (0·5 M) and ATP ($1·98 \times 10^{-3}$ M) was sonically treated. For identification and determination of ethylene he used a gas chromatographic apparatus capable of detecting 0·004 μl. of ethylene per litre of air, in a 1-ml. gas sample. This amount of ethylene can be expected to be present as an air contaminant[5] and under Meigh's experimental conditions about 9 ml. of room air are inadvertently trapped inside the closed reaction vessel. In our collection system we pass a moving stream of purified air over the particulate suspension, and in addition routinely run blanks on room air, purified air and the mercuric perchlorate. Under such conditions we could detect no ethylene production by the reaction mixture in the absence of sub-cellular particles. It should perhaps be noted that Meigh, who can determine 0·004 mμl. C_2H_4/ml. gas sample in comparison with our 0·01 μl./ml., analyses gas from about one-eighth the amount of particles we use, and at such levels possible minor contributions from the sonication process, air contamination, etc., may assume greater importance.

In view of the fact that many steps in the preparation of sub-cellular particles cannot be performed quantitatively, in all our experiments conclusions have been drawn only from comparisons made within the same experimental run. When both halves of a reaction mixture containing sonicated particles from one preparation are treated identically, reasonably comparable amounts of ethylene are obtained during the early periods of production (Table 1 and Table 2, runs 1, 2).

An additional reason for variation in the rates of ethylene production among different experimental runs could be variations in an 'endogenous' production (enzymatic and/or non-enzymatic) of ethylene, independent of the presence of added substrate and co-factors. That this is indeed the case is clear from the fact that ethylene was produced when the particles were suspended only in sucrose–phosphate buffer (Table 2).

Results of experiments evaluating the effects of DPN on ethylene evolution by the sonicated sub-cellular particles are summarized in Table 3. Addition of DPN to mixtures containing ATP (runs 1–4) resulted in a considerable lowering of ethylene production, both during the first 2 h and the subsequent 18-h collection periods. Possibly the presence of DPN promotes the utilization in other reactions of intermediates involved in ethylene production or even the further metabolism of ethylene itself. When DPN was added to particles suspended only in sucrose–phosphate, no consistent effect on the initial evolution of ethylene was observed (Table 3, runs 5–7) as if, in fact, the endogenous production was uninfluenced by the DPN supply.

Results assembled in Table 4 show that ATP enhanced production of ethylene, particularly during *in vitro* ageing. (While the stability of ATP during the entire ageing period may be questionable, other substances formed by virtue of its presence may continue to exert their effects over long periods of time.) These results, illustrating the stimulatory effects of ATP on ethylene production, are consistent with conclusions reached from investigations of the effect of dinitrophenol on ethylene production by whole tomatoes[9] and tissue slices[10].

Table 4. Effect of ATP ($1·98 \times 10^{-3}$ M) on Production of Ethylene by Sonicated Sub-cellular Particles from Tomatoes

Ethylene, mμl./mg N

Reaction mixture	Time (h)	Run No. 1 +ATP	1 −ATP	2 +ATP	2 −ATP
0·5 M sucrose, 0·125 M KH_2PO_4, *p*H 7	0–2	59·2	21·6	34·6	27·7
	2–20	81·6	21·6	59·5	11·9

		4 +ATP	4 −ATP	4 +ATP	4 −ATP
As above, + $MgSO_4$ (10^{-3} M)	0–1	9·3	9·3	59·8	48·2
	1–2	18·6	18·6	↓	↓
	2–20	22·6	*T*	66·4	25·0

		5 +ATP	5 −ATP	6 +ATP	6 −ATP
As above, + $MgSO_4$, (10^{-3} M), $MnSO_4$ (10^{-3} M) and L-malic acid (0·05 M)	0–2	36·0	28·8	35·8	25·8
	2–20	48·0	6·0	53·5	1·3

Reference to results in Table 5 shows that magnesium in the concentrations used in our experiments appears to assist in ethylene evolution, while ethylenediamine tetraacetate inhibited it. Since manganese was precipitated in the sucrose-buffer solution, its effect on ethylene evolution could not be satisfactorily tested.

Table 5. Effect of Mg^{++} and EDTA on Production of Ethylene by Sonicated Sub-cellular Particles from Tomatoes

C_2H_4, mμl./mg N

Time (h)	Run No. 1 +Mg	1 −Mg	2 +Mg	2 −Mg
0–1	58·6	36·5	56·0	39·4
1–2	↓	↓	2·1	2·1
2–20	40·5	24·3	—	—

	3 +EDTA	3 −EDTA	4 +EDTA	4 −EDTA
0–2	24·9	29·6	27·0	36·0
2–20	12·2	26·0	25·0	45·0

Reaction mixtures: Runs 1, 2, 3, as in Table 2, + $MgSO_4$ (10^{-3} M) or EDTA (0·008 M). Run 4, as in Table 1, + EDTA, sodium salt (0·008 M).

These investigations indicate the existence of a complex situation governing the evolution of ethylene by sonicated sub-cellular particles from tomatoes. Initial evolution of the gas on fragmentation of the particles occurs whether they are suspended in sucrose phosphate buffer or in the complete reaction mixture. Certain substrate components do, however, influence the level of ethylene evolution. During ageing, further disintegration of the particles undoubtedly takes place, with possible liberation or production of enzymes, precursors or co-factors necessary for ethylene production.

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INHIBITION OF 6-MERCAPTOPURINE-RESISTANT CANCER CELLS IN CULTURE BY *BIS*(THIOINOSINE)-5′,5‴-PHOSPHATE

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ONE of the stumbling-blocks to major advancement of the status of clinical cancer chemotherapy, particularly with respect to the leukæmias and lymphomas, appears to be the development of drug resistance *in vivo*; that is, chemical selection and overgrowth of specific drug-resistant mutant cells from large, originally heterogeneous populations of neoplastic cells.

An effort has been made to gain knowledge at the molecular level regarding the nature of the altered metabolism within specific drug-resistant cells which makes possible their uninhibited growth in the presence of levels of drug which are lethal to the parent, drug-sensitive cells from which they arose. It might be hoped that such basic knowledge would eventually allow for more rational drug design and synthesis aimed at circumventing the specific metabolic mechanisms responsible for, or associated with, resistance to particular classes of chemotherapeutic agents.

It has been demonstrated in a convincing manner that associated with development of cellular resistance to purine antagonists, such as 6-mercaptopurine, 8-azaguanine, 6-thioguanine, 8-azaadenine, 8-azahypoxanthine, 8-azaxanthine, 2-fluoroadenine, and 2,6-diaminopurine, there is often a marked decrease or a loss of the pyrophosphorylase activity responsible for converting the specific purine antagonist to its respective ribonucleotide, presumably the inhibitory form of this class of metabolite antagonists[1–4].

Loss of specific purine ribonucleotide pyrophosphorylase activities represents only one mechanism of resistance to purine antagonists. It cannot be said that such loss of enzymatic activity is the cause of resistance since these biochemical alterations could be only fortuitously associated with resistance to purine antagonists. However, the consistency of the metabolic evidence provided a logical basis for a rational hypothesis. A demonstration that 6-mercaptopurine ribonucleotide is indeed an effective inhibitor of cells resistant to 6-mercaptopurine that lack inosinic acid pyrophosphorylase activity* would strengthen the view that there exists a causal relationship between the loss of this enzymatic activity and resistance to 6-mercaptopurine. Evidence of this nature cannot be obtained readily because purine ribonucleotides, which can be biosynthesized intracellularly or in appropriate cell-free systems, apparently do not gain entry into many types of intact cells[5]. Presumably for this reason 6-mercaptopurine ribonucleotide fails to inhibit cells resistant to 6-mercaptopurine[6,7] even though it does inhibit cells sensitive to 6-mercaptopurine†.

Although the general problem of cell permeability is complex and poorly understood‡, the observations discussed here led to the preparation of a number of derivatives of 6-mercaptopurine ribonucleotide[9,10] in the hope that some of them might be able to penetrate cancer cells and there be split by phosphodiesterase action to 6-mercaptopurine ribonucleotide, thus circumventing the loss of inosinic acid pyrophosphorylase activity in 6-mercaptopurine-resistant cells. Such a result would provide strong evidence for the mechanism of resistance to purine antagonists which has been proposed; such a compound might be an effective inhibitor of neoplasms resistant to this class of compounds provided it was not extensively degraded *en route* to such resistant neoplastic cells.

The cells used in these studies were *H.Ep* No. 2 (Toolan[11]), which are quite sensitive to 6-mercaptopurine and are designated *H.Ep* No. 2/*S*, and a subline of the parent sensitive line, which is approximately 1,000 times more resistant to 6-mercaptopurine than the *H.Ep* No. 2/*S*. The development and characteristics of this resistant line, designated *H.Ep* No. 2/*MP*, have been described previously[12].

Inhibition investigations were carried out following the procedure of Eagle and Foley[13] as modified by the Cancer Chemotherapy National Service Center for cytotoxicity screening in cell culture[14]. Cell growth was measured by the protein determination procedure of Oyama and Eagle[15]. After preliminary studies using serial one-log dilutions of compound, subsequent experiments were carried out using 0·3-log dilutions in order to obtain a more exact ED_{50}. The ED_{50} is defined as that concentration of drug in μg/ml. or μmoles/ml. which will inhibit the growth of cells to 50 per cent of cell control growth.

Simultaneous experiments were carried out with the *H.Ep* No. 2/*S* and *H.Ep* No. 2/*MP* cells and were termin-

* In all experience to date, loss of inosinic acid pyrophosphorylase activity has been accompanied by the loss of capacity to convert 6-mercaptopurine to 6-mercaptopurine ribonucleotide; presumably the same enzyme is responsible for both reactions.

† In this case inhibition is undoubtedly due to cleavage of the ribonucleotide to the ribonucleoside and to the base, which can enter the cells and there be converted to the ribonucleotide.

‡ It is perhaps pertinent to the present work that, whereas sodium D-glucuronate is not metabolized by human beings, the non-ionized glucuronic acid lactone is metabolized. This difference may be due to the ability of the lactone to penetrate the cell membrane and then be hydrolysed to the acid, which can be metabolized intracellularly[8].

ated when cell controls showed an increase in protein of seven- to ten-fold over the initial base line controls. The two cell-lines grow at essentially the same rate (22–24-h generation time) so that it was possible to terminate the experiments at the same time. Experiments were terminated after either 72 h or 96 h. Since 72 h is the minimum length of time for a seven- to eight-fold increase in growth it was sometimes necessary to continue an experiment an extra day in order to achieve the desired growth increase. When it was necessary to continue an experiment the cultures were re-fed with fresh medium containing the proper concentrations of compound.

The syntheses of the compounds used in these investigations, 6-mercaptopurine ribonucleoside[16], 6-mercaptopurine ribonucleotide[17], 2′-deoxy-5-fluorouridylyl (5′→5′) thioinosine, 2′-deoxy-5-fluorouridylyl (3′→5′) thioinosine, and *bis*(thioinosine)5′,5‴-phosphate[10], have all been reported.

Although none of a series of simple ester derivatives of 6-mercaptopurine ribonucleotide[9] showed more than minimal ability to inhibit *H.Ep* No. 2/*MP* cells in culture (G. G. Kelley, unpublished results), three dinucleoside phosphates, 2′-deoxy-5-fluorouridylyl (5′→5′) thioinosine, 2′-deoxy-5-fluorouridylyl (3′→5′) thioinosine, and *bis*-(thioinosine)5′,5‴-phosphate, have proved to be quite effective inhibitors of both *H.Ep* No. 2/*S* and *H.Ep* No. 2/*MP* cells. The inhibitory action of the 5-fluorouracil derivatives could be due simply to their breakdown to 5-fluorouracil, itself an effective inhibitor of *H.Ep* No. 2/*MP* cells. The action of the third compound, *bis*(thioinosine)5′,5‴-phosphate, obviously cannot be explained on a similar basis. Since 6-mercaptopurine, 6-mercaptopurine ribonucleoside, and 6-mercaptopurine ribonucleotide are all ineffective inhibitors of *H.Ep* No. 2/*MP* cells except at high levels, inhibition by *bis*(thioinosine)5′,5‴-phosphate can only occur if this molecule penetrates these cells intact. Inside the cell this compound itself might be an effective inhibitor or its inhibitory activity might result from phosphodiesterase cleavage to 6-mercaptopurine ribonucleotide (Fig. 1), probably the metabolic product of 6-mercaptopurine that inhibits the growth of cells sensitive to 6-mercaptopurine.

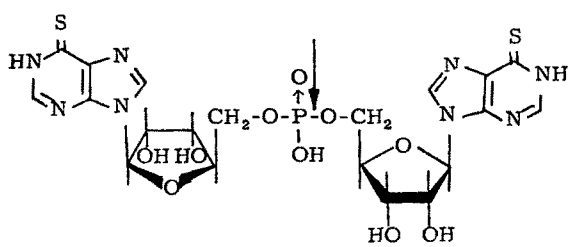


Fig. 1. Possible phosphodiesterase cleavage point of *bis*(thioinosine)-5′,5‴-phosphate

Since in a preliminary experiment at one concentration *bis*(thioinosine)5′,5‴-phosphate showed only minimal activity against *H.Ep* No. 2/*MP* cells[7], repetitive experiments at several concentrations were carried out on two different preparations of this compound. The ED_{50} values determined from these seven individual experiments, given in Table 1, show the reproducibility of this inhibitory effect. In each experiment 6-mercaptopurine was also evaluated to check the degree of resistance of this cell line. *Bis*(thioinosine)5′,5‴-phosphate and 6-mercaptopurine were tested against *H.Ep* No. 2/*S* cells in concurrent experiments, and these results are also given in Table 1. The data from these individual experiments were plotted on semilog paper to obtain the average ED_{50} values for 6-mercaptopurine and *bis*(thioinosine)-5′,5‴-phosphate against *H.Ep* No. 2/*S* and H.*Ep* No. 2/*MP* (Fig. 2 shows the plot for the *H.Ep* No. 2/*MP* cell line). These average values are compared in Table 2 with the ED_{50} values for 6-mercaptopurine ribonucleoside, 6-mercaptopurine ribonucleotide, and the two 5-fluorouracil derivatives. These results indicate that, whereas *bis*(thioinosine)5′,5‴-phosphate is of the same order of effectiveness as 6-mercaptopurine in the inhibition of *H.Ep* No. 2/*S* cells, it is 240 times as effective as 6-mercaptopurine in the inhibition of *H.Ep* No. 2/*MP* cells. (These comparisons are on a molar basis. Since the *H.Ep* No. 2/*S* cells are inhibited by 6-mercaptopurine, 1 mole of *bis*(thioinosine)5′,5‴-phosphate is equivalent to 2 moles of 6-mercaptopurine for the inhibition of this cell line, but not for the *H.Ep* No. 2/*MP* cell line.)

Table 1. Inhibition of Growth of *H.Ep* No. 2/*S* and *H.Ep* No. 2/*MP* Cells in Culture by 6-Mercaptopurine and *Bis*(Thioinosine)5′,5‴-Phosphate: ED_{50} Values from Individual Experiments

N.S.C. No.	Compound	Experiment 1	2	3	4	5	6	7
		ED_{50} (μg/ml.) *H.Ep* No. 2/*MP*						
755	6-Mercaptopurine	100	15	1,000	500	500	400	860
407427	*Bis*(thioinosine)-5′,5‴-phosphate	3	3	6	11	10	11	3
		ED_{50} (μg/ml.) *H.Ep* No. 2/*S*						
755	6-Mercaptopurine	0·2	0·3	0·96	0·14	0·29	> 1·6	0·15
407427	*Bis*(thioinosine)-5′,5‴-phosphate	< 1	0·3	0·55	0·37	> 1·6	0·55	0·2

Table 2. A Comparison of the Capacity of Several Derivatives of 6-Mercaptopurine to Inhibit the Growth of *H.Ep* No. 2/*S* and *H.Ep* No. 2/*MP*

N.S.C. No.	Compound	ED_{50} *H.Ep* No. 2/*S* μg/ml.	*H.Ep* No. 2/*S* μmoles/ml. (× 10³)	*H.Ep*/No. 2/*MP* μg/ml.	*H.Ep*/No. 2/*MP* μmoles/ml. (× 10³)
755	6-Mercaptopurine	0·5*	2·94	570*	3,350
4911	6-Mercaptopurine ribonucleoside	0·4	1·41	> 100†	> 352
46024	6-Mercaptopurine ribonucleotide	0·6	1·47	> 100†	> 245
409289	2′-Deoxy-5-fluorouridylyl (5′→5′)thioinosine	< 0·1	< 0·16	< 1	< 1·6
409841	2′-Deoxy-5-fluorouridylyl (3′→5′)thioinosine	< 0·1	< 0·16	< 1	< 1·6
407427	*Bis*(thioinosine)5′,5‴-phosphate	0·4*	0·63	8·8*	13·9

* Average of seven experiments (see Fig. 2).
† Non-inhibitory at this, the highest dose tested.

LePage *et al.*[18] have shown that azaserine-induced accumulation of formylglycinamide ribonucleotide (FGAR) was inhibited by purine analogues such as 6-mercaptopurine and 6-thioguanine, presumably by pseudo-feedback inhibition of 5-phosphoribosylamine synthesis. Using this same technique it was found that 6-mercaptopurine and 6-thioguanine inhibited formylglycinamide ribonucleotide formation in sensitive *H.Ep* No. 2 cells in culture but not in sublines resistant to these purine analogues[19]. It was demonstrated previously[20] that the 6-mercaptopurine-resistant cells lacked inosinic and guanylic acid pyrophosphorylase activities and thus lacked the enzymatic capacity to form ribonucleotides from the base analogues. McCollister *et al.*[21] have shown recently that the ribonucleotides of mercaptopurine and thioguanine inhibited the enzymatic synthesis of 5-phosphoribosylamine, the first irreversible reaction unique to purine biosynthesis, and that the free bases and the corresponding ribonucleosides were inactive as inhibitors of this reaction. In view of the observed inhibition of the growth of *H.Ep* No. 2/*MP* cells by *bis*(thioinosine)5′,5‴-phosphate, we were led to examine this compound for its effect on formylglycinamide ribonucleotide formation.

Table 3. Effect of Nucleotide Derivatives of 6-Mercaptopurine on Azaserine-Induced Formylglycinamide Ribonucleotide (FGAR) Accumulation in *H.Ep* No. 2/*MP* Cells in Culture

Inhibitor	FGAR-^{14}C accumulation in the soluble fraction (% of azaserine control)
Azaserine control	100*
+ 6-Mercaptopurine ribonucleotide 0·29 μmoles/ml.†	77
+ *Bis*(thioinosine) 5′,5‴-phosphate 0·14 μmoles/ml.‡	9
0·014 μmoles/ml.	56

* Activity of FGAR-^{14}C = 73,146 counts per minute/10^7 cells.
† Concentration = 131 μg/ml.
‡ Concentration = 93 μg/ml.

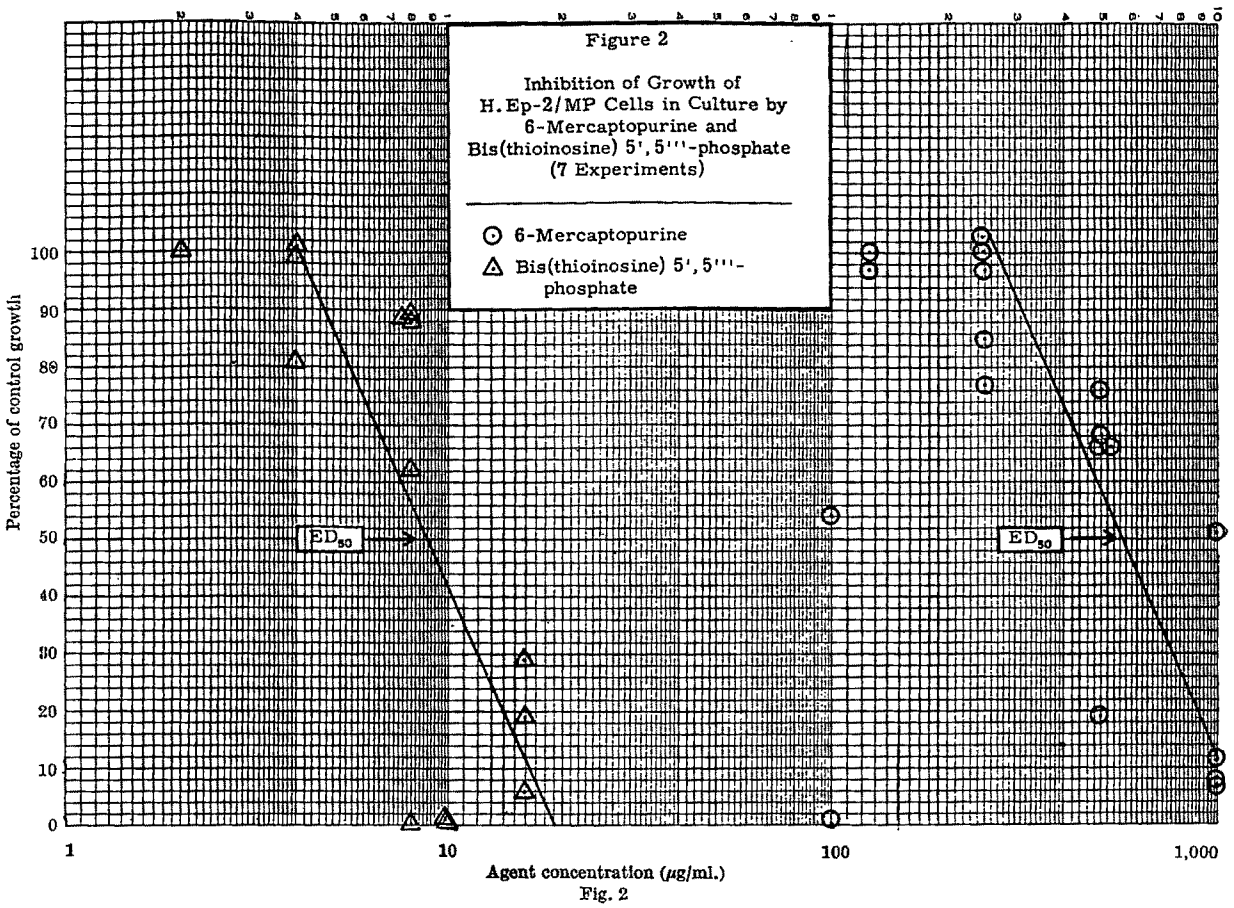


Fig. 2

H.Ep No. 2/*MP* cells in suspension culture were treated with azaserine (10 μc./ml.) followed 1 h later by sodium formate-^{14}C (25 μg/100 ml. culture medium containing approximately 4 × 10^7 cells). 2 h after addition of the radioactive substrate *H.Ep* No. 2/*MP* cells were collected by centrifugation and soluble extracts of the cells were prepared for analysis by two-dimensional chromatography-autoradiography as described previously[20]. The purine nucleotide analogues were added to suspensions of *H.Ep* No. 2/*MP* cells at the indicated concentrations (Table 3) 30 min after the addition of azaserine; 30 min later sodium formate-^{14}C was added, followed 2 h later by collection of cells, as described for the azaserine control culture.

Radioactive FGAR was located on the paper chromatograms by exposure of the paper to 14 in. × 17 in. sheets of X-ray film (Eastman Kodak, single emulsion, blue sensitive) for one week. Quantitative determinations of radioactivity were made by counting disks cut from paper chromatograms in a Packard Instrument Co. liquid scintillation spectrometer by modification of procedures previously described[22].

The azaserine pretreatment of *H.Ep* No. 2/*MP* cells caused significant accumulation of formate-labelled FGAR (Table 3). Addition of a relatively high concentration of 6-mercaptopurine ribonucleotide, 131 μg/ml., resulted in only 23 per cent inhibition of FGAR accumulation whereas *bis*(thioinosine)5′,5‴-phosphate at a molar concentration one-half that of 6-mercaptopurine ribonucleotide caused 91 per cent inhibition of accumulation of FGAR. Even at a molar concentration one-twentieth that of 6-mercaptopurine ribonucleotide the diester phosphate produced 44 per cent inhibition of FGAR accumulation. It might be postulated that *bis*(thioinosine)5′,5‴-phosphate gained entry into the resistant cells where it was afterwards cleaved intracellularly to yield 6-mercaptopurine ribonucleotide. The relative inactivity of 6-mercaptopurine ribonucleotide may be a consequence of extracellular cleavage to the ribonucleoside and the purine base.

Razzell and Khorana[23] found that venom phosphodiesterase hydrolysed thymidylyl(5′→5′)thymidine in addition to cleavage of simple esters of nucleoside-5′-phosphates and certain oligonucleotides. Preliminary results obtained in this laboratory have indicated that venom phosphodiesterase readily cleaved *bis*(thioinosine)5′,5‴-phosphate and, furthermore, that the soluble supernatant fraction prepared by high-speed centrifugation of disrupted *H.Ep* No. 2 cells also brought about hydrolysis of this compound (R. W. Brockman, Sue Chumley and Frank Hays, unpublished observations). In view of previous observations in this laboratory that simple esters of 6-mercaptopurine ribonucleotide were not inhibitory to *H.Ep* No. 2/*MP* cells, it is significant that Anderson and Heppel[24] found that a partially purified leukæmia cell phosphodiesterase was inactive in hydrolysing simple esters of nucleoside-5′-phosphates and that the presence of a nucleoside group on each side of the phosphodiester bond seemed requisite for cleavage by this enzyme.

The results obtained in this work provide good evidence in support of the mechanism of resistance discussed and have encouraged us to pursue the synthesis of a sufficient quantity of *bis*(thioinosine)5′,5‴-phosphate to evaluate this compound against neoplasms resistant to 6-mercaptopurine in experimental animals. Furthermore, in view of the repeatedly observed decrease in the capacity of cells that are resistant to pyrimidine antagonists to form pyrimidine ribonucleotides[25], it will be of interest to evaluate the effectiveness of the *bis*(ribonucleoside)5′,5‴-phosphates of bases such as 5-fluorouracil, 5-bromouracil and 5-iodouracil, and the azapyrimidines against appropriate drug-resistant cells.

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CAPACITATION OF RAM SPERMATOZOA AND PENETRATION OF THE OVINE EGG

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THE need for rat and rabbit spermatozoa to spend a "capacitation" period of 2 h and 4–6 h, respectively, in the female genital tract before attaining full fertilizing ability was first demonstrated by Austin[1] and Chang[2]. Capacitation of spermatozoa probably occupies 2–4 h in the hamster[3] but may be developed in less than 1 h in the mouse[4]. The observation by Dauzier and Thibault[5] that fertilization of sheep ova can be achieved *in vitro* with spermatozoa recovered from the genital tract of mated ewes, but not with freshly ejaculated semen, indicates that ram spermatozoa may also undergo capacitation in the genital tract of the ewe. The following observations made at this laboratory on the penetration of the ovine egg *in vivo* support this conclusion.

Laparotomy was performed in multiparous Merino ewes 18–30 h after the observed onset of œstrus. If ovulation had occurred, approximately 0·02 ml. of freshly ejaculated ram semen was deposited in the ampulla of the appropriate Fallopian tube(s). Ewes were killed by intra-cardiac injection of pentobarbitone solution at various intervals after insemination. Eggs were recovered from the Fallopian tubes within 10 min after death and mounted in saline[6].

During preliminary examination by phase-contrast microscopy (× 200 – × 500), eggs were rolled on the slide by slight movement of the coverslip. The tail of a penetrating spermatozoon lying free within the perivitelline space was clearly discernible in unfixed eggs but, until the eggs were fixed and cleared, it was impossible to determine whether or not the head of the spermatozoon had entered the vitellus or was merely attached to the vitelline membrane. Each egg was flooded with 5 per cent formol saline for 2–5 min, washed with saline and fixed and cleared in acetic-alcohol[7] for 2–5 h. Treatment of the eggs with formol saline delayed the breakdown of the zona pellucida and enabled the exact location of the head of the penetrating spermatozoon to be determined in the partially cleared egg while the zona remained intact. When completely cleared of yolk, the eggs were stained for 2–15 h with 1 per cent aceto-orcein prepared according to Ferguson[7]. Excess stain was removed with 45 per cent acetic acid before the eggs were re-examined using bright-field microscopy (× 200 – × 800).

The results of the examination of 44 eggs are shown in Table 1. Spermatozoa were attached to the zona pellucida in every egg recovered. Nevertheless, penetration of the zona pellucida was not found to have occurred until 1·5 h after insemination. It would appear, therefore, that the lower limit of the time required for capacitation of ram spermatozoa in the Fallopian tube of the ewe is approximately 1·5 h.

In all eggs recently penetrated, the entire spermatozoon was contained within the perivitelline space and the head was attached to the vitelline membrane. Active flagellation of the tail of the spermatozoon within the perivitelline space, resulting in oscillatory movements of the vitellus within the zona pellucida, was observed in 3 of the 4 penetrated eggs recovered at 1·5 h and in 2 of the 3 penetrated eggs recovered at 1·75 h after insemination. Movement of the tail of the penetrating spermazotoon was not seen in any of the eggs recovered at longer intervals. As the presence of the head of the penetrating spermatozoon within the vitellus was not observed until

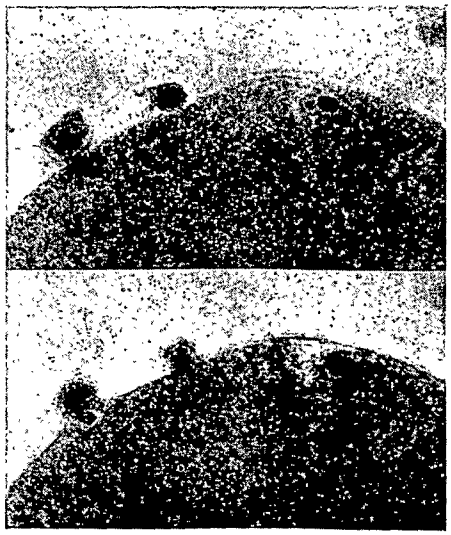


Fig. 1. A fertilized sheep ovum 3·25 h after tubal insemination showing entire spermatozoon within vitellus at the time of abstriction of the second polar body

Top, spermatozoon head deeply stained but showing only slight change in shape and volume; bottom, same ovum: chromosomes aggregated near periphery of vitellus at completion of second maturation division of ovum

Table 1. Examination of Eggs recovered From The Fallopian Tubes of Ewes at Different Times after Tubal Insemination

Time after tubal insemination (h)	No. of eggs				
	Examined	With zona pellucida penetrated	With sperm head within vitellus	With two polar bodies	With two pronuclei
½	3				
1	3				
1¼	4				
1½	5	4			
1¾	3	3			
2	4	4			
2¼	3	3	1		
2¾	4	4	2		
3	3	3	3		
3¼	4	4	4	1*	
3½	4	4	1*	4	3
4	4	4		4	4

* Second polar body abstricted but individual chromosomes in vitellus still distinguishable.

2·5 h after insemination, it is likely that 1–1·5 h is required for the absorption of the spermatozoon head into the vitellus of the ovine egg compared with approximately 30 min in the rat[8] and mouse egg[4].

Spermatozoon heads which were attached to the zona pellucida or were in the perivitelline space did not stain readily, but those that had entered the vitellus exhibited a strong affinity for the orcein stain. However, little change was seen in the size or shape of the spermatozoon head within the vitellus before the second polar body had been abstricted at the completion of the second maturation division of the egg nucleus (Fig. 1). This appeared to take place approximately 0·5 h after the spermatozoon head was absorbed into the vitellus. Thereafter, development of the male and female pronuclei appeared to be rapid. Small but discrete male and female pronuclei were observed in 3 of 4 eggs recovered 3·5 h after insemination and two large pronuclei were present in each of the 4 eggs recovered 4 h after insemination.

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VARIATION IN THE SENSITIVITY TO GAMMA-AMINO-BUTYRIC ACID OF DIFFERENT REGIONS OF THE MAUTHNER NEURONE

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Mauthner cells occur paired in the medulla of most teleost fishes and certain amphibia. The cell has attracted the attention of biologists because of its large size, its unusual shape, and the distinctive grouping and morphology of the nerve endings which make synaptic connexions on it (Fig. 1, taken from ref. 1). Furshpan and Furukawa[2] have established a basis for the placing of a microelectrode tip at various predetermined regions of the cell, which lies 1–2 mm below the surface. Their findings thus made it possible to devise experiments to demonstrate regional differences in chemical sensitivity of the Mauthner cell. In this article such differences are described, and tentatively correlated with known morphological and functional features.

Substances were tested by applying them ionophoretically to the cell membrane from micropipettes (tip diameters of 0·5–1·5μ) while recording the internal potential of the resting and excited cell. Attention has been directed, in particular, to γ-amino-butyric acid (GABA) and L-glutamic acid. GABA was passed as a cation from a 4 M solution of *p*H 2·5, glutamic acid as an anion from a 2 M solution, *p*H 7·3 or 8·6. Intracellular electrodes were micropipettes, tip diameters of 0·5–1·5μ, filled with either 3 M potassium chloride or 0·6 M potassium sulphate. Goldfish, initially anæsthetized in 2 per cent urethane, were perfused through the mouth with 0·3 per cent urethane in water, and prepared similarly to those of Furshpan and Furukawa[2]. The head was clamped, the brain exposed and the cerebellum displaced to reveal the dorsal surface of the medulla. Wire electrodes inserted alongside the vertebral column in the tail excited the Mauthner cells antidromically via their large low-threshold axons in the cord. Similar electrodes were inserted through the skull for orthodromic activation of the cells via the VIII nerves in the region of the vestibular apparatus. Initial intramuscular injections of *d*-tubocurarine prevented movements caused by Mauthner cell excitation.

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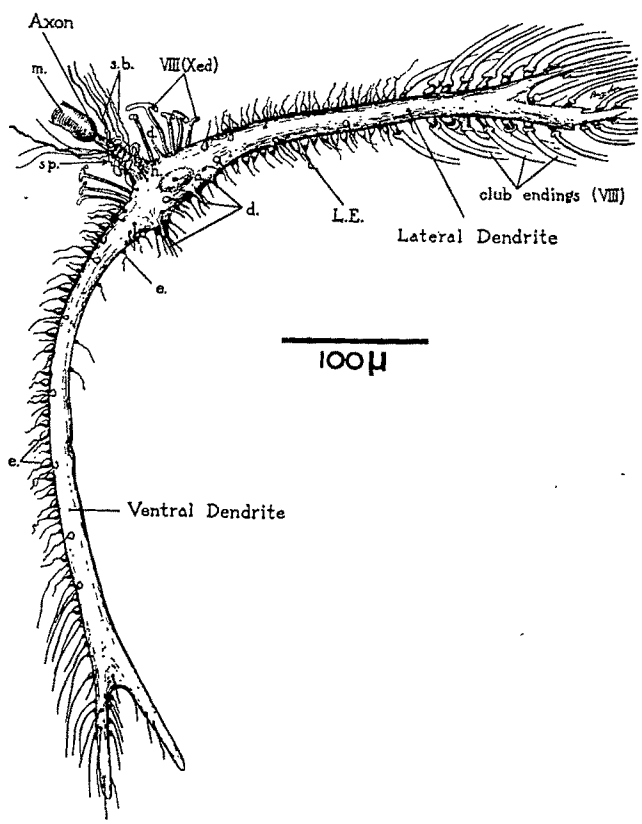


Fig. 1. Semi-schematic representation of the right Mauthner cell and associated nerve endings in the goldfish (reproduced ref. 1). VIII (*Xed*), and *LE*, refer to endings of fibres of the contralateral VIII nerve, though the large end-bulbs (*LE*) may include endings of secondary vestibular fibres (ref. 1). Club endings (VIII) are ipsilateral in origin. *h* = axon-hillock

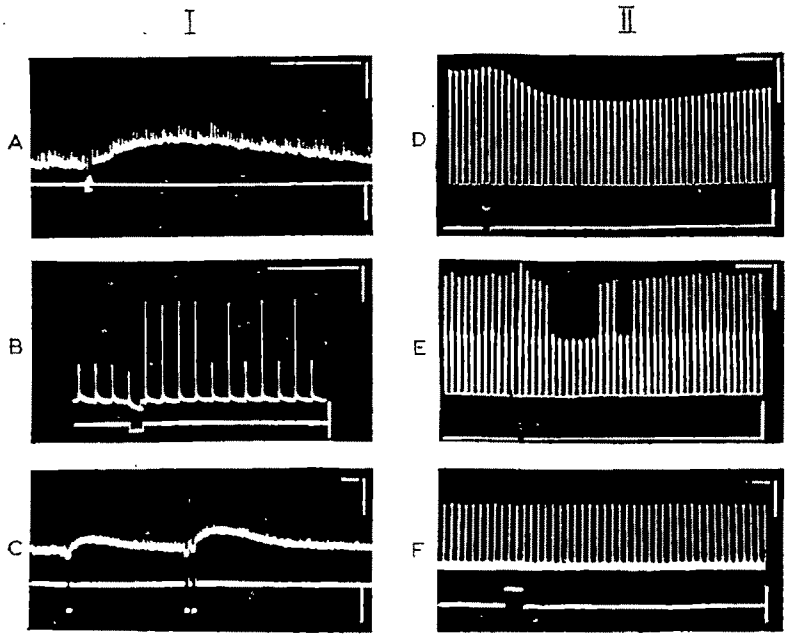


Fig. 2. (I) Effects of L-glutamic acid: (*A*) glutamate pipette at cell body. − current pulse causes downward deflexion on lower trace; (*B*) glutamate applied during sub-threshold ipsilateral VIII nerve stimulation at 10/sec; (*C*) glutamate pulses applied through pipette on lateral dendrite, 550μ lateral to axon-hillock. (II) Effects of GABA: internal recordings with potassium sulphate electrode. (*D*) GABA pipette at cell body. + current pulse (upward deflexion on lower trace) applied during antidromic excitation at 10/sec; (*E*) GABA pulse applied to cell body during orthodromic excitation at 10/sec; (*F*) GABA pulse applied through pipette on lateral dendrite, 400μ lateral to axon-hillock. Vertical bar in *A* = 2 mV; in *C* = 5 mV; in all other records = 20 mV. Horizontal bars = 500 msec in all records. Vertical bar at end of current trace = 10^{-6} amp in *A* and *B*, and 10^{-7} amp in *C*, *D*, *E*, *F*

Micropipette tip positioning was determined as follows. First the axon hillock of the Mauthner cell (which served as a reference point) was located in the medulla as described by Furshpan and Furukawa. The electrode tip lies within a few μ of this point when it records, some 0·5 msec after a spinal cord stimulus, the characteristic highly localized negative spike of 20–50 mV. The electrode, or drug pipette, could then be withdrawn from the brain and moved laterally and posteriorly along the approximate line of the cell body and lateral dendrite for a measured distance, and re-inserted near the desired spot. Re-insertions along an antero-posterior line were continued until the shape and size of the extracellular potential indicated a close positioning to a chosen cell region. The recording electrode was usually inserted into the cell body, while the position of the drug electrode was frequently varied during the course of the experiment. An appropriate 'braking' voltage was applied to this pipette, just adequate to prevent the effects of drug diffusing from the tip[3]. Fig 2*A* shows the effect of glutamate applied just outside the cell body. The addition of sub-threshold EPSP's (evoked orthodromically) to such a sub-threshold response caused the cell to fire (Fig. 2*B*). The effects of GABA applied at the same place are shown in Fig. 2*D* and 2*E*. It diminishes the amplitude of the intracellularly recorded spike (Fig. 2*D*), and can prevent orthodromically evoked firing (Fig. 2*E*, which shows the (reduced) EPSP's remaining when the spikes are blocked). During the effects of GABA there was often no change in resting membrane potential when a potassium sulphate electrode was used, but with a potassium chloride electrode GABA frequently caused a depolarization. It seems, therefore, that GABA acts principally by increasing the membrane conductance to chloride and possibly other ions. In all these respects, GABA closely mimics the effects, described by Furshpan and Furukawa, of the natural inhibitory transmitter. Controls using pipettes filled with hydrochloric acid or sodium chloride plus hydrochloric acid (*p*H 2·0) have shown that these effects of GABA are clearly distinguishable from any due to concomitantly released H^+ ions.

Figs. 2*C* and 2*F* show corresponding records made with the drug pipettes at the distal part of the lateral dendrite. This region of the cell shows a striking difference from the cell body in its sensitivity to GABA, but not to glutamate. That this difference was not due to difficulty in placing the GABA pipette tip close to the membrane was shown by the results obtained with double-barrelled pipettes (tips totalling 1–4μ), having GABA in one barrel and glutamate in the other. The response to glutamate was a critical indicator of the proximity of the tip to the membrane, and was a convenient one to use, since it was followed by little or no desensitization. It was always possible to find a 'critical' pipette position, which gave responses to glutamate like those of Fig. 2*A* and *C*. This position must have been fairly close to the membrane, for small movements often resulted in penetration of the cell, and the response to glutamate was very sensitive to electrode shifts of only a few μ. In regions sensitive to GABA, the position for optimum GABA response coincided with this position for optimum response to glutamate. Using this method for positioning double-barrelled pipettes at the cell surface, systematic surveys were made of the distribution of sensitivity to the two substances. The results of the most complete mapping experiment are shown in Fig. 3 in which the 'threshold charge' (that required for a just-detectable response), for glutamate and GABA applied successively, is plotted for various distances along the cell up to 400μ from the axon-hillock. Each point represents a critical located position as described above. Large current pulses introduce errors due to local *p*H changes, and to diffusion of drug to adjacent regions; therefore the sensitivity to GABA of the distal region is likely to be over-estimated. An internal electrode within 75μ of the distally located drug pipette

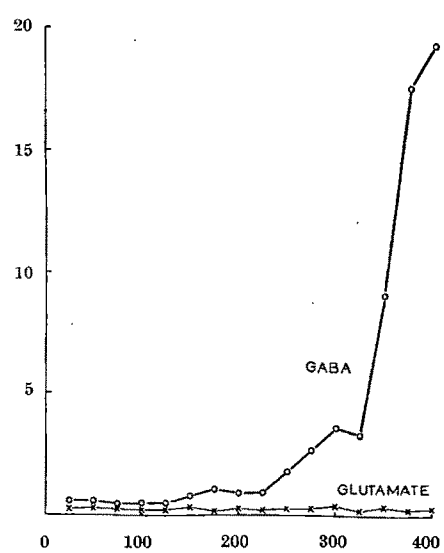


Fig. 3. Sensitivity of cell body-lateral dendrite regions of Mauthner cell to GABA and to L-glutamic acid. See text for explanation. Ordinate, threshold charge (coulombs × 10^{-8}), Abscissa, distance (μ) lateral to axon-hillock, which is at the origin

also failed to detect a significant response to GABA, that to glutamate being normal. The threshold charge for glutamate was remarkably constant, not only from one region to another (Fig. 3), but even from cell to cell.

The conclusion from these results is that while the Mauthner cell membrane is uniformly sensitive to glutamate there is a regional variation in sensitivity to GABA, the action of which mimics that of the natural inhibitory transmitter. This variation may be correlated with other known features of the cell. The GABA-insensitive region corresponds with that which receives the large-club endings of the ipsilateral VIII nerve (Fig. 1, which was not corrected for shrinkage; this must have been in the region of 30 per cent (Bodian, personal communication)). These endings are almost certainly excitatory[2]. On the other hand, the axon-hillock, cell body, and probably the proximal part of the lateral dendrite—the regions where GABA has an inhibitory effect—receive fibres from the contralateral VIII nerve[1]; stimulation of this nerve inhibits the Mauthner cell, probably directly[3].

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SINGLE-CELL ISOLATION AND PRESERVATION OF BACTERIAL CULTURES

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CULTURES originating from single cells are usually recognized as a minimum precaution to ensure culture purity in studies on bacterial variation and transformations; moreover, derivatives of these clones isolated as single cells often increase the confidence in the results of such investigations. Frequently the difficulties encountered with direct methods, where single cells are seen before isolation, have encouraged the use of simpler indirect methods where single colonies used for isolations are assumed to arise from single cells.

The poured-plate technique, an indirect method, is probably used most commonly because of its speed and simplicity. McNew[1] has presented evidence to support the assumption that, with the poured-plate technique, colonies can be selected that have single cells as their origin. However, several authors[2-6] have pointed out or demonstrated the inadequacy of this method for single-cell isolations. In a comparison of the poured-plate method with a direct method involving mechanical manipulation, Riker *et al.*[7] found they could not separate the cells of *Agrobacterium tumefaciens* (Smith and Townsend) Conn. and *Agrobacterium rhizogenes* (Riker *et al.*) Conn., from a mixed culture using poured plates whereas the bacteria were separable by the direct or mechanical method.

Hildebrand[4,5] has classified the direct methods for isolating single cells into two categories, semi-mechanical and mechanical. The semi-mechanical procedures, although not considered as precise as the mechanical methods, have been used effectively[4,5]; they usually involve the use of a microscope to locate a single cell or a gelatin or agar surface. The location of the cell is marked and when a small colony has developed a transfer is made to an appropriate medium, or the cell may be transferred immediately along with a small amount of medium on which it is resting. The mechanical method commonly utilized is essentially the same as that introduded by Barber[8]. A micromanipulator equipped with a needle or micropipette is used to handle directly an individual cell and to effect its isolation usually into a microculture chamber. The special equipment and skill that are required to use such a method effectively have minimized its use. Thus the need for a method of handling a bacterium directly with the precision of a micromanipulator and the simplicity of a semi-mechanical method has been apparent.

In 1954, Lederberg[9] described a modification of the de Fonbrune oil-chamber technique, a method the features of which approach the desired simplicity and ease of use. The surface of a ruled glass slide is layered with mineral oil and, by means of a capillary pipette, microdroplets containing bacterial cells are deposited under the thin layer of oil. The drops, which spread to a diameter of 0·1–0·2 mm, are examined microscopically. Those that contain a single bacterium are either recovered immediately by flushing them repeatedly in and out of a capillary pipette with subsequent transfer to a suitable medium, or they are left to incubate until a large bacterial clone develops.

Frequent use of Lederberg's[9] technique in examination of plant pathogenic bacteria has resulted in further modifications reported here that have improved its ease of use and effectiveness without the loss of its simplicity. Additional data are presented on the preservation of bacterial clones in distilled water, adapted from the method of Kelman[10,11], in which long-term storage is accomplished with preservation of desired traits such as pathogenicity.

Isolation of single bacterial cells. A major difficulty often encountered in the method described by Lederberg[9] was the spreading of the droplets under the oil on clean glass slides until their definition was lost and recovery of single cells was impossible. On occasion when the droplets were found that contained single bacterial cells, only small amounts of them could be aspirated into capillary pipettes because they adhered to the surface of the glass slides, often resulting in the loss of the bacterial cell; better results were achieved when the slides were first coated with silicone. Later it was found that 'Plexiglass' (acrylate and methacrylate resin) slides provided an ideal surface without the use of silicone on which the droplets retained a desired form under the oil and could be examined critically with the microscope.

Moreover, the use of spherical droplets rather than spreading or flattened droplets enabled the operator to work with droplets 50–70μ in diameter that could be viewed in their entirety in each plane during search of the droplet with low-aperture objectives. Flattened droplets 0·1–0·2 mm in diameter, although allowing use of phase or dark-field microscopy, required a shift in gaze to search different quadrants in each plane of the droplet and increased the possibility of overlooking one or more additional bacterial cells. It was also found that heat sterilization of the mineral oil gave assurance of an aseptic system and did not affect the viability of single cells of *Agrobacterium tumefaciens*, *Pseudomonas syringae*, van Hall, or *Xanthomonas malvacearum* (Erw. Smith) Dowson.

Materials. (*a*) Bacterial cell dilutions are prepared from cultures 10–12 h old grown in 5 ml. of nutrient broth and seeded with a loop of cells from an agar slant. Dilutions are made in nutrient broth beginning with a sample withdrawn from the centre of the broth culture until a dilution is obtained that yields 1 or 2 cells per microdrop.

(*b*) Microscope slides 7·5 × 2·5 cm are cut from 'Plexiglass' stock, 3 mm thick. A grid of 5 mm squares is scratched on the top of the slides leaving a margin of approximately 5 mm. The slides can be used repeatedly and are sterilized in 70 per cent ethanol, then dried in sterile Petri dishes at 45° C.

(*c*) A heavy mineral oil, non-medicated, is dispensed into a 125-ml. Erlenmeyer flask and sterilized by heating on a hot plate for 2 h at approximately 300° C. A glass rod inserted in a cork makes a convenient applicator.

(*d*) Capillary pipettes are drawn from glass tubing (2 mm inside and 3·5 mm outside diam.). Pieces approximately 6 in. are fire polished on both ends then heated in the centre and drawn to give a capillary 4–5 in. long and about 0·5 mm in diameter. The pipettes are cut apart and then the capillaries are heated about 0·5 in. from the larger diameter section and drawn to a smaller size. The fine capillary can best be drawn after heating the larger capillary in a flame about 1/8 in. long. An eye-dropper with a small tip makes a convenient burner. The fine capillary should be about 1 in. long and have a bore about 50μ in diameter for making droplets of approximately the same diameter. Frequently capillaries are drawn with a bore of about 100μ; these pipettes are useful for recovering the droplets containing a single bacterium. The pipettes are plugged with cotton and sterilized by autoclaving them at 121° C for 15 min.

(*e*) Rubber tubing 2 ft. long (3 mm inside and 4 mm outside diam.) is used to control the deposition of droplets on to the slides and also for their recovery.

(*f*) A microscope equipped with a 5-power objective and an eyepiece micrometer is used when depositing the droplets with bacteria on the 'Plexiglass' slides (Fig. 1). The microscope stage should be level to prevent movement of the oil layer and the droplets.

(*g*) A level surface on which to rest the 'Plexiglass' slides is used when the thin layer of oil is applied to the area over the grids. Drawing a line with a wax pencil around the margin of the slide's surface will prevent the oil from flowing over the edge of the slide if it is not level.

(*h*) Also needed are nutrient agar slants or other substrates that are best for culturing the single cells.

Method. Approximately 1 ml. of mineral oil is placed on the surface of a 'Plexiglass' slide and spread over the area above the grid scratched on top of the slide. The slide is then positioned on a level mechanical stage. A small sample (50 μl.) of the dilute bacterial suspension is taken up in a capillary pipette the delivery of which is controlled using a length of rubber tubing extending to the operator's mouth.

Holding the pipette in a stationary lateral position with the hand resting on the corner of the stage, the tip of the pipette is then positioned just above the oil layer and over a square on the slide's grid (Fig. 1). A high aperture objective (× 5) allows ample working distance and sufficient resolution so that the pipette can be manipulated and the tip dipped into the oil. With slight air pressure a microdroplet is delivered into the oil layer and the tip of the pipette is then raised just out of the oil. Without moving the pipette further, another square is positioned under the pipette's tip by means of the mechanical stage and the process is repeated until several droplets have been deposited under the oil. The droplets, about 50μ in diameter, will settle to the surface of the slide and retain a spherical shape. Under bright field, the droplets are then examined for the presence of bacteria using a low aperture objective (× 43). By focusing through the droplet, each plane is seen in its entirety and the bacterial population is determined. If more than one cell is present



Fig. 1. Deposition of broth microdroplets on a 'Plexiglass' slide. A capillary pipette is attached to a rubber tube extending to the operator's mouth. The high-aperture objective (× 5) is useful during this manipulation. After the droplets are deposited under the layer of oil on the slide they are examined for single cells with a low-aperture (× 43) objective

per droplet, the dilution of the bacterial suspension is adjusted to give 1 cell per droplet, and a new series of droplets are deposited under the oil.

When droplets are found that contain a single cell they are immediately recovered and transferred to a nutrient agar surface or other substrate favourable for their growth. For recovery, a capillary pipette the tip of which was too large (approximately 100μ) for delivering the original droplet, is charged with about 50 μl. of sterile broth. With the same procedure used in delivering the droplet, the tip of the pipette is brought up to the droplet and the droplet is gently aspirated into the pipette. The contents of the pipette are then transferred on to an agar surface as a series of small droplets. Within 3–4 days a microcolony should be evident.

Preservation of single-celled clones. A simple procedure for preserving the resulting single-celled clones consists of allowing the colonies to develop until a loop (2 mm diam.) of the cells can be transferred to 5 ml. of sterile glass-distilled water in a screw-cap tube. The cells are then dispersed in the water and the tubes are stored at 10° C. When subsequent transfers are needed, a loop of cells from the water suspension is streaked on nutrient agar and the tube re-stored. Hundreds of transfers are thus possible from the tube in which the initial population of cells was stored. So far this method of storage has been found suitable for *Corynebacterium insidiosum* (McColloch) Jensen, *Agrobacterium tumefaciens* and *Pseudomonas* sp.

One hundred single-celled isolates of *P. syringae* originally obtained from soil and diseased plants were stored in this manner in March 1961. At the time of storage and following at 6-month and 20-month intervals, populations of the viable cells were determined. The results of these tests on 15 representative isolates are given in Table 1. The pathogenicity of the isolates on peach seedlings and their production of a wide-spectrum antibiotic was not lessened after 20 months in storage.

In an earlier investigation 1 culture of *A. tumefaciens* and 9 cultures of *P. syringae* were stored in 5 ml. of sterile distilled water at room temperature that varied from 22° to 32° C. After 40 and 30 months, the cultures of *A. tumefaciens* and *P. syringae*, respectively, showed no marked decrease in cell viability or change in pathogenicity.

Discussion

It is generally accepted that the poured-plate method of isolating single cells is frequently unsatisfactory because: (1) bacterial cells tend to clump naturally; (2) cells often occur in pairs; (3) agar has an agglutinating effect on the cells; (4) single cells of some bacterial species will not grow in agar. With this method, it is assumed that certain individual colonies have developed from single cells. Thus it is a gamble and never gives the assurance of single-cell origin that is obtained with mechanical methods where the single cell is observed before isolation. Although the disadvantages of the poured-plate method are apparent, the method is still widely used because of its simplicity and speed when compared with other methods requiring more equipment and skill.

The method described in this report most closely approaches the simplicity and accuracy required of a technique for isolating viable single cells. It overcomes the disadvantages of manipulations encountered with the method described by Lederberg[9] and gives better assurance of single-cell isolation.

Other advantages and checks on accuracy absent in other methods include, first, the use of droplets that retain a spherical shape and are about 50μ in diameter. Such droplets enable the operator, during microscopic examination, to scan them vertically without shifting the eyes to search a droplet in different areas of each horizontal plane. Secondly, the entire droplet can be easily recovered immediately after it is found to contain a single cell. Thirdly, by delivering the recovered droplet, mixed with additional broth, as a series of separate drops on an appropriate medium, an additional check on the cell population of the recovered droplet is obtained; usually if an additional cell were present, it would give rise to another colony. Although droplets containing more than one cell are rejected, this manner of transferring the recovered droplet constitutes an additional check. The number of isolated single cells of *Agrobacterium tumefaciens*, *Pseudomonas syringae* and *Xanthomonas malvacearum* that were viable was approximately 4 or 5 out of 6 in each series. In our experience this is much above the ratio of viable cells isolated with other mechanical methods probably because with other methods the cells are lost during the recovery and transfer procedure. The potential use of this method in the purification of other microbial forms is apparent.

In regard to the preservation of bacterial cells, layering cultures with mineral oil, lyophylization or the storage of cultures at low temperatures on agar slants, are methods commonly used. Often, however, these methods are overly time-consuming and inconvenient. A rapid decline in cell viability or a subsequent loss of cells having desired characters such as pathogenicity due to selection pressures of the substrate or storage method may also occur.

Table 1. VIABILITY OF CELLS OF *Pseudomonas syringae* AFTER STORAGE IN DISTILLED WATER AT 10° C

Isolate	Viable cells/ml. Zero time	6 months	20 months
B-3	$1{\cdot}0 \times 10^8$	$2{\cdot}0 \times 10^5$	$8{\cdot}1 \times 10^8$
B-15	$5{\cdot}5 \times 10^7$	$1{\cdot}3 \times 10^5$	$9{\cdot}5 \times 10^5$
B-59	$1{\cdot}2 \times 10^8$	$5{\cdot}4 \times 10^6$	$1{\cdot}3 \times 10^7$
B-60	$2{\cdot}7 \times 10^8$	$3{\cdot}0 \times 10^6$	$8{\cdot}8 \times 10^6$
B-61	$1{\cdot}1 \times 10^8$	$1{\cdot}4 \times 10^6$	$1{\cdot}3 \times 10^8$
B-68	$3{\cdot}5 \times 10^8$	$2{\cdot}9 \times 10^6$	$1{\cdot}9 \times 10^8$
B-70	$3{\cdot}6 \times 10^8$	$8{\cdot}4 \times 10^6$	$8{\cdot}6 \times 10^7$
B-98	$7{\cdot}4 \times 10^8$	$5{\cdot}8 \times 10^5$	$9{\cdot}3 \times 10^7$
B-99	$1{\cdot}0 \times 10^9$	$1{\cdot}2 \times 10^6$	$4{\cdot}1 \times 10^8$
B-100	$9{\cdot}6 \times 10^8$	$2{\cdot}4 \times 10^6$	$4{\cdot}2 \times 10^7$
B-101	$6{\cdot}7 \times 10^8$	$1{\cdot}3 \times 10^9$	$3{\cdot}4 \times 10^7$
B-104	$1{\cdot}2 \times 10^9$	$2{\cdot}8 \times 10^7$	$5{\cdot}0 \times 10^8$
B-105	$8{\cdot}7 \times 10^8$	$2{\cdot}3 \times 10^7$	$7{\cdot}6 \times 10^7$
B-123	$4{\cdot}4 \times 10^7$	—	$3{\cdot}2 \times 10^9$
B-124	$7{\cdot}7 \times 10^7$	$2{\cdot}0 \times 10^4$	$2{\cdot}4 \times 10^5$

Recently, the use of sterile glass-distilled water as a storage medium has overcome many of the disadvantages of other methods and appears to be an ideal way of maintaining and preserving bacterial cultures[10,11]. No special facilities are needed for its use, and large numbers of cultures can be quickly processed. Reconstitution of cultures can be accomplished repeatedly with a random and representative sample of cells each time. So far the method has been extended to relatively few bacteria, but it has proved effective for *Agrobacterium tumefaciens*, *Corynebacterium insidiosum* and *Pseudomonas* sp. In the work recorded here, 100 isolates of *P. syringae* were stored at least 20 months with little or no decrease in viable cells. The stored cells also retained their pathogenicity and the ability to synthesize a wide-spectrum antibiotic. An increase in the number of viable cells was frequently observed in the stored populations. This may be explained by the manner in which the cells were suspended in the water before they were sampled. When sampled originally and after 6-months storage, the cells were suspended by swirling the contents of the tubes by hand motion, whereas the sampling at 20 months was made from cell populations agitated on a 'Vortex, Jr.' mixer.

No marked decrease on cell viability was evident from temperature fluctuations and the temperatures of storage within the limits of these experiments.

Investigations are now in progress on the physiological changes in cells that occur during their long-term storage in distilled water.

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DIFFERENCES IN THE UTILIZATION OF TRITIATED LEUCINE BY SINGLE NEURONES IN NORMAL AND EXERCISED RATS: AN AUTORADIOGRAPHIC INVESTIGATION WITH MICRODENSITOMETRY

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IT is commonly believed, though not adequately established, that the neural control of specific behavioural processes is dependent on the activity of circumscribed and localizable brain regions. In an attempt to verify this belief, various techniques have been devised to correlate changes in the physiological condition or behavioural activity of experimental animals with concomitant alterations in the functioning of the brain. One common procedure is the recording of changes in the electrical activity of the brain as a consequence of such

variables as sleep, induced sensory activity, drug action and the like. A less-common but relatively old approach is the cytological one, which attempts to correlate changes in the appearance or staining property of nerve cells in specific regions of the brain with prior manipulation of the physiological state or behaviour of the animal. The attempt to investigate functional brain localization by means of a morphological technique was prompted by Nissl's[1] demonstration, and subsequent confirmation by others, of chromatolytic changes in neurones as a consequence of such pathological processes as severance of the axon (retrograde degeneration) or structural de-afferentation (transneuronal degeneration). Can cytological changes be demonstrated in nerve cells as a consequence of behavioural activation or inactivation ? Several investigators[2–5] stimulated one eye of animals photically and blindfolded the other, and then compared the size and staining properties of retinal cells of the 'activated' eye with the 'inactivated' one. Unfortunately, the majority of investigators failed to obtain reliable results[6]. By now it is generally accepted that staining with conventional Nissl stains is not sufficiently sensitive or reliable for the demonstration of chromatophilic changes, if any, produced by normal physiological or behavioural activation[6–8]. More recently some investigators have introduced better stains[9] or applied direct biophysical techniques[10,11] to demonstrate changes in the nucleic acid and protein concentration of 'activated' nerve cells[12–17]. These results, however, have also been criticized[18,19].

Though we cannot rule out the possibility that alterations in the activity-level of neurones are accompanied by structural changes, a more promising approach to the problem may be the investigation of transient metabolic changes accompanying activation or inactivation. This rationale may underlie the recent attempts of Hydén *et al.*[20,21], using microchemical assaying procedures on single nerve cells in 'activated' animals, to show alterations in the concentration and composition of nucleic acids and proteins. Another promising approach, which we have used in this work, is the *in vivo* administration of radioactively labelled metabolic precursors, combined with fine-resolution autoradiography for accurate localization of the sites of altered metabolism. The use of autoradiography was prompted by our recent finding that if this technique is combined with microdensitometry one may obtain reliable quantitative indices of the utilization of tritium-labelled precursors of proteins[22] or nucleic acids[23].

In this experiment 8 young adult Long–Evans hooded rats weighing about 250 g were used. Pairs of animals were randomly assigned to three experimental groups and one control group. All the experimental animals were exercised in a motor-driven wheel, 14 in. in diameter, for 2 h; two groups at the speed of 7 r.p.m., one group at 12 r.p.m. All the animals, controls included, were injected intraperitoneally with 2 mc. of DL-leucine-4,5-^{3}H (specific activity 5·4 curies/millimole; the chemical dissolved in 2 ml. of sterile isotonic saline). The injections were made in two 1-mc. doses with 30 min elapsing between the two injections, and the animals were killed after a 1-h exchange period by cardiac perfusion with 10 per cent neutral formalin. Of the animals exercised at 7 r.p.m., the injection was made in one group immediately after completion of their 2-h exercise period and in the other group during exercise. The 2 animals which ran at 12 r.p.m. were injected immediately after their exercise. The control animals were resting both before and during injection. The removed brains were embedded in paraffin and serial sections were cut at 7μ. The deparaffinized sections were coated by the dipping technique[24,25] with Kodak '*NTB*-3' photographic emulsion, diluted at the ratio of 2 : 1 with water. The coated sections were exposed in lightproof slide-boxes containing a desiccant for 19 weeks at 5° C, and were developed in Kodak '*D*-19' at 20° C for 6 min, fixed and washed at the same temperature and left unstained. Alternate sections, which were not prepared for autoradiography, were stained with gallocyanin chromalum. Qualitative assessment of the regional density of the autoradiograms was accomplished with a Shea comparison microscope (American Optical Co.), which permits simultaneous comparison of a pair of sections, such as a control and an experimental section. Quantitative determination of the grain density of single neurones or surrounding 'neuropil' was carried out with a microdensitometer, consisting of a Zeiss photomicroscope, a vertical projection attachment with interchangeable slides of varying apertures, and a Photovolt model 501*M* photometer. Details of the apparatus and the procedure utilized are reported elsewhere[22], where it is shown that density readings obtained with this apparatus and grain counting are linearly related. In the investigation recorded here, density measurements were made on projected circular areas 12μ in diameter, at a magnification of about 320.

The speed of 7 r.p.m. (about 25 ft./min) is an apparently comfortable walking pace for rats, though they are forced to co-ordinate their locomotor rhythm so that they can step on the thin cross-bars spaced 0·5 in. apart. The distance covered at this speed is a very small fraction of what rats were reported to cover spontaneously in activity wheels[26]. Nor is the speed of 12 r.p.m. excessive; however, some animals encounter difficulties in maintaining a steady rhythm at this speed and may prefer a discontinuous jumping movement, taking occasional free rides by hanging on to the bars with their forepaws, and may be exhausted at the end of the 2 h of exercise. It is reasonable to assume, however, that locomotion at both speeds represents a 'normal' though possibly stressful pattern of behaviour.

As shown in Fig. 1, there is considerable increase ($P < 0{\cdot}001$) in the uptake of radioleucine in the motor

Table 1. Mean Grain Density of Single Neurones in Several Brain Structures in the Control Group and the Animals Exercised at 7 r.p.m. and Injected during Exercise

Rank ordering of structures in terms of percentage increase of uptake in the exercised animals

Structure	Density						Increase (per cent)	t	P
	Control			Exercised					
	N	Mean	$S.D.$	N	Mean	$S.D.$			
Motor cortex, neurones in second layer	40	0·185	± 0·036	40	0·307	± 0·043	66	13·6	< 0·001
Cervical cord, ventral horn, neuropil	80	0·063	± 0·024	80	0·099	± 0·034	57	7·2	< 0·001
Motor cortex, pyramidal neurones in fifth layer	40	0·282	± 0·041	40	0·421	± 0·055	49	12·6	< 0·001
Posterior cortex, neurones in second layer	40	0·215	± 0·053	40	0·315	± 0·037	47	10·0	< 0·001
Cervical cord, ventral horn cells	40	0·476	± 0·042	40	0·671	± 0·062	41	16·2	< 0·001
Posterior cortex, neurones in fifth layer	40	0·274	± 0·044	40	0·374	± 0·044	36	10·0	< 0·001
Lumbar cord, ventral horn cells	40	0·512	± 0·063	40	0·693	± 0·113	35	8·6	< 0·001
Mammillary body, neurones	40	0·331	± 0·046	40	0·442	± 0·084	34	7·4	< 0·001
Cerebellum, Purkinje cells	40	0·458	± 0·052	40	0·612	± 0·061	34	11·8	< 0·001
Medulla, large reticular neurones	40	0·512	± 0·058	40	0·674	± 0·080	32	10·1	< 0·001
Choriold plexus (third ventricle)	40	0·333	± 0·052	40	0·426	± 0·062	28	7·2	< 0·001
Hippocampus, pyramidal neurones	40	0·262	± 0·040	40	0·330	± 0·039	26	7·6	< 0·001
Deiter's nucleus, neurones	40	0·573	± 0·051	40	0·718	± 0·044	25	13·2	< 0·001
Hippocampus, granule cells	40	0·176	± 0·027	40	0·218	± 0·035	24	6·0	< 0·001
Cochlear nucleus, neurones	40	0·639	± 0·064	40	0·760	± 0·095	19	6·7	< 0·001

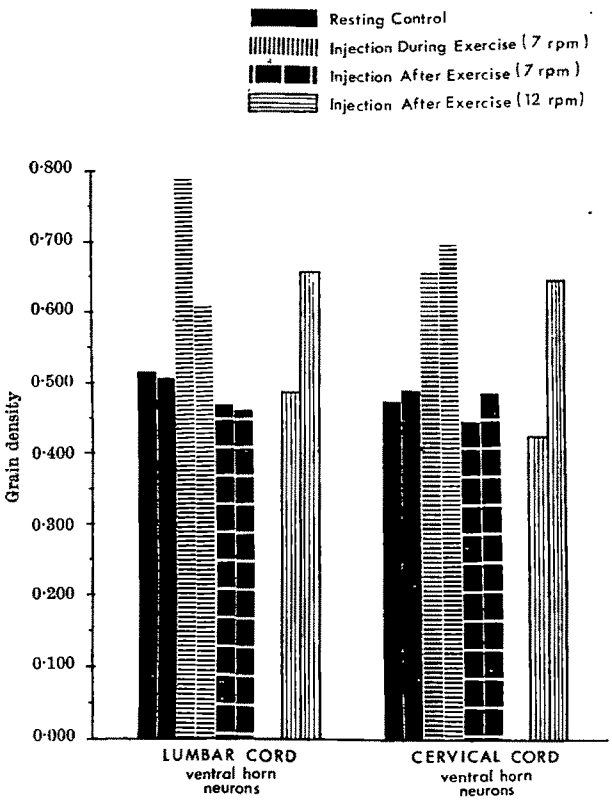


Fig. 1. Mean density of single neurones (N = 20) in the lumbar and cervical spinal cord. Each bar represents one animal

neurones of the lumbar and cervical spinal cord in the animals exercised at 7 r.p.m. with the chemical injected during exercise. In contrast, a slight decrease in uptake ($P < 0{\cdot}001$ in the lumbar motoneurones; $P < 0{\cdot}10$ in the cervical motoneurones) was seen in the animals exercised at the same speed with the chemical injected after exercise. A somewhat different result was obtained in the animals which ran at 12 r.p.m. and were injected after exercise. In one of the two animals (which showed no difficulty in keeping up with the higher speed) a small drop in uptake was seen, in a manner similar to the other two animals exercised at a lower speed and injected afterwards, while in the other animal (which virtually collapsed from fatigue at the end of the exercise period) a considerable increase in uptake was obtained. In summary, these findings brought evidence of appreciable increase in utilization of amino-acid in motor cells during mild activity and a slight decrease in uptake (possibly returning to normal) immediately after activity. The evidence of the effect of more strenuous exercise on amino-acid uptake after cessation of locomotion remains ambiguous since the two animals studied showed different reactions.

The increased utilization of amino-acid during mild exercise was further investigated in 15 different brain regions and the grain density of over a thousand neurones in these structures was measured. As presented in Table 1, there is a significant increase ($P < 0{\cdot}001$) in the density of all cell types, not only in motor but also in sensory regions. The rank ordering of structures in terms of percentage increase of leucine uptake during exercise shows that the relative increase is highest in some motor structures (such as neurones of the motor cortex and the ventral horn of the spinal cord), but the same applies to some sensory regions, such as the posterior (visual) cortex. Lowest increase in uptake was observed in other sensory structures (Deiter's nucleus, cochlear nucleus) and in the hippocampus. The increase in uptake does not appear to be related to the total density of neurones; the smallest percentage increase was seen in two structures (hippocampus and cochlear nucleus) the neurones of which are distinguished in the list by the lowest and highest grain density, respectively.

These findings indicate that fine-resolution autoradiography, combined with microdensitometry, is a suitable technique for demonstrating changes in the amino-acid utilization of single nerve cells during exercise. Whether such changes can be demonstrated by conventional histological techniques is controversial[27,28]. The autoradiographic technique may, therefore, be used as an indicator of chemical changes in nerve cells as a consequence of behavioural 'activation'. The exact interpretation of the changes obtained, however, poses some difficulties which we cannot resolve without further experimentation. While it is reasonable to assume that what was retained after perfusion and histological processing represents amino-acids incorporated into proteins, we cannot infer from this that exercise induces a higher rate of protein turnover in nerve cells. First, the direct effect of exercise may be a change in the local amino-acid pool with a consequent change of the specific activity of the pool (such an assumption would imply that the amino-acid pool is decreased as a consequence of exercise). Secondly, owing to previously demonstrated increase in local blood

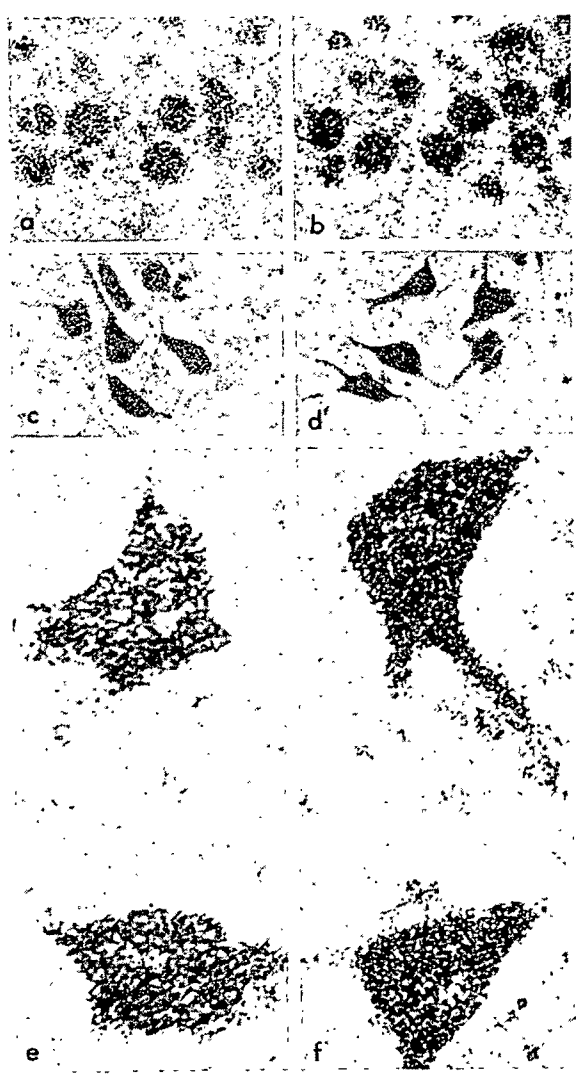


Fig. 2. Photomicrographs of unstained autoradiographs from a control animal (left column) and an animal exercised at 7 r.p.m. and injected during exercise (right column): *a–b*, Purkinje cells from cerebellum (× *c.* 256); *c–d*, motor horn cells from cervical spinal cord (× *c.* 128); *e–f*, higher power photomicrographs of motor horn cells (× *c.* 512)

circulation as a result of functional activation[29,30], the higher uptake of tritiated leucine may merely reflect greater availability. On the other hand, it could be argued that either explanation (depletion of local amino-acid pool or increase in local circulation) may indirectly reflect higher rate of metabolism. Indeed, evidence was recently obtained[31] of increased incorporation of methionine labelled with sulphur-35 into proteins of the brain stem and cerebral hemispheres of rats during the first few hours of forced swimming. Contrary to expectation, the changes of utilization of amino-acid were observed in this work not only in motor cells (or cells in motor structures) but also over the entire brain. Whether this is due to the non-specific nature of the behavioural task utilized (exercise might involve activation of sensory as well as motor structures) or whether any specific task produces a general metabolic activation remains to be investigated.

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LOW-VOLTAGE ELECTRON BEAM IRRADIATION OF NORMAL AND 5-BROMOURIDINE DEOXYRIBOSIDE-TREATED *L*-P_{59} MOUSE FIBROBLAST CELLS *IN VITRO*

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NUCLEAR or chromosomal damage may be a major factor in radiation killing of mammalian cells[1–3]. The contribution of cytoplasmic effects, either directly or by induced nuclear effects, is not clear. It is known that the incorporation of 5-bromouridine deoxyriboside (BUdR) into the chromosomal deoxyribonucleic acid (DNA) of a cell[4,5] increases or potentiates both radiation-induced killing[6,7] and chromosomal damage[8]. It is of interest to determine whether the BUdR-effect occurs when only the cytoplasm is irradiated, that is, whether migration of radiation energy or effect from cytoplasmic to nuclear material occurs. Such an investigation may be carried out using electron beams to irradiate to selective depths within a cell[9–14].

Fig. 1 illustrates the electron-beam irradiation system. Accelerated electrons passed through a collimator slit (0·25 × 2 mm) into either a beam measuring system *in vacuo* or the biological sample. A collodion foil of about 1·5 μg/cm² mounted on the slit isolated the gun vacuum from the lower chamber. Collimator current was used as a reference for setting the beam current during irradiations. A mechanical scan system moved the sample 1 mm below the slit in a raster pattern covering a 0·5-in.² area in 17 sec (3 cm² in 55 sec in initial investigations). Each point on the specimen passed under the beam slit about 5 times during a complete raster pattern. The beam overlapping and the scattering by foil and gas were considered sufficient to provide a uniform exposure over the sample. The sample and mounting systems were cooled to 2° C before and during the irradiation. Air was evacuated from the lower chamber leaving an equilibrium water-vapour pressure of 6 mm mercury, that is, irradiation was carried out under anoxic conditions. The pre-absorption path of the foil and water vapour was about 2·5 μg/cm² Samples could be inserted, evacuated, irradiated and removed in a 1-min period (3 min in earlier work). Dehydration of the cells was limited by the low temperature and short exposure time.

L-P_{59} mouse fibroblast cells (2 × 10⁶ inoculum) were grown at 37° C as monolayers in stoppered *T*-60 flasks containing McCoy's 5*a* medium[15] supplemented with 10 per cent calf serum. Following a 24-h initial incubation, BUdR (2·0 μg/ml.) was added to one group of flasks and both the control and BUdR-treated groups were incubated for 3 more days. The BUdR treatment did not reduce plating efficiency or induce observable chromosomal effects. Cells were removed from the glass by treating with 0·2 per cent trypsin in medium, centrifuged, and re-

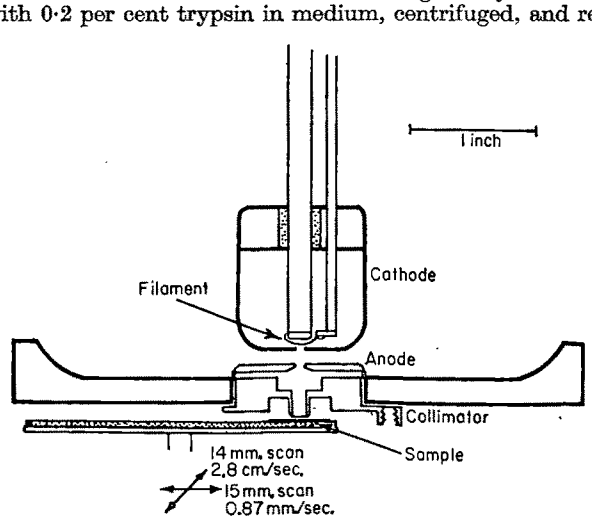
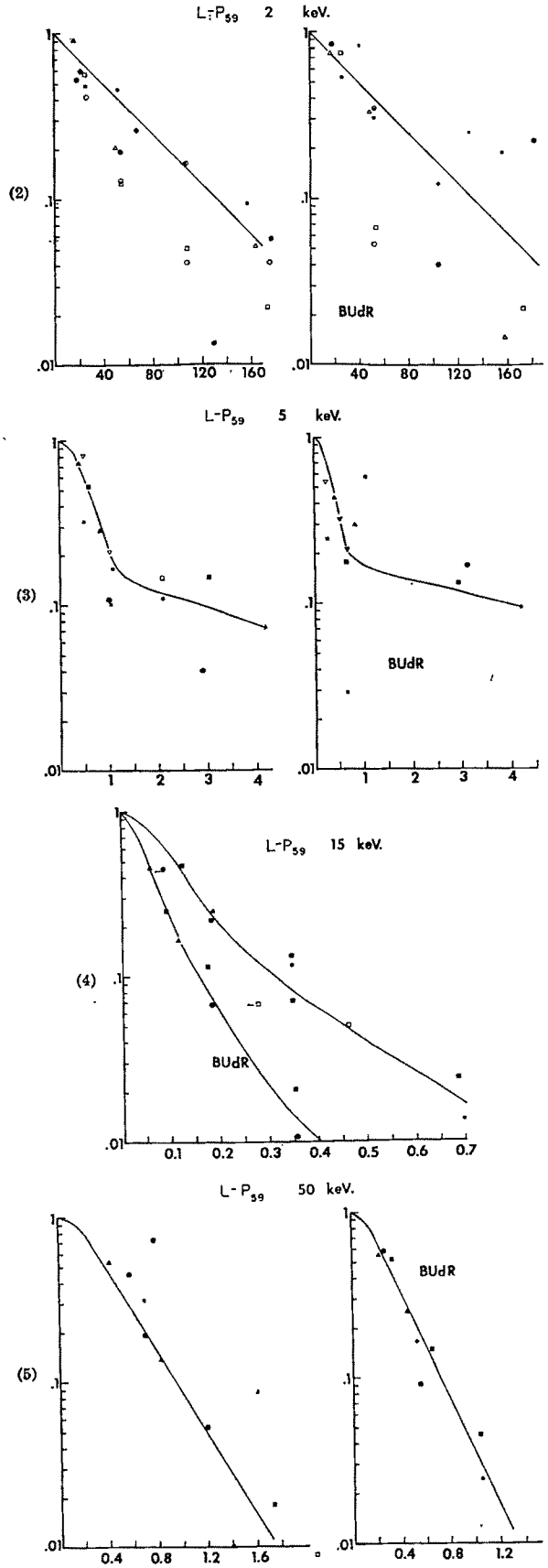


Fig. 1. Electron beam irradiation system



Figs. 2–5. Semilogarithmic plot of surviving fraction versus incident electron beam exposure in units of 10^{-9} coulombs/cm² for untreated and BUdR-treated L-P_{59} cells. A number of experiments are represented. Fig. 2, 2-keV beam; Fig. 3, 5-keV. beam; Fig. 4, 15-keV beam; Fig. 5, 50-keV beam

suspended in medium (2×10^5 cells/ml.). 40 μl. of this suspension were pipetted on to 0·5-in. diameter, 100-mμ porosity, membrane ('Millipore') filters placed on medium-soaked underpads. The fluid passed through the filters in about 5 min, leaving the cells exposed on the surface. The wet underpads prevented dehydration of the filter and specimen during the subsequent irradiation at the reduced pressure. Following irradiation, the filters were placed in medium and the cells released from the filter by sonication for 15 sec at the minimum operable power-level of a Raytheon 10-kc/s, 250-W sonic oscillator (this sonication had no effect on control plating efficiency). A number of cells sufficient to establish about 100 colonies were then plated into Petri dishes containing medium plus a feeder layer of L-P_{59} cells which had been irradiated 24 h previously with 4,000 rads of cæsium-137 γ-rays. The plates were incubated 10 days at 37° C in 5 per cent carbon dioxide, fixed and stained and the colonies counted. (The feeder layer formed giant cells but no colonies.) Control cells were treated as already described except that the cells were not irradiated. Additional controls included non-evacuated filter samples and direct plating of the initial suspension. Equal plating efficiencies were observed (~ 70 per cent) for all control systems.

Representative plots of the survival data are shown in Figs. 2–5. Exposure dose is given in units of 10^{-9} coulombs/cm². A summary of these results and those reported for 220-kVp. X-ray exposures[16] is given in Table 1.

Table 1

Voltage (keV)	Cell penetration (μ)	Treatment	Beam exposure (10^{-9} coulombs/cm²)	LD_{50} Cell dose (kilorads)	Local dose (kilorads)	LD_{50} dose reduction BUdR treatment
1·5 (0·75 residue)	0·07		~200	~13·5	~2,200	
2·0	0·15		~40	~5·2	~400	
		BUdR	~40	~5·2	~400	~0·95
3·0	0·28		~6	~1·3	~53·5	
5·0	0·73		0·57	0·245	3·8	
		BUdR	0·38	0·164	2·54	1·5
15·0	5·2		0·11	0·142	0·316	
		BUdR	0·06	0·077	0·173	1·83
30·0	17·0		~0·3	~0·58	~0·58	
50·0	45·0		0·39	0·50	0·50	
		BUdR	0·28	0·36	0·36	1·4
90·0	125·0		0·75	0·45	0·45	
220-kVp. X-ray		N_2 saturated		0·475	0·475	
		N_2 plus BUdR		0·325	0·325	1·46

Penetration depths and dose calculations were based on studies in air and collodion foils of the radiation properties of low-voltage electron beams[16,17] which included determinations in plastic foils of the penetration range, R (defined here as the thickness which transmits 2 per cent of the incident energy), the initial average rate of energy loss per electron per unit path length on dE/dx, and the rate of energy loss per electron per unit penetration depth or dE/dt. The following semi-empirical relations were fitted to the data between 20 eV and 100,000 eV:

$$\frac{dE}{dx} = 14{\cdot}5\,(E + 0{\cdot}4)^{-0{\cdot}761} \qquad (1)$$

$$R = 0{\cdot}0385\,(E + 0{\cdot}4)^{1{\cdot}77} - 0{\cdot}0075 \qquad (2)$$

E and dE are in keV and R and dx are in units of 100 μg/cm² or μ at unit density.

Fig. 6 shows a plot of dE/dt (5- to 50-keV beams) normalized in units of the dE/dx and R appropriate to the energy considered. The radiation dose at any depth in an absorber exposed to a broad electron beam becomes:

$$\text{Dose (kilorads)} = \frac{dE}{dt}\ (\text{keV}/\mu) \times \text{beam exposure } (10^{-9}\ \text{coulombs/cm}^2) \qquad (3)$$

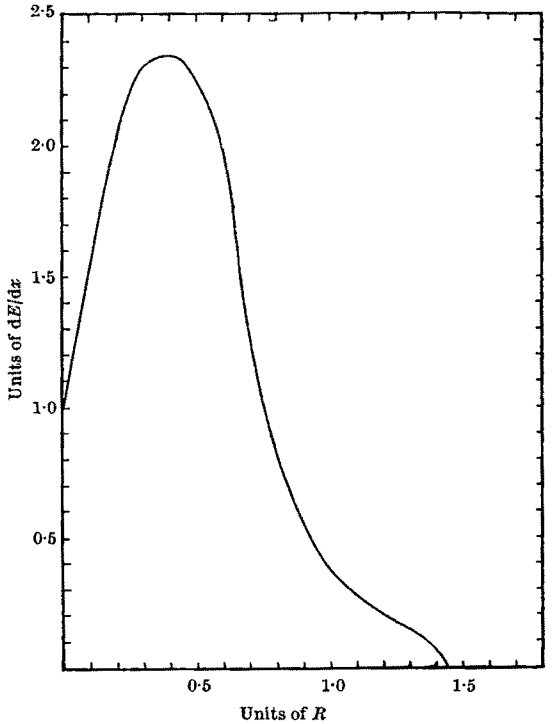


Fig. 6. Plot of the average energy deposited per electron per unit penetration depth in keV/μ (unit density) versus penetration depth for 5- to 50-keV electron beams. The plot is normalized in units of the dE/dx (initial rate of energy loss) and R (range) appropriate to the beam-energy considered

The cell LD_{50} listed in Table 1 represents, for 50 per cent survival, the energy deposited per cell divided by the cell mass while the local LD_{50} represents the actual average dose in the irradiated region.

The survival curves of Figs. 3–5 are sigmoidal with extrapolation numbers in the order of 1·5 or greater. The 'tailing' or upward concavity of the curves prohibits obtaining accurate extrapolations. The tailing is believed to be caused by a shielding of a small fraction of the cells by water, dirt, fragments, or by cell clumping, or overlapping, or a variable depth of the sensitive region.

Nuclear and cellular sizes were measured microscopically to be approximately 10 and 17μ respectively. The cell maintains a spherical shape in suspension, but it is uncertain exactly what shape the cell adopts during the 5- to 10-min period it remains on the membrane filter surface. It is reasonable to assume that some flattening of the cytoplasm occurs as is shown in Fig. 7.

Strictly reproducible results were not obtained with the 2-keV beams (Fig. 2). The 2- and 3-keV beams yielded cell LD_{50} of about 5 krads and local LD_{50} of about 400 krads (Table 1) for both untreated and BUdR-treated cells. The local dose to the cell surface or membrane was in the order of 1,500 krads. It was observed in initial studies that 1·5-keV beams (with residue energy of about 0·75 keV and residue penetration of about 70 Å) would induce cell fragmentation and death within a short period for local doses in excess of 2,000 krads or cell doses in excess of 13 krads. Although the 2-keV results, shown in Fig. 2 for seven experiments, are scattered, a comparison of the results with non-treated and BUdR-treated cells for each experiment gave no consistent difference in the radiation responses, that is, the ratio of the slopes of the log survival versus dose plots varied from 0·8 to 1·5. An exponential best fit to all the points yielded a BUdR dose reduction factor of 0·95 $\pm$ 0·20.

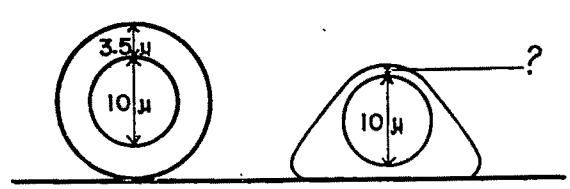


Fig. 7. Diagram illustrating uncertainty of cytoplasm thickness above nucleus when cell settles on the surface of the membrane filter

The 5-keV beam gave a cell LD_{50} dose of about 245 rads for untreated cells and about 164 rads for BUdR-treated cells. Thus, a BUdR dose reduction factor of 1·5 was obtained. The 5-keV beam penetrates only 0·75μ. If BUdR potentiation is to be associated with a nuclear effect, these results require an unexpectedly thin cytoplasmic covering. Flattening of the cell and dehydration of the cell surface during the evacuation may have appreciably reduced the absorption thickness of the upper cytoplasm.

Results obtained with the 15-keV beam are interesting in that cell LD_{50} were only 140 rads for untreated cells and 77 rads for BUdR-treated cells. This beam, which irradiated the upper one-fourth of the cell, was 3–4 times as efficient for radiation killing as fully penetrating beams. The result implies that the local energy was more favourably located in relation to a radiation sensitive region. The BUdR dose reduction factor of 1·83 is believed to be significantly larger than the values of 1·0–1·5 obtained for the other irradiations and implies that a radiation effect directly associated with DNA is more important at this energy. It is of interest that over one-half of the energy of a 15-keV beam is expended between 1–3 microns penetration depth. The peak local LD_{50} in this region (which are greater than the average local doses listed in Table 1) for the 15-keV beam were about 460 rads for untreated cells and 250 rads for treated cells which approximates the LD_{50} for beams which irradiated the whole cell.

Results obtained for fully penetrating beams (30, 50 and 90 keV) are in essential agreement with X-ray irradiation results[18]. LD_{50} of 450–500 rads for untreated cells and 330–360 rads for BUdR-treated cells and BUdR dose reduction factors of 1·40–1·46 were obtained.

If the BUdR potentiation effect is directly associated with DNA, these results suggest that one mechanism for radiation killing involves cell chromatin located on or near the nuclear membrane but that another mechanism for radiation killing, which is not subject to BUdR potentiation (and, therefore, does not involve the DNA or involves it indirectly), is significant when an appreciable fraction of the radiation energy is expended in the cytoplasm or nucleoplasm. Thus, the net BUdR potentiation observed will depend on the fraction of radiation energy distributed between the two mechanisms.

This work was supported in part by the U.S. Atomic Energy Commission contract *AT*-(40–1)–2832 and the National Institutes of Health grant *C*–4484.

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BEHAVIOUR OF MAMMALIAN HÆMOGLOBIN AFTER FREEZING AND THAWING

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IT was observed in the course of a routine procedure to lyse defibrinated blood by freezing and thawing that two changes occurred with rat blood. One was a change in colour, and the other the appearance of an amorphous deposit. Experiments were afterwards undertaken to investigate these two observations.

Blood was obtained from Wistar rats and defibrinated by shaking it with glass beads for 5 min. The defibrinated blood was then frozen in an alcohol/carbon dioxide bath, and when completely frozen allowed to return to room temperature. This procedure was repeated six times. Blood was also obtained and treated in a similar manner from the following examples of the class Mammalia:

Order	Examples tested
Primates	Monkey and man
Carnivora	Dog and cat
Perissodactyla	Horse
Artiodactyla	Cow and sheep
Rodentia	Mouse, rat, hamster, gerbil, guinea pig
Lagomorpha	Rabbit
Insectivora	Hedgehog

All the blood samples tested, with the exception of those from rats. When the defibrinated blood was frozen, the blood changed colour as it solidified. The colour produced was that of red lead (approximately British Standard Colour 0–005). On thawing, the blood resumed the normal colour of lysed red cells (approximately British Standard Colour 0–006) and the solution was clear and gave the absorption band of oxyhæmoglobin. In only one blood specimen did crystallization occur, and that was with guinea pig. Repeated freezing and thawing six times resulted in the same colour change when the material was in the solid state; but each time there was a reversion to the normal colour when the blood was allowed to thaw.

Rat hæmoglobin. On freezing, the defibrinated rat blood took on the colour of red lead as described here when it was in the solid-state, and this colour persisted when the blood was thawed, with some drop in the intensity of the colour. Repetition of the process produced intensification of this colour change when the blood was in the liquid state (Fig. 1). This change in colour was stable and persisted for at least 24 h. After the first thawing, it was clear that a precipitate had formed, which increased in amount as the procedure was repeated. After centrifugation the supernatant had the normal colour of a solution of oxyhæmoglobin (approximately British Standard Colour 0–006) and gave the typical absorption bands of oxyhæmoglobin, while the deposit had the red lead colour. Microscopic examination of the deposit showed it to be amorphous, as illustrated in Fig. 2. There was no evidence of crystallization. The deposit gave essentially the same reflectance spectrum as a sample of crystalline oxyhæmoglobin precipitated from whole defibrinated rat blood by the addition of one-sixteenth of its volume of ether[1] (Table 1 and Fig. 3).



Fig. 1. *A*, guinea pig blood, lysed by freezing and thawing; *B*, normal rat blood; *C*, rat blood frozen and thawed; *D*, lysed rat blood (water) frozen and thawed

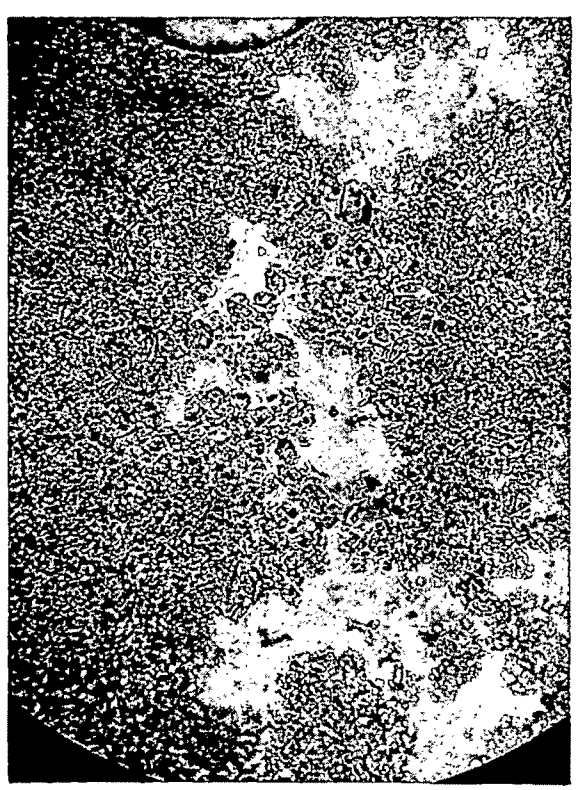


Fig. 2. Photomicrograph showing the amorphous deposit of oxyhæmoglobin after freezing and thawing defibrinated rat blood (× *c.* 750)

Table 1. Reflectance Spectra (Maxima and Minima (mμ)) of Normal Rat Hæmoglobin Crystals and 'Frozen' Rat Hæmoglobin

	Absorption in mμ	
	Normal rat blood	Frozen rat blood
Maximum	415	420
Minimum	510	510
Maximum	543	542
Minimum	563	563
Maximum	575	576

The spectra were determined on a Unicam *SP*'500 spectrophotometer with a reflectance attachment. The samples were diluted with water and filtered through Whatman No. 1 paper with gentle suction to obtain an even layer of hæmoglobin. Reflectance measurements were made on the samples covered with a microscope slide to exclude air. Wet filter paper similarly covered with a microscope slide was used as a blank.

The small differences in the shape of the reflectance curves (Fig. 3) are attributed to difficulties in obtaining reproducible values because of the low reflectivity of the samples, while the difference in optical densities between the two curves arises because of the different amounts of hæmoglobin on the filter paper in the two cases. It is the area of the reflectant surface which is the significant factor determining the optical density, but this is not easily controllable or measurable, since equal weights of the two samples do not possess equal surface areas, owing to the difference in particle size. The results indicate that there is no chemical difference between the normal rat blood and the frozen rat blood, and would suggest that the apparent difference to the eye is a difference of colour saturation (optical density) and not of hue (colour), arising from the differing particle sizes in the two cases.

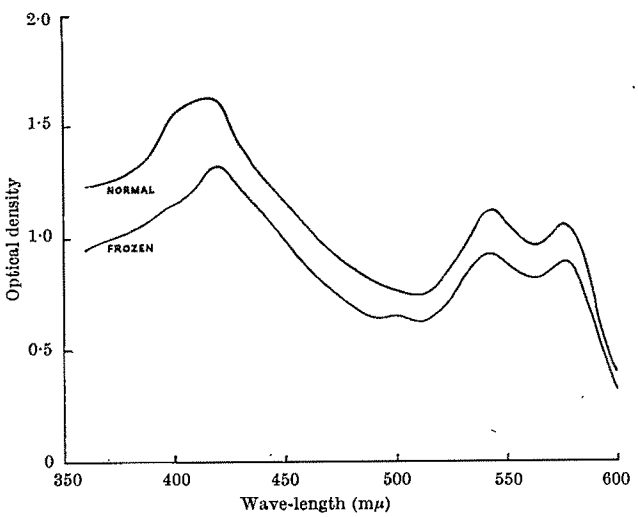


Fig. 3. Reflectance spectra of the amorphous rat hæmoglobin and crystals of rat oxyhæmoglobin

These changes also occurred with rat cells which had been washed three times in saline and finally suspended in saline, and in defibrinated blood previously lysed with water. Similar changes were also observed with blood from two further groups of Wistar rats from other breeders, and from a group of hooded rats. Freezing and thawing in an ice/salt bath, a much slower procedure, produced similar changes.

The addition of saponin or water to defibrinated cells resulted in rapid crystallization of the oxyhæmoglobin.

The addition of a reducing agent, such as sodium hydrosulphite, to a suspension of the amorphous material resulted in the material going into solution. Re-oxidization caused the reappearance of the amorphous deposit, in contrast to normal rat hæmoglobin, which gave a crystalline oxyhæmoglobin on standing. The addition of a reducing agent before freezing and thawing inhibited both the change in colour and the appearance of the precipitate, and resulted solely in a solution of reduced hæmoglobin with typical absorption bands.

We have been unable to find any reference in the literature to the change in colour which appears on freezing blood or to the deposition of amorphous oxyhæmoglobin after freezing and thawing solely in the case of rat blood. Haurowitz and Harden[2] state that "it is remarkable that hæmolysis in some cases, such as in the red cells of the rat, leads directly to the crystallization of oxyhæmoglobin. Evidently the concentration of hæmoglobin and oxyhæmoglobin in the red cell is higher than that of a saturated aqueous solution". Direct crystallization was not observed by the freezing technique, although crystals, indistinguishable from normal rat hæmoglobin, tended to occur afterwards if the amorphous deposit was left overnight on a microscope slide. These crystals did not appear if the amorphous material was left standing in its own mother liquor.

We thank Prof. C. Rimington for his advice and interest in this work, and Mr. Threlfall, of the Applied Physical Chemistry Division of these Laboratories, for carrying out the spectrophotometric measurements.

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IMMUNOASSAY OF HUMAN GROWTH HORMONE IN PLASMA

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IMMUNOASSAY of circulating human growth hormone (HGH), utilizing hæmagglutination inhibition[1] or HGH labelled with iodine-131 (ref. 4), has been hampered by insufficient sensitivity[2,3] or by non-specific interference from substances in plasma[2–4] that can be removed only by extraction procedures that do not always ensure complete recovery of hormone. In a recent abstract[5], Hunter and Greenwood have reported a radioimmunoassay of improved sensitivity for HGH in plasma extracts, but a detailed description of their methods and results has not yet appeared.

The present report describes a radioimmunoassay that provides a high degree of specificity and sensitivity, and permits determination of HGH in unextracted plasma (Table 1). By application of this assay acute changes in endogenous plasma HGH-levels have been demonstrated for the first time[6,7].

The hormone-^{131}I immunoassay method, originally used for measurement of endogenous plasma insulin[8], has been modified for the determination of endogenous plasma HGH. This method, like the previously reported HGH-^{131}I immunoassay methods[4,5], exploits the ability of endo-

Table 1. IMMUNOASSAY METHODS AND RESULTS: ADULTS

Assay method* and adult cases reported	Samples used for assay	Sensitivity (mμg/ml.)	Non-specific reaction with plasma	Plasma HGH concentration Acromegaly cases	Acromegaly HGH	Normal cases	Normal HGH
Dominguez and Pearson (ref. 2): hæmagglutination inhibition; 24 patients total	TCA-acetone extracts (ref. 2) of plasma	20·0	Yes	4 active 3 inactive	35–300 < 20	7	< 20
Utiger, Parker and Daughaday (ref. 4): HGH-^{131}I–antibody complexes precipitated by goat anti-rabbit γ-globulin; 63 patients total	Ethanol extracts (ref. 13) of plasma	3·0	Yes	22 untreated 6 treated; inactive	15–260 0–27	18 5 7	< 2 3–15 15–50
Hunter and Greenwood (ref. 5): HGH-^{131}I–antibody complexes separated by electrophoresis on cellulose acetate; 14 patients total	Acetic acid acetone extracts (ref. 14) of plasma†	1·0†	Not reported†	7	25–67	7	2–6
Present method: HGH-^{131}I–antibody complexes separated by paper chromatoelectrophoresis; 212 patients total	Plasma (unextracted)	0·1	No	3 10 17	2–5·5 10–30 30–250	57 5 2	0–5 5–9·9 10–15

* Results obtained with the hæmagglutination inhibition method utilizing unextracted plasma samples are not included because of evidence that the method lacks specificity (refs. 2, 3).

† Hunter and Greenwood have recently reported (personal communication) that plasma can be assayed directly but that concentrated plasma extracts are utilized for measurements in a large proportion of adults. Present sensitivity is 0·1 mμg/ml. Non-specific reaction with plasma does not occur.

genous HGH in plasma to inhibit competitively, in a quantitative manner, the binding of HGH-^{131}I to specific antibody.

Antisera are produced in rabbits and in guinea pigs by the administration of HGH (Wilhelmi *HS* 269), emulsified in complete Freund's adjuvant, by any of a variety of routes and dosage schedules. To maintain a completely soluble system at all concentrations of antiserum the antiserum is digested with papain by the method of Porter[9].

HGH is labelled with iodine-131 at specific activities of 400–500 mc./mg by the method of Hunter and Greenwood[10]. Unreacted iodine-131 is removed by dialysis. HGH-^{131}I is purified by electrophoresis on starch-gel (borate buffer 0·3 M, *p*H 8) (ref. 11). The fraction of HGH-^{131}I eluted from the pre-albumin region of the gel binds completely to HGH antibodies and competes for binding to HGH antibodies in a manner quantitatively identical with that of unlabelled HGH.

For assay purposes, we use a dilution of antiserum in which 50–65 per cent of a small tracer of HGH-^{131}I (approximately 0·03 mμg/ml.) is bound to antibody at equilibrium (about 5 days at 4° C). Thus, the ratio (*B*/*F*) of antibody-bound HGH-^{131}I (*B*, bound) to unbound HGH-^{131}I (*F*, free) is about 1 or 2 in the absence of unlabelled HGH; the *B*/*F* ratio falls progressively with increasing concentrations of unlabelled HGH. A 'standard curve' is obtained by plotting *B*/*F* vs. HGH concentration in known 'standard solutions' (Fig. 1). Plasma sample samples are assayed by preparing mixtures that contain the same concentrations of HGH-^{131}I and antibody as in the standard solution, but in which the unknown plasma is substituted for standard HGH. The plasma concentration of HGH in the unknown is determined from the observed *B*/*F* ratio by comparison with the standard curve (Fig. 1).

Free HGH-^{131}I and antibody-bound HGH-^{131}I are separated by chromato-electrophoresis[8] in strips of *ECTEOLA*, DEAE, or Whatman 3 *MM* paper. (Variations in the adsorptive qualities of different batches of these papers make it advisable to test each batch before large-scale use.) Free HGH-^{131}I is adsorbed at the site of application whereas antibody-bound HGH-^{131}I migrates anodally with the γ-globulins. After the γ-globulins have moved about 3 in. (1–1½ h at 4° C) the strips are dried and scanned for radioactivity in an automatic strip counter (Fig. 1).

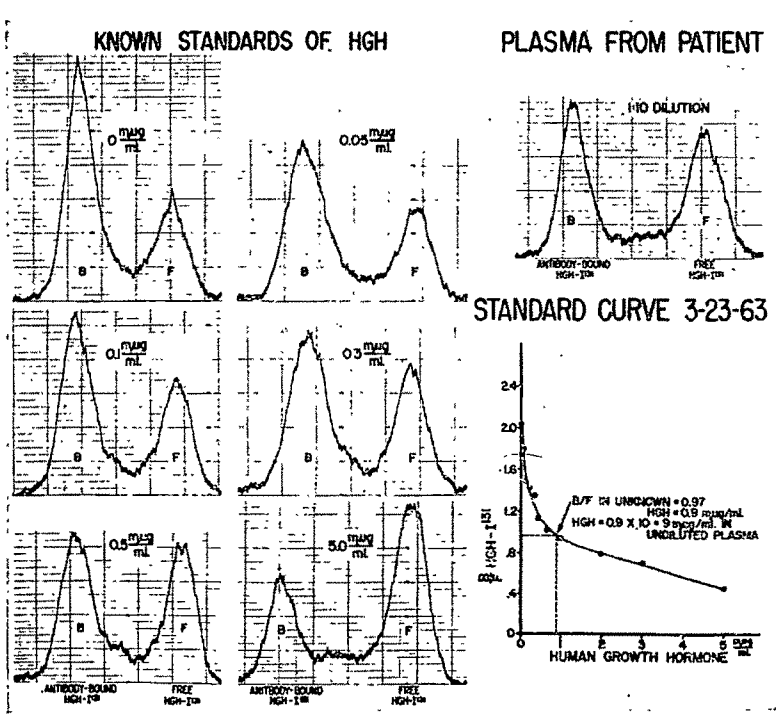


Fig. 1. Standard curve and scans of radioactivity of several chromatoelectrophoretograms. Antibody-bound and free HGH-^{131}I were separated by chromatoelectrophoresis on strips of *ECTEOLA* paper that were then scanned for radioactivity. Areas under the peaks corresponding to bound (*B*) and free (*F*) HGH-^{131}I were determined by planimetry. All mixtures contained identical concentrations of HGH-^{131}I and of antibody. Standard solutions contained known concentrations of unlabelled HGH as shown (left). HGH concentration in a patient's plasma is obtained from the observed *B*/*F* ratio (upper right) by comparison with the standard curve (lower right)

Small amounts of labelled hormone that may become damaged during incubation with plasma also migrate away from the site of application. From control mixtures prepared without antiserum the damage is corrected, as previously described for insulin[8].

In general, plasma was assayed at 1 : 10 dilution. For greater accuracy in certain samples we have used dilutions from 1 : 2 to 1 : 500.

It has been found that the HGH concentration in heparinized plasma is not significantly altered by storage at −20° C for several months or by repeated freezing and thawing. The HGH concentration in serum is identical with that in plasma.

With selected antisera, HGH concentrations of 0·1 mμg/ml. are detected by this method. HGH added to plasma is recovered quantitatively. Purified HGH preparations from several sources (Raben homo 6, 13, 14; Wilhelmi *HS* 269; British Medical Research Council) react identically. More recently, Wilhelmi has supplied a more highly purified preparation (372*B*) which on immunoassay is about twice as potent as the others

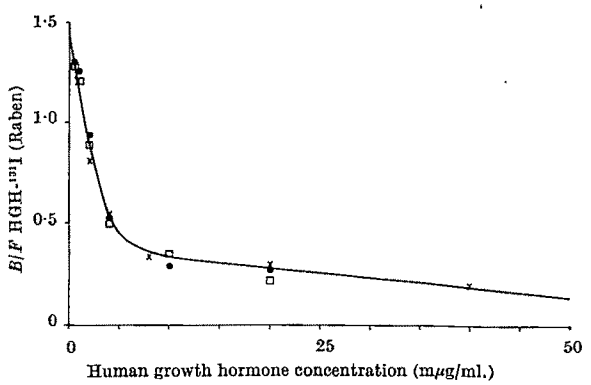


Fig. 2. Comparison of pituitary and plasma HGH. B/F ratio of HGH-^{131}I (Raben) as function of concentration of unlabelled HGH. Known concentrations of unlabelled Raben HGH were used for the pituitary curve (solid line). The plasma samples, diluted 1 : 50 for assay, yielded HGH concentrations, in undiluted plasma, of 100 mμg/ml. (normal adult following stimulation by hypoglycæmia, □), 90 mμg/ml. (umbilical cord, ●), and 200 mμg/ml. (acromegalic, ×). From the values obtained by assay, each of the three plasmas (diluted from 1 : 5 to 1 : 250) was used as the source of known concentrations of unlabelled HGH. The results demonstrate that endogenous plasma HGH and purified pituitary HGH react identically in this system

listed. HGH concentrations reported here were assayed using Raben No. 6 as standard.

In the system reported here endogenous plasma HGH and purified pituitary HGH react identically (Fig. 2). In two cases studied HGH concentrations in blood from the internal jugular vein were 40 and 110 per cent higher respectively than in blood simultaneously obtained from the inferior vena cava. HGH has not been detected in plasma from twenty-one patients, totally hypophysectomized for non-pituitary disorders (diabetic retinopathy, carcinoma) (Fig. 3). Four of these subjects had been studied pre-operatively at which time two had measurable plasma HGH. Bovine and porcine growth hormone preparations, and whole plasma from several non-primate

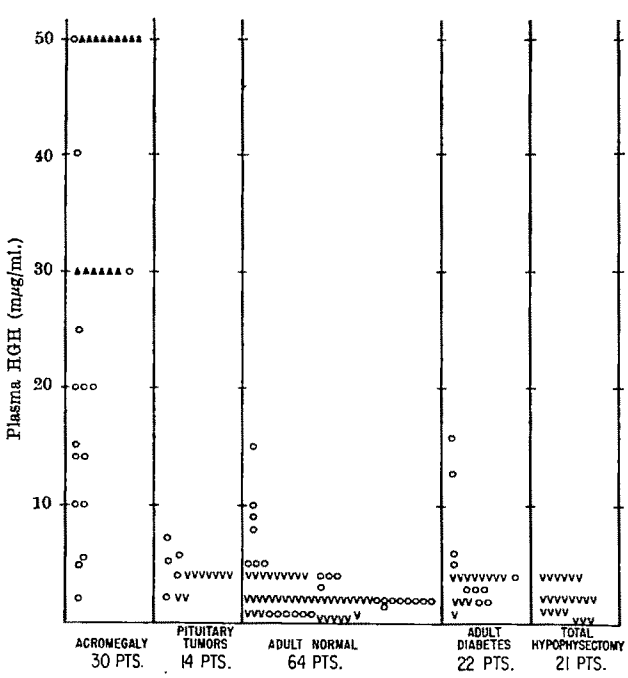


Fig. 3. HGH concentrations in plasma samples randomly obtained from 150 adult subjects. Since the sensitivity of the assay increased during the course of these studies, HGH concentrations, when measureable, are plotted as "less than . . ." the lower limit of sensitivity of the particular assay. In recent investigations (not shown), HGH was detectable (≥ 1 mμg/ml.) in seven of eight normal subjects after an overnight fast. Most of the acromegalic patients had been treated by radiation or surgery; clinically active and inactive cases are included V, Less than; ▲, more than; O, equal to

species (cattle, hog, rat, mouse, horse, sheep, rabbit and guinea pig), did not inhibit binding of HGH-^{131}I to antibody. Simian growth hormone (Wilhelmi *M655A*) inhibited the binding of HGH-^{131}I but not so strongly as did HGH. Thus, the assay is specific for primate growth hormone and free from interference by non-specific components of plasma.

Plasma HGH concentrations have been determined in 212 adults (Fig. 3, Table 2). Most plasma specimens were obtained from 1 to 3 h after the patients had eaten. Recent investigations of this laboratory indicate that hypoglycæmia and prolonged fasting result in elevation of plasma HGH (ref. 6), and that feeding or glucose ingestion is followed by an abrupt fall in plasma HGH (refs. 6 and 7). Assays of plasma from normal subjects fasted for 12–18 h generally yield moderately higher levels of HGH than reported here in non-fasting subjects. The occasional very high level obtained in non-acromegalic subjects (for example, in a patient with anorexia nervosa, plasma HGH was 35 mμg/ml.) may be directly related to a prolonged period of under-nutrition.

Table 2. Plasma HGH Concentration

	Total No. of patients	No. of cases with concentrations in range indicated < 5 mμg/ml.	5–9·9 mμg/ml.	> 10 mμg/ml.
Graves's disease (active)	6	6	0	0
Graves's disease (treated)	5	4	0	1
Hyperfunctioning thyroid adenoma	1	1	0	0
Unilateral exophthalmos (euthyroid)	1	1	0	0
Hypoparathyroidism	1	1	0	0
Hypercalciuria (idiopathic)	1	1	0	0
Myxœdema (untreated)	1	0	0	1
Myxœdema (treated)	2	2	0	0
Anorexia nervosa	3	2	0	1
Sheehan's syndrome	1	1	0	0
Klinefelter's syndrome	2	2	0	0
Turner's syndrome	1	1	0	0
Pregnancy	6	4	1	1
Post-partum (0–3 days) œstrogen treated	5	4	1	0
Normal lactation	3	3	0	0
Post-partum (1 month) normal lactation	1	1	0	0
Cervical carcinoma	3	1	2	0
Endometrial hyperplasia	5	3	1	1
Endometrial carcinoma	3	1	0	2
Other	11	9	2	0
	62	48	7	7

Plasma HGH concentrations (umbilical cord venous blood) in two new-born babies were 40 and 100 mμg/ml.; in the former case HGH concentration in arterial cord blood obtained simultaneously was also 40 mμg/ml. In four infants, two months to one year old, plasma HGH concentrations were 2–8·5 mμg/ml. A small number of plasma specimens from normal children at other age-levels does not permit further conclusions regarding plasma HGH-levels in childhood.

In view of the great changes in plasma HGH which have recently been shown to occur after fasting, hypoglycæmia, glucose administration and exercise[6,7], a comparison of values obtained by different methods would have greater significance if plasma samples had been obtained under standardized conditions. Nevertheless, even for samples obtained at random, such comparison is of interest (Table 1).

Non-specific substances in plasma have been reported to interfere with previous immunoassay methods for determination of HGH in plasma[2–4]. In particular, bovine and porcine plasmas appear to contain HGH (refs. 2–4), although pure growth hormone or pituitary extracts from these species do not inhibit the HGH–antibody reaction or show any cross-reaction with the human hormone in a variety of immunological test systems[12]. Previous workers have reported that serums from totally hypophysectomized[2] and hypopituitary[3,4] human subjects appear to contain HGH and have therefore resorted to a variety of extraction procedures to remove the interfering substances from plasma (Table 1). In the present method, non-specific inhibition does not occur. As little as 0·1 ml. of plasma can be assayed conveniently, permitting serial determinations even on infants. The method is ideally

suited for processing large numbers of specimens. As many as 200 plasma samples have been assayed in a single experiment.

The separation of antibody-bound and free hormone by chromato-electrophoresis on DEAE and *ECTEOLA* papers appears to be superior to the separation by electrophoresis on cellulose acetate[5]. The present technique not only separates the two peaks more clearly but also permits analysis of volumes up to 0·4–0·5 ml., compared with 0·025 ml., the maximum that can conveniently be applied to cellulose acetate; the potential sensitivity of the method is thereby considerably enhanced. We thank the following investigators for materials supplied: J. Fisher, R. Fraser, M. Graff, M. Grumbach, A. Pazianos, O. Pearson, M. Raben, A. Rudolph, M. Sonenberg and A. Wilhelmi.

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EFFECTS OF AMPHETAMINE AND PENTOBARBITONE ON EXPLORATORY BEHAVIOUR IN RATS

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EXPERIMENTS have shown that many mammals reveal a persistent tendency to explore the environment in which they live and, in doing so, they are often exposed to unfamiliar objects and situations which evoke investigatory responses. The study of exploratory behaviour and curiosity has attracted much attention in recent years; even so, the determinants of this kind of behaviour are not fully understood[1]. A central problem in this area has been concerned with the extent to which exploratory behaviour is affected by conditions that evoke fear or anxiety. One approach has been to suggest that novel stimuli may elicit fear as well as an exploratory drive simultaneously, thus generating an approach–withdrawal conflict[2]. According to this view, exploration will be inhibited or checked as a function of the intensity of fear aroused by the situation.

In a recent investigation, Barry and Miller[3] examined the effects of several drugs on an approach–avoidance conflict in rats. It was found that an avoidance tendency induced by electric shocks could be mitigated by alcohol and also by a barbiturate drug. According to Steinberg, Rushton and Tinson[4], results of this kind suggest that drugs which can alleviate fear or anxiety would increase exploratory activity in an unfamiliar environment; such drugs might have little effect, however, on exploration of a familiar environment where responses induced by fear are likely to have been eliminated. Steinberg *et al.* tested the prediction by investigating the effects of amylobarbitone (15 mg/kg) and amphetamine sulphate (0·75 mg/kg), separately and as a mixture, on the exploratory activity of rats in an enclosed maze. Some of the animals (the 'experienced' rats) were exposed to the maze repeatedly before drug tests were made and others (the 'inexperienced' rats) were given the drugs prior to the first maze trial. The results showed that both drugs, when given separately, tended to increase the activity of inexperienced rats but only to a limited extent. When the drugs were given in combination, however, a significant increase in activity was observed. Neither of the drugs administered separately, nor the mixture, produced any significant change in the activity of the 'experienced' rats, when compared with controls.

The experiment recorded here was undertaken partly with the view of extending previous research by myself as well as the findings reported by Steinberg *et al.* The effects of a central depressant (pentobarbitone) and a central stimulant (amphetamine) on a similar measure of exploration have been investigated. Although the drugs have been administered separately, it was thought that by varying the dosages of each drug and by increasing the length of individual trials, a clearer appraisal of their effects might be made.

35 male and 35 female black-hooded rats[5], aged 56–71 days, were used; the rats had not been used for any previous experiment nor were they deprived of food or water during this experiment.

The apparatus consisted of an enclosed symmetrical Y-maze; the arms were 24 in. long, 6 in. wide and 6 in. high, and they were covered by wire-mesh lids. The maze was made of wood and unpainted except for a thin black line that divided each arm into two 12-in. segments; it was situated within an enclosure made of angled steel and covered with butter-muslin to afford a uniform extra-maze environment. Five 6-in. loudspeakers were mounted 4 ft. above the maze and they transmitted masking white-noise at a uniform intensity of 70 db s.p.l. (ref. 0·0002 dyne/cm^2), as measured by a sound level meter. Illumination was provided by a 15-W red lamp suspended 8 in. above the intersection of the maze pathways and, apart from a reading lamp, the room was in darkness. Subjects were weighed individually and, after injection, a rat was placed in a restraining box in the experimental room for 20 min before testing. A trial began when a rat was placed into the end segment of one arm of the maze; observations of activity were then made by counting the number of segments entered in each minute of a 10-min period. Two such trials were given with an inter-trial interval of 24 h.

Subjects were assigned at random to seven groups each consisting of 10 rats (5 males and 5 females). One group (controls) received saline injections according to the ratio of 1 c.c./100 g body-weight. Three other groups were given pentobarbitone in the dosages 3·5 mg/kg, 7·0 mg/kg, and 10·5 mg/kg, respectively; the remaining groups received amphetamine sulphate at dosages of 0·75 mg/kg, 1·5 mg/kg, and 2·25 mg/kg. All injections were made via the intraperitoneal route 20 min before testing and each injection was adjusted to individual weights according to the ratio of 1 c.c./100 g body-weight. Each group received the same drug treatment on both maze trials.

The main results are shown in Figs. 1 and 2; data points refer to the mean activity of the groups derived by calculating the mean of the two trial totals for individual rats on which group means were then based. Tests were made to determine whether the separate variances fulfilled the condition of homogeneity of variance; Bartlett's

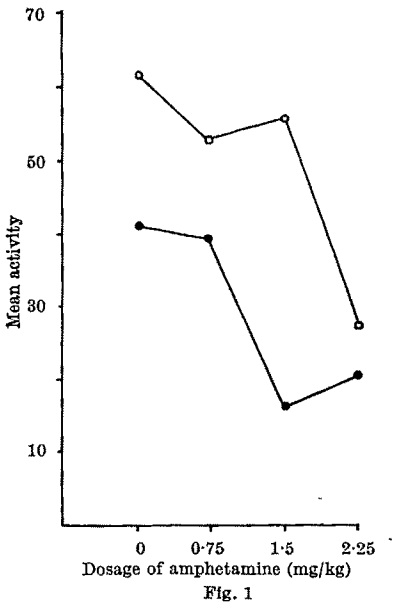


Fig. 1

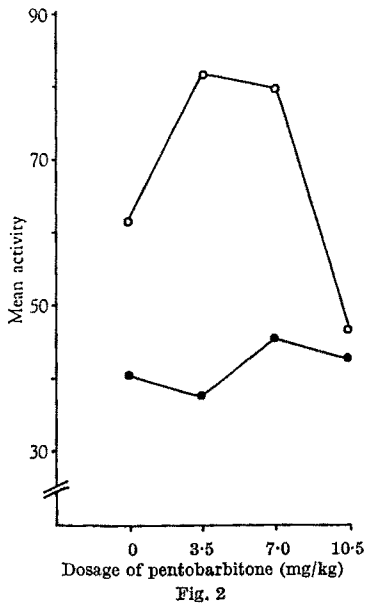


Fig. 2

Figs. 1 and 2. Graphs showing the effects of amphetamine and pentobarbitone at different dosages on exploratory activity; closed circles (●) denote the mean activity of males, and open circles (○) that of females

test indicated that the data fulfilled this requirement (amphetamine: $\chi^2 = 17{\cdot}8$ (uncorrected), $D.F. = 7$, $P > 0{\cdot}01$; pentobarbitone: $\chi^2 = 6{\cdot}6$, $D.F. = 7$, $P > 0{\cdot}3$).

Two analyses of variance, involving the data summarized in Figs. 1 and 2 respectively, were then carried out. (Control observations, based on the results obtained from 5 males and 5 females that received saline injections throughout, are common to each of the analyses and also to Figs. 1 and 2.) In each case, the sex-dosage interaction proved to be significant at beyond the 0·01 level and, therefore, simple effects analyses were carried out with respect to the sex and dosage variables separately. These analyses established: (1) amphetamine reduced the activity of males ($F = 1197{\cdot}5/229{\cdot}5$, $D.F. = 3$ and 32, $P < 0{\cdot}01$) and females ($F = 3523{\cdot}2/229{\cdot}5$, $D.F. = 3$ and 32, $P < 0{\cdot}001$); (2) pentobarbitone was not found to affect the activity of males ($F = 161{\cdot}3/81{\cdot}7$, $D.F. = 3$ and 32, $P > 0{\cdot}05$); (3) females, with respect to both sets of data, were more active than males (pentobarbitone analysis: $F = 6566{\cdot}4/81{\cdot}7$, $D.F. = 1$ and 32, $P < 0{\cdot}001$; amphetamine analysis: $F = 4110{\cdot}8/229{\cdot}5$, $D.F. = 1$ and 32, $P < 0{\cdot}001$).

Reference to Fig. 2 suggests that although pentobarbitone exerted no effect on the activity of males, the drug increased the activity of females at the two lower dosages and decreased activity at the third dosage as compared with controls. It appears, therefore, that these results may be described by a curvilinear relationship and the significance of such a trend was examined by means of F-tests involving the use of orthogonal polynomials[6]. The analysis showed (1) that a significant effect attributable to dosage was present ($F = 1349{\cdot}8/94{\cdot}6$, $D.F. = 3$ and 16, $P < 0{\cdot}001$); (2) that this effect was represented mainly by the quadratic component of trend ($F = 3458{\cdot}4/94{\cdot}6$, $D.F. = 1$ and 16, $P < 0{\cdot}001$) since the F-ratio for the linear and cubic components failed to reach significance.

Finally, an analysis of activity scores during the first 3 min of the first trial was undertaken with respect to rats which received amphetamine to determine whether these data were consistent with those of Steinberg *et al.* The results showed no significant sex-dosage interaction nor did they reflect any significant difference attributable to sex. A significant difference attributable to dosage was found, however ($F = 5{\cdot}07$, $D.F. = 3$ and 32, $P < 0{\cdot}01$). Further analysis by the Scheffé method established that no significant difference was present between the activity scores of controls and rats that received 0·75 mg/kg amphetamine ($t = 2{\cdot}76$, $K = 2{\cdot}92$ at $P = 0{\cdot}01$); a significant reduction occurred, however, with respect to the higher dosages of the drug.

On the basis of several experiments, Montgomery[2] proposed that novel stimuli evoke an approach–withdrawal conflict through the simultaneous arousal of fear and exploratory drives. If this hypothesis is accepted, predictions may be made concerning the effects of drugs on exploratory behaviour. As already noted, Steinberg *et al.* suggested that a depressant drug known to alleviate fear will increase exploration in a novel environment. The results of their experiment were consistent with the prediction and essentially the same result has been found in the work recorded here with another barbiturate drug. By varying the dosage of pentobarbitone it was observed that the drug increased the activity or females at two lower dosages but, at a higher dosage, activity was reduced as compared with controls. Although the drug did not affect the activity of males, a second study (to be reported elsewhere) has shown that their activity is affected in a manner similar to that of females when the intensity of masking noise is raised from 70 to 90 db s.p.l.

No definite prediction was made by Steinberg *et al.* concerning the effects of amphetamine on exploratory behaviour and, although there was a slight tendency for the drug to increase the activity of 'inexperienced' rats in that study, the present results show that the drug leads to a reduction of exploratory activity during the same period. The effect was slight at 0·75 mg/kg but, at two higher dosages, a marked reduction of activity was observed as compared with controls—a trend that was confirmed over the experiment as a whole. An interesting feature of the present experiment was the finding that female rats are more active than males and this result agrees with several previous reports[7].

Although one might account for the effects of drugs on exploratory behaviour in terms of the dual-drive hypothesis[2], more information concerning the conditions under which investigatory tendencies are evoked is needed. In so far as these conditions have been incompletely specified, there is a danger of circularity in this formulation since the presence of an exploratory drive is sometimes inferred from, and at the same time used to explain, the approach of an animal towards an unfamiliar object. To circumvent the difficulty, an alternative concept has been proposed. The idea that exploratory behaviour represents an attempt on the part of an animal to maintain an optimal level of excitement or arousal was first suggested by Hebb and Thompson[8] and has appeared in various formulations[1]. For example, an experiment by Glickman[9] originated with the assumption that the optimal level of arousal is maintained centrally but is dependent on peripheral sensory receptors for neural input and modification. The author argued that depriving an animal of one of its main sources of sensory input would modify the course of exploratory behaviour; specifically, he suggested that blind rats might be more active in an exploratory behaviour test than rats without impairment of vision as they would have to cover more territory to attain an equivalent degree of arousal through the remaining sensory modalities. The results of the experiment were in agreement with the prediction since it was found that blind rats engaged in more ambulatory activity than controls.

To account for the present findings, and by analogy with Glickman's proposal, the suggestion may be made that

any drug that raises arousal thresholds for exteroceptive stimuli will lead, within certain limits, to an increase in exploratory behaviour; conversely, a reduction in perceptual thresholds through the action of a stimulant drug may mean that an equivalent level of arousal can be derived from less exploratory activity as compared with controls. This suggestion appears to be consistent with recent neurophysiological findings. Glickman has pointed out that the role of the reticular activating system of the brain-stem in maintaining the non-specific activity of the central nervous system[10] and as a system involved in habituation to novel stimuli[11] leads one to suppose that it may constitute a threshold mechanism underlying exploratory behaviour. Furthermore, Bradley *et al.*[12] have shown, using the *encéphale isolé* preparation in cats, that amphetamine leads to a progressive reduction in the threshold for arousal responses produced by direct stimulation of the reticular formation. Bradley states that when a dosage of 1·0 mg/kg was reached, the preparation remained permanently alert in terms both of behaviour and of electroencephalograph activity and he suggests that the stimulant effect of the drug may be largely independent of environmental stimulation since the full EEG arousal response occurred irrespective of whether extraneous stimuli were excluded or not. Conversely, the thresholds for behavioural and EEG arousal were raised by pentobarbitone at 3·5 mg/kg and when the dosage was increased beyond 8–10 mg/kg, arousal produced by direct stimulation was blocked completely, even though high stimulating voltages were used.

If the suggestion that exploratory behaviour represents a tendency to maintain "an optimal influx of arousal potential"[1] proves to be essentially correct, Bradley's results afford an explanation of the present data in so far as the drugs involved have been shown to raise or lower thresholds for central and behavioural arousal. It should be noted that the general conception is not necessarily at variance with Montgomery's proposal since the concept of an optimal level of arousal implies a continuum ranging from behavioural states of drowsiness and inactivity to states of acute emotional disturbance and hyperactivity. In a recent investigation[13], it was found that rats which have had a painful shock avoid an unfamiliar area immediately afterwards, preferring instead a familiar place; on the other hand, rats not exposed to shock but given the same choice between a familiar and an unfamiliar place tended to choose the latter. The experiment seems to suggest that investigatory behaviour is likely to occur when the alerting effects of novel stimuli are superimposed on a relatively low level of central arousal whereas behavioural withdrawal may be associated with higher levels of arousal induced by stressful stimulation.

I thank Prof. O. L. Wade and Miss D. Bell (Department of Therapeutics and Pharmacology, Queen's University) for providing the drugs. I also thank Prof. G. Seth for criticism of an earlier draft of the paper, Mr. M. Moore for statistical advice, and Miss E. Bradbury for assistance.

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DRONE PRODUCTION IN HONEY-BEE COLONIES (*APIS MELLIFERA* L.)

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In summer a honey-bee (*Apis mellifera* L.) colony normally has a single fertile female (queen) and perhaps thirty thousand infertile females (workers); the number of males (drones) varies considerably but may be as high as several thousand in colonies allowed to develop without interference from the beekeeper[1]. Biologically this great number of males is interesting because their only important function seems to be the fertilization of the very few young queens (in temperate climates) which may be reared during the summer. However, this situation may be a survival from an environment where many swarms were produced every year, or it may serve some other function such as increasing the chances of cross-mating. In any event, though, one might think that it would be more advantageous for the colony to use its resources in producing more workers, which require less food as larvæ than the same number of drones[2].

Drone brood normally makes its first appearance in late April in Scotland and continues until approximately late August, with maximum amounts in early July[3], but the factors governing its production are not known. Colonies vary very greatly in their output of drone brood[3–6], and it appears likely that adverse environmental conditions, such as food shortage or bad weather, rapidly reduce the amounts present. Another factor thought to influence the extent of drone rearing is the area of drone comb available for laying. In the natural course of events the worker bees frequently replace limited areas of worker cells with the larger drone cells and may also repair damaged worker comb with drone comb, causing a gradual increase in the number of drone cells. But it is a common beekeeping practice to remove such 'deteriorating' comb, with the aim of discouraging drone rearing and thereby encouraging the rearing of workers which will increase the foraging strength of the colony.

The present account gives results from the first year of observations designed to provide information on the precise effect of different amounts of drone comb in the colonies, and on the influence of different levels of drone rearing on the production of worker brood and honey.

The work was begun on April 30, 1962, using 17 colonies which remained queenright throughout the experimental period. All the colonies, which were sited near Aberdeen, were housed in modified National hives, consisting initially of 2 brood chambers (22 combs). As the colonies expanded other brood boxes containing empty combs were added as required, in order that the amount of space available for laying was not restricted at any stage. There were two treatments: (1) Nine 'treated' colonies (*T*) were given one British Standard frame of drone base foundation (approximately 100 in.^{2} on each face). (2) Eight 'control' colonies (*C*) were given one British Standard frame of worker base foundation. In both groups the foundation was built out by the workers into the appropriate type of comb. All other combs used were carefully selected so

that only a minimum area of drone cells (a few square inches) was present on any of them.

From the start of the experiment until swarming or supersedure occurred (resulting in a natural change of queen) examinations involving the measurement of brood areas with a grid[7] were made on each colony, once a fortnight in May and weekly thereafter. Apart from these examinations there was no interference with the natural course of events.

For the first few weeks of the experiment, as Table 1 shows, there was no significant difference between the area of drone brood in the treated and control colonies, but by May 30 the mean amount in the treated colonies was higher than in the controls, and from June 13 onwards there was significantly more drone brood in the treated colonies (t test). The results, therefore, indicate that a reduction in the amount of drone comb in the spring causes a considerable decrease in the amount of drone brood reared. Furthermore, by July 12, all the treated colonies had almost completely filled their single drone comb with brood (185–200 in.^{2}), which makes it clear that shortage of comb remained a limiting factor even in these colonies. Further experiments are planned in which a greater area of drone comb will be added.

Table 1. Areas of Drone Brood (All Stages) in Treated and Control Colonies, 1962

Date	Treatment	No. of colonies	Mean area of drone brood (sq. in.)	S.E.	P
30.4.62	T	9	4	± 2·96	N.S.
	C	8	5		
15.5.62	T	9	13	± 5·61	N.S.
	C	8	13		
30.5.62	T	9	24	± 11·83	N.S.
	C	8	15		
6.6.62	T	9	76	± 25·97	N.S.
	C	8	26		
13.6.62	T	9	146	± 23·85	< 0·001
	C	8	47		
20.6.62	T	9	164	± 30·04	< 0·01
	C	8	59		
27.6.62	T	9	188	± 22·57	< 0·001
	C	8	61		
4.7.62	T	9	212	± 21·56	< 0·001
	C	8	85		
12.7.62	T	9	217	± 14·79	< 0·001
	C	8	78		
18.7.62	T	9	214	± 16·49	< 0·001
	C	8	84		
26.7.62	T	8	170	± 18·68	< 0·001
	C	7	55		

The treated and control colonies showed no significant difference in their production of worker brood at any time (Fig. 1), and therefore at the level of drone production in the treated colonies it appears that there was no appreciable loss of effective colony strength as a result of the greater production of drones.

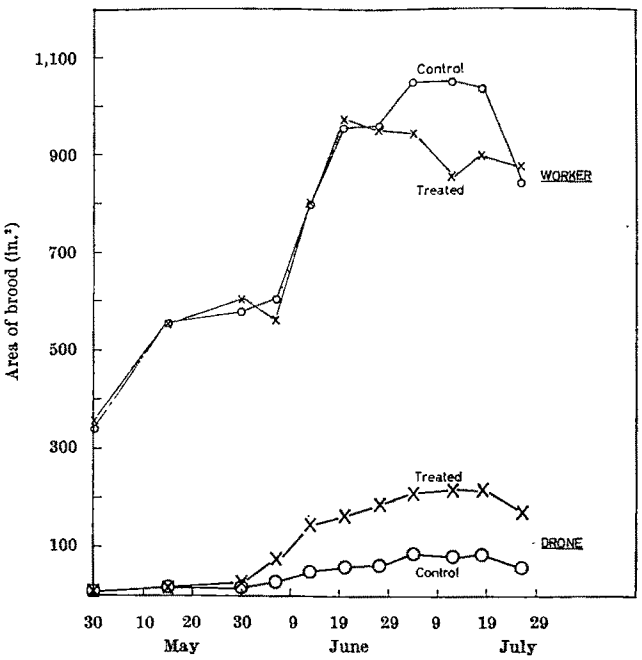


Fig. 1. Areas of drone brood and worker brood in treated and control colonies
1 sq. in. of drone brood, approx. 15 cells; 1 sq. in. of worker brood, approx. 26 cells

Similarly, honey production was not significantly different in the two groups (treated mean = 39 lb.; control mean = 50 lb.; $S.E.$ ± 15·4 lb.). This result is, of course, in accordance with the lack of difference in the production of worker brood in the two groups, since this would be reflected in the foraging strength of the colonies and their consequent honey-producing ability.

In conclusion, it should be mentioned that the summer of 1962 was exceptionally poor from the beekeeping point of view and it is possible that the results given here may have been somewhat affected by this. Nevertheless, the total production of brood (worker and drone) was similar to that of colonies studied in 1956, which was regarded as having a quite normal summer[2]. Results from future summers should show to what extent meteorological factors affect brood rearing.

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MATRIX ANALYSIS AND ARCHÆOLOGY

SINCE Mr. J. Matthews, in his recent article[1], has apparently misunderstood my preliminary report on certain experimental aspects of matrix analysis and archæological data I would like to take this opportunity to reply pending the full publication of my work, especially as the practical difficulties and highly experimental nature of work in this field are clearly unrecognized in the article by Matthews.

The paper by Matthews falls into three sections: first, a lengthy summary of the Brainerd[2] and Robinson[3] articles: secondly, a lengthy summary of my preliminary article[4]; and finally, five original paragraphs. These summaries, besides being almost as long as the originals, are full of errors and in particular the "salient features of his [Brainerd's] work summarized here" bear little resemblance to the important contribution made by Brainerd. The following misrepresentation and errors are taken in sequence from Matthew's work:

(1) In paragraphs 4, 26 and elsewhere Matthews claims to quote the definitions of 'artefact' and 'type' from Brainerd and Clarke, using the term "constantly" recurrent groups of traits. The actual terms used by Clarke and Brainerd define artefact types as "consistently recurrent groups of humanly imposed traits . . . ". If indeed the recurrent trait groups were "constant" then all the artefacts in a given class would have to

be identical whereas there is always variation within limits[4].

(2) Similarly, it was not the primary aim of my paper to "place the kinds of beaker pottery in chronological order[1]" but to "examine the material for significantly recurrent groups of traits[4]". Admittedly I spent some time trying to explain one of the curious but least useful feature of archæological matrices, namely, their independently confirmed chronological linearity (with Brainerd's provisos[2]).

(3) Matthews further states that "Clarke has not made any adaptations of Robinson's method". This is not so. The sorting technique and programme details of the matrix in my article have not yet been published, and it is therefore surprising to read this statement. Of course they are based on Robinson's advance, but since he is concerned with types in assemblages and I was concerned with traits and types there are important differences. This is clearly stated in my article, in which I make it clear that several years work in collaboration with Mr. W. Easterbrook, Mr. J. Grant and Dr. R. Needham have considerably developed the programme.

(4) Again Matthews states that no coefficient has been used in the beaker matrices. I assume he means by this some coefficient to compensate for trait numerical rarity. This was quite deliberate since experiment showed that it was more desirable to compensate in the interpretive phase bearing in mind the total occurrence of the traits, this having the added advantage of presenting concisely on one diagram the actual situation regarding the data. This does not mean that compensated diagrams were not produced; in fact, several were produced for these data using the compensating factor developed by T. T. Tanimoto[5]. These confirmed the general pattern of the uncompensated matrix but in many ways were less clear for the following reason. Consider the case of beakers with cord decoration and decoration inside the lip. Roughly stated the data show that most internally decorated beakers are cord decorated; however, only a few corded beakers are internally decorated. In other words, the correlation is directional, a very high correlation one way, a very low correlation the other. The result under schemes like Tanimoto's is to average out the two and lose the significant relationship. Doubtless an efficient compensating or trait weighting scheme could be evolved and such work is going on, but it will be rather complicated and may involve prejudicing the information we are trying to gain from the matrix.

(5) Matthews once again wrongly assumes that my system of ordering and sorting are identical with that of Robinson. In any event, if Robinson's method "only applies to . . . assemblages for which the constituent types have been defined" then my method applies to assemblages of traits for which the constituent traits have been defined. The data may be heterogeneous in the quantitative and qualitative sense but are from a single artefact type varying with time, usually with more shared traits between beakers than otherwise.

(6) The concern with 'which' beakers are showing 'which' association of traits is a recognized limitation of trait correlation study. This simply means that the aspect must be dealt with by alternative methods; in this case the 'clumping' technique evolved by Dr. R. Needham (Cambridge) has successfully sorted part of these data in terms of groups of vessels with similar trait associations. This analysis confirms the breakdown suggested by the original sorted matrix and taking the three methods together—uncompensated matrix, Tanimoto matrix and clumping technique, the agreement is impressive.

(7) Matthews further alleges that there is no indication of the total number of individual beakers used for the analysis. The number of complete beakers used is in fact stated in my article, that is, 760.

(8) There remain the two original contributions put forward by Matthews. The first allegation suggests that the appearance of the trait correlation 141 in three separate parts of the matrix "cannot arise through coincidence; the suspicion is aroused that . . . the same 141 pots possess all six characteristics". This statement can be refuted on three independent accounts:

(*a*) Simple examination of the original data shows this statement to be wrong. The data are freely available for examination at Cambridge, and it is hoped will be published at some later date.

(*b*) It is impossible for these three pairs of traits to apply to the same 141 vessels since two of them, Nos. 37 and 38, are different ranges of the same rim height/waist diameter ratio. This would mean that each of the 141 vessels must have two shapes. It seems that Matthews did not check the trait key for these correlations[4] which likewise he refers to anonymously under *A/B,C/D,E/F*; they refer to my traits 5/37,11/38,15/20.

(*c*) Statistically it is wrong to suggest that because the number 141 occurs three times in a 39 × 39 matrix that these must refer to one and the same individuals. Since the matrix presents something like 700 pigeon-holes for frequencies of 0–244 (highest frequency 244 for traits 15/23) it would be statistically very surprising if some numbers in that range did not occur several times as they do. Why choose 141 ? One notes that 101 occurs four times and 11 no less than eleven times.

(9) This leaves the case of the hypothetical matrix, Tables 3 and 4. Matthews's main point seems to be that two different artefact assemblages can produce the same matrix pattern and that the groupings in these sorted matrices are not those in the imaginary initial grouping. The first point is quite well known and means nothing more than the pattern or relationship underlying often quite unrelated data maybe, and often is, similar just as two graphs of different material may be bimodal. There is no reason why unrelated material should not produce similar matrices when separately treated, so that some matrices of bacteria taxonomy show patterns very like that for the beakers, itself not unlike Tugby's Australian axe analysis. Brainerd even used this feature to produce a series of hypothetical matrix surface models illustrating various types of relationship in order that actual matrices could be compared with them[2]. I suggest that the matrix of Matthews's Table 4 shows a more proper classification of his Table 2 than the arbitrary allocation of the classes to groups shown therein. Compare Table 2, class 5, of group *B* and class 1 of group *A*; these two classes are called different 'kinds of artefact' at one point; class 5 shares no less than ten out of eleven traits with class 1 and yet they are said to belong not only to two "different kinds of artefact" but are even then attributed to two different type groups *A* and *B*. If group *A* class 1 is compared with group *B*, class 8, we find the reverse situation with puzzlingly the same allocation as before, that is, class 1 has 14 traits all mutually exclusive to the six traits defining class 8. In other words, on the basis of the trait disposition given in Table 2, I can see no evidence that the initial two-fold division into groups *A* and *B* was correct; therefore it would not be surprising if it was not borne out by the matrix sort, Table 4. In archæological terms the classes 1–8 might well be eight classes of one type, for example, beakers; hence the matrix would naturally be the same, especially since the figures used were identical for *A* and *B* leaving only three possible correlations, namely, 0, 10 or 20. Alternatively this theoretical matrix represents entirely diverse material, not compatible for examination on one matrix as is the case with a single type varying, for example, beakers; this would mean that classes one and eight on Table 2, which have no overlapping traits, might just as well be bronze shields on one hand and flint axes on the other. Meaningless questions often receive meaningless answers.

Most puzzling of all is Matthews's assumption that the sorting method he has used to produce Table 4 is necessarily the same as the unpublished programme technique used in my figures, even though they may both be *R*-matrices. Both *R* and *Q* matrices provide useful information about the data fed into them; they merely provide different aspects of the same material.

The mistaken view underlying the whole of Matthews's approach to my work seems to be his belief that I am relying entirely on this one experimental approach to classification. In fact, my whole thesis is devoted to the integration of various unrelated approaches to the same set of material in order to define the measure of agreement and the likely area of the 'real' situation. Any one of the methods used may be, and probably will be, biased by its line of approach. Another basis for misunderstanding is Matthews's apparent lack of comprehension of the pyramidal structure of archæological definitions so that the relationship trait/type is of the same nature as type/assemblage; thus matrices can be used at two different but similar levels (compare Clarke and Tugby with Brainerd). Hence the interchange as compared with Robinson's articles which was concerned with assemblages, the traits defining types show an exactly similar lenticular development and are subject to the same arguments. Just as identical assemblages of types are believed to define a single group or cultural facet so identical sets of traits are believed to define single types.

Finally, I would like to stress that the useful classification of data is the outstanding problem in many different disciplines providing, as it does, the model with which the student is taught and suggesting aspects for research.

The constant revision of classification is an essential task if old teaching models are not to conceal and condemn as ridiculous those lines of thought that imperil their validity and promise useful advance.

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I was interested to read the preceding communication commenting on my recent article in *Nature*. There are one or two points that I should like to make. The numbered paragraphs refer to the original paragraphs in Clarke's letter.

I think that anyone who is interested can read the papers by Brainerd and Robinson to which Clarke refers, and so judge the validity of Clarke's statements about the length and accuracy of my summary.

(1) Clarke is wrong. Brainerd[1] writes of "the criteria of *constant* combination of attributes in the defining of types". Neither Brainerd nor Robinson, in the articles to which Clarke refers, mentions types in terms of "consistently" recurring groups of traits.

(2) Clarke misquotes me here. I wrote[2], "The chronological purpose is only part of Clarke's endeavour. He is primarily concerned to define kinds and ranges of pottery". But if Clarke's intention is not to place kinds of beaker pottery in a chronological order, why has he concerned himself with Robinson's statistical technique ? This, in Robinson's words, is a method for chronologically ordering archæological deposits. The first part of Clarke's article[3] is a description of the method devised by Robinson, and the second part describes an application of this method to a collection of beaker pottery.

(3) In his article, Clarke states that he has made use of Robinson's method. He does not describe any adaptations that he may have made. He does mention the use of "an electronic brain" (*sic*) to sort his Tables, but this does not entail any modification of the statistical technique. Clarke does not mention either Dr. Needham or Messrs. Easterbrook and Grant in his article.

(4) On reading Clarke's article, I assumed that no coefficient had been entered in the unsorted matrix, and that the numbers of individuals observed to possess two characteristics had been entered directly. There are many suitable coefficients which can be used in this sort of situation[4]. Robinson has a very good coefficient, specially devised for his own method.

(5) This paragraph neatly summarizes Clarke's basic misunderstanding. The assemblages to which Robinson refers are archæological deposits which are compared in terms of the proportions of the various types which they contain. If Clarke were to arrange the beakers into assemblages from various archæological deposits, and then compare these assemblages in terms of the proportions of the various traits they contain, then he might be able to place those deposits in a chronological sequence.

(6) I am not impressed by the agreement which Clarke mentions here. The presentation of the data is in question, not the possible sorting techniques.

(7) Clarke misrepresents me here. I allege nothing in this context. I point out that there is no indication of the number of beakers used in the analysis in either of his two matrices. I also point out that the numbers of individual pots which possess each trait (which would occupy the main diagonal) are also missing from the Tables.

(8) Clarke is right and I am wrong. There is information contained in the matrix which demonstrates that the example to which I refer, concerning the number 141, is not appropriate for the point that I wish to make. I wish to submit the following errata to my article in *Nature*: p. 933, lines 35, 37, 38, 40, p. 934, line 1, for "141" read "149" ; p. 933, line 39, for *E* read *A*, for *F* read *E*; p. 934, line 1, for "six" read "five".

If my suggestion (it is only a suggestion) is inappropriate, then I shall be pleased to examine the beakers which demonstrate its inappropriateness.

(9) Is Clarke wise in his comment about imaginary figures and matrices ? The matrix which he gives in his article as Fig. 2, on which pivot the arguments substantiating his method, is filled with imaginary figures.

Clarke's comments on the matrices which I use as an illustration and which are included at the end of my article are quite interesting—although I am not sure that the point that Clarke is trying to make is very clear; but I am sure that Clarke has missed the point of my illustration.

In his article Clarke describes the statistical technique devised by Robinson for the chronological ordering of archæological deposits; he then applies this method to a collection of beaker pottery, and proceeds to arrange this collection in types and ranges of types which are presumed to have a chronological order. In my article[2] I have mentioned some of the reasons why Robinson's technique cannot be applied to collections of beaker pottery in this way. If Clarke is using some other method to analyse the collection of pottery in terms of significantly recurring groups of traits, then perhaps he could explain the principle on which this method is based and how this method can be expected to achieve his stated purpose.

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LETTERS TO THE EDITOR

PHYSICS

A New Explanation of Domain Wall Creep in Thin Magnetic Films

We suggest an alternative explanation of the creep effect in thin magnetic films, and briefly outline experimental results which support it.

Creep is the phenomenon whereby domain wall motion in a thin magnetic film occurs when a number of magnetic field pulses are applied in a direction other than the easy direction, even though their magnitude is less than the d.c. threshold value. In our experiments we find it convenient to apply a d.c. field in the easy direction and pulse fields in the hard direction.

It has been suggested[1] that the transition of the domain wall from cross-tie or Bloch to Néel type, which occurs when a transverse field is applied, could itself be a cause of creep. However, the exact mechanism is not clear. According to the wall transition theory, creep would not be evident in a very thin film of thickness less than about 400 Å since no wall transitions can occur and the walls are always of the Néel type. However, we have observed that, whereas creep does not occur in these very thin films if the transverse pulses are all in the same direction, nevertheless the effect can be induced by applying the transverse pulses alternately in one direction and then the other. Fig. 1 shows typical thresholds for a 250 Å film with a d.c. field along the easy axis, and, in the hard direction, either (*a*) a d.c. field, or (*b*) a large number of transverse pulses (> 100,000) all applied in the same direction, or (*c*) 500 transverse pulses applied alternately in opposite directions. It is difficult to reconcile this result with the wall-transition theory.

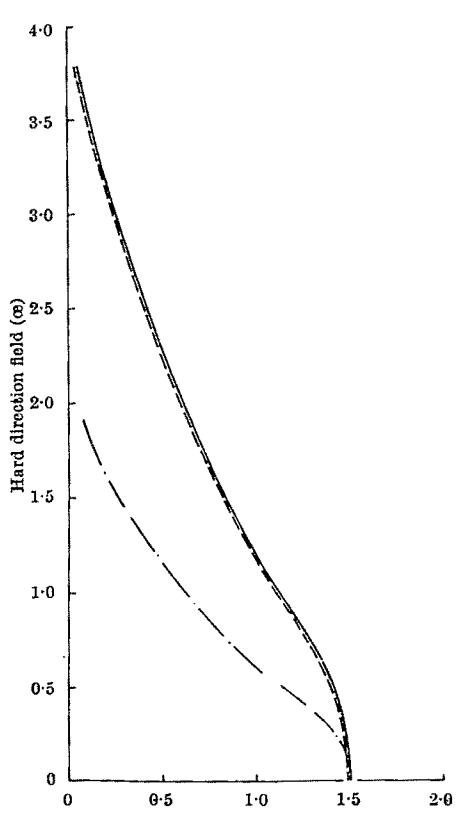


Fig. 1. Influence of bi-directional pulse fields on creep effect on thin magnetic film (250 Å). ——, d.c. threshold; – – –, uni-directional pulse threshold (> 100,000 pulses); —·—, bi-directional pulse threshold (500 pulses)

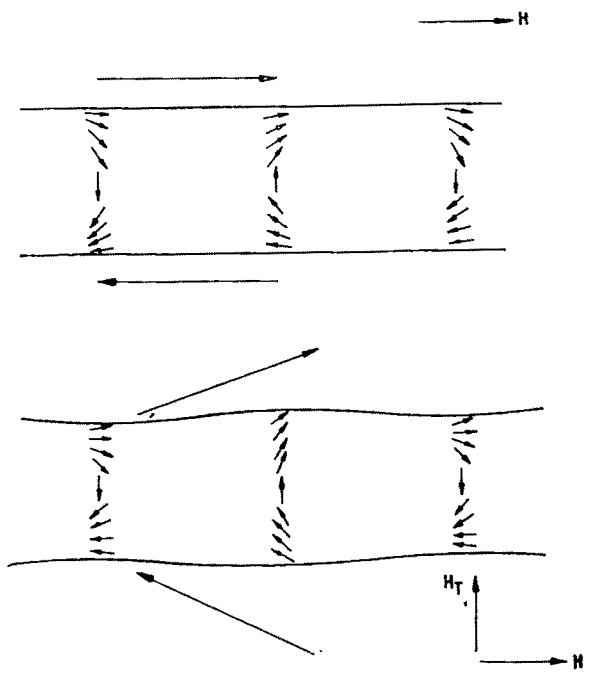


Fig. 2. Above, postulated Néel wall with alternate sections having different senses of rotation of magnetization vectors; below, effect of application of transverse field H_T

An alternative mechanism which we suggest, which does offer an explanation of creep, can be based on the change in structure of Néel walls when transverse fields are applied. Suppose the sense of rotation of magnetization in the Néel wall differs in alternate sections. When a transverse field is applied in conjunction with a d.c. field in the easy direction such that at no time is the d.c. threshold exceeded, then, as shown in Fig. 2, the transverse field creates greater or less divergence of the magnetization vector in alternate sections of the wall depending on whether the magnetization along the centre of the wall reinforces or opposes the applied field. The less divergent parts of the wall are comparatively unrestrained and can advance across the film under the influence of the easy direction field; nevertheless, any motion of the wall as a whole is prevented because the more divergent parts are unable to pass faults and inclusions. So long as the transverse field is in the same direction this state of affairs persists and the wall as a whole remains stationary. However, if the transverse field is reversed then the more divergent parts of the wall become less divergent and can now move past obstructions. The movement continues until again stopped by the retarding effect of those sections of the wall which have just become more divergent when the field reversed and are unable to progress past faults. Therefore, every time the transverse field is reversed so alternate parts of the domain wall advance; the overall effect is that the entire wall creeps.

We have observed visually using colloid techniques in conjunction with low-pulse repetition rates that alternate sections of Néel walls do indeed advance in the way we suggest when the field is reversed and the wall as a whole moves across the film.

If, as we suggest, creep is more likely when there are Néel walls in the film, then creep may be expected in those field configurations which result in the formation of Néel walls. Here we may well have the explanation of Middelhoek's experimental results which show that creep does indeed seem in some way related to wall transitions from either Bloch, or cross-tie, to Néel.

Although the mechanism we put forward is concerned primarily with Néel walls, there is nevertheless the possibility that a similar, though smaller, structural change may occur involving only Bloch or cross-tie walls. This aspect remains to be investigated in later work.

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Primary and Subsidiary Condon Loci of Molecular Spectra

The Franck–Condon principle[1] predicts that the vibrational quantum numbers v', v'' of the strongest bands of a molecular spectrum lie on a family of open double-limbed quasi-parabolic curves in the $v'v''$ plane which are often roughly coaxial about $v'=v''$. The curves are called the primary (outermost) and subsidiary (inner) Condon loci. The loci are indicated on a number of recently published Franck–Condon factor arrays[2–4]. The smaller the difference Δr_e between equilibrium internuclear separations r'_e and r''_e of the molecular potentials involved, the narrower are the loci and the closer is the apex of the primary locus to (0, 0). The greater Δr_e, the more open the loci, and the further the apex of the primary locus is from (0, 0) down the diagonal $v'=v''$. The apices of the subsidiary loci are often spaced roughly two quantum numbers apart down the diagonal axis.

Apart from brief semi-classical discussions of the shape of the primary locus[5,6], little comment has been made on their geometry from a quantum point of view. The geometry of the primary and subsidiary loci, which is therefore discussed here, has obvious applications to identifications of molecular spectra in addition to being of general spectroscopic interest.

Intense bands have large Franck–Condon factors $q_{v'v''}=|\int\psi_{v'}\psi_{v''}dr|^2$ for which the non-cancelling overlap between vibrational wave functions ψ_v is large. Large overlap usually occurs when an antinode of one wave function and an antinode of the other wave function lie in the same region of r. The primary Condon locus passes through those v', v'' values for which the largest contribution to $q_{v'v''}$ occurs by strong overlap between pairs of terminal (outer) antinodes. Similarly the subsidiary loci pass through v', v'' values for which the largest contribution to $q_{v'v''}$ occurs by overlap between terminal and subsidiary antinodes.

The Franck–Condon principle, which in classical terms calls for equality of oscillator turning points[6], can thus be recast in quantum terms by calling for equality between the positions of an appropriate pair of antinodes, one for each of the levels involved.

Pairs of antinodes of order p are equally spaced about r_e for potentials which are symmetrical about r_e. There are $w+1$ such pairs of antinodes for even ($v=2w$) and for odd ($v=2w+1$) vibrational levels. The p'th antinodes of the v'th level of a symmetrical potential thus lie at $r_{v_1}(p)$ and $r_{v_2}(p)$ where:

$$r_{v_1}(p)=r_e-F_v(p) \tag{1a}$$

$$r_{v_2}(p)=r_e+F_v(p) \tag{1b}$$

$F_v(p)$ is the separation between the p'th antinode and r_e. It depends on the specific form of the potential. For a simple harmonic potential it is possible to show that:

$$F_v(p)=(k\omega)^{-1/2}\left\{\frac{v-b_p}{a_p}\right\}^{\frac{1}{n_p}} \tag{2}$$

where $1<a_p<3{\cdot}5$; $b_p=2(p-1)$, $1<n_p<2$, $0<p<10$, $k=4\Pi^2\,\mu c/h$ and ω is the oscillator frequency in cm^{-1}.

As the Franck–Condon principle calls for equality of antinode positions, the primary locus is:

$$r_{v'_{1,2}}(1)=r_{v''_{1,2}}(1) \tag{3a}$$

and the p'th subsidiary locus lies in the region where:

$$r_{v'_{1,2}}(1,p)=r_{v''_{1,2}}(p,1) \tag{3b}$$

For a simple harmonic potential, equation (3a) may be rewritten, using equations (1a), (1b) and (2), as:

$$\pm k^{1/2}a_1^{\frac{1}{n_1}}\Delta r_e=\pm\frac{(v'-b_1)^{\frac{1}{n_1}}}{\omega'^{1/2}}\pm\frac{(v''-b_1)^{\frac{1}{n_1}}}{\omega''^{1/2}} \tag{4}$$

where Δr_e is the magnitude of $r'-r''_e$. Equation (4) represents the primary locus in the v', v'' plane.

The choice of signs implied in equation (4) allows it to take the following physically meaningful forms:

$$\Delta r_e=V_1'^{a_1}+V_1''^{a_1} \tag{5a}$$

$$\Delta r_e=V_1'^{a_1}-V_1''^{a_1} \tag{5b}$$

$$\Delta r_e=V_1''^{a_1}-V_1'^{a_1} \tag{5c}$$

where $\alpha_1=1/n_1$ and $V_1=(k\omega)^{-1/2}a_1(v-b_1)/a_1$. The gross regional overlap between the two potentials involved determines where each of these equations is used to represent segments of the primary Condon locus as follows.

When Δr_e is sufficiently large that only one limb of one potential curve (for example, $r<r'_e$ or $r>r'_e$) lies in the same range of r as the other limb of the other potential curve ($r>r''_e$ or $r<r''_e$) equation 5a represents the primary Condon locus. It is a broad curve, well separated from (0, 0).

On the other hand, when Δr_e is small so that there is complete overlap between all of each potential, three segments (a, b, c) have to be treated separately. They are:

(i) If $r'_e>r''_e$; (a) $r<r''_e$, (b) $r''_e<r<r'_e$, (c) $r>r'_e$

(ii) If $r''_e>r'_e$; (a) $r<r'_e$, (b) $r'_e<r<r''_e$, (c) $r>r''_e$

Equation (5a) represents segments (ib) and (ii$b$). Equation (5b) represents segments (ia) and (ii$c$). Equation (5c) represents segments (ic) and (ii$a$). In each of these cases, equation (5b) represents the apex of the locus and equations (5a) and (5c) represent the limbs.

Equations 5a, 5b, 5c have been plotted for a number of band systems and have been found to represent the primary locus quite well for moderate values of quantum numbers ($v\sim10$) in spite of the obvious limitations of the potential used. Similar treatment of equation 3b has led to adequate representation of the subsidiary loci.

This work was supported in part by research grants from the National Aeronautics and Space Administration, the Air Force Office of Scientific Research, the National Research Council of Canada and the Ontario Research Foundation and in part by contracts with the Department of Defence Production of Canada and the Air Force Cambridge Research Center.

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Predissociation and Perturbation in the Selenium Molecule

It is known that the predissociation produced by the radiationless transition from an excited stable molecular state to an unstable one is responsible for the broadening of the absorption rotational lines and the increase in intensity of the perturbed bands. In the emission spectrum, the bands vanish in the region of perturbation. This usual case is well illustrated in the band system $B^3\Sigma_u^- - X^3\Sigma_g^-$ of S_2. In the progression $v''=0$, the absorption spectrum shows sharp band heads up to $v'=9$, while the $v'=10, 11\ldots$ bands are very diffuse. At $v'=10$ a strong intensity increase occurs. This predissociation corresponds to the case I(c) of Herzberg[1] due to an interaction between two excited states one of which is stable and the other of repulsive type. The crossing of these two potential energy curves occurs above the dissociation limit of the repulsive level. So far it is the only well-established case of this kind.

The foregoing appearance is not found in the similar transition $B\Sigma - X\Sigma$ of Se_2. In the progression $v''=0$, absorption bands are observed up to $v'=22$, and are followed by a short-range continuous spectrum. The difference with S_2 is that in the small dispersion spectrum there is no apparent intensity increase. The perturbation seems so strong that the nearest diffuse bands have the aspect of a continuous background. The extrapolation of this progression gives for the short wave-length limit the value $\lambda_1=3200$ Å. On the other hand, the short wave-length limit of the continuous spectrum is $\lambda_2=2750$ Å. Thus, the continuous spectrum is actually made up from the broadened bands and a true short range continuum. The crossing point of the two interacting states is located near the dissociation limit of the stable state. Among the numerous sub-cases of predissociation of diatomic molecules discussed by Mulliken[2], only two may be considered here. In both cases the internuclear distance r_c of the crossing point is larger than r_e. If the perturbing state is a stable one, the case C^+ occurs, where both potential curves have a common asymptote and both molecules originate from the same atoms $S(^3P)+S(^1D)$. In the second case the perturbing state is of repulsive type and originates from different atoms. In order to obtain more information concerning the broadening of the line, we photographed again the absorption spectrum near the limit of the progression $v''=0$. Fig. 1 shows a part of the spectrum taken in the region 3200 Å–3600 Å with a JAco-Ebert spectrograph having a linear reciprocal dispersion of 0·6 per mm. The absorbing layer was 14·5 cm long and the pressure of selenium about 15 mm mercury. It is seen that the width of the rotational lines of the (18·0) band (Fig. 1, below) is about 3 times smaller than that of the (20·0) band (Fig. 1, above). In the same spectral region the rotation structure is simple and seems to correspond to a $^1\Sigma$–$^1\Sigma$ transition. The rotational lines are well separated, and the effect of the perturbation is to broaden the lines in consequence of the decrease of the life-time of the excited state; but the broadened lines do not overlap in the (20·0) band. This is why there is no intensity increase of that band.

The following (21·0) band shows no rotational structure with the dispersion here used. Under small dispersion this band does not seem to be stronger than the (19·0) band. Therefore, we have to conclude that in the present case the decrease of the lifetime is associated with a greater width of the lines but not necessarily with a higher intensity of the perturbed band. It is known indeed that for a single line the total intensity absorbed is not sensitive to the shape of the line profile and basically depends on the product of the oscillator strength and the concentration of absorbing particles. The increase of the band intensity could only originate from the overlapping of the neighbouring lines.

To sum up, the perturbations in the $B\Sigma - X\Sigma$ transition are basically similar for the two molecules, Se_2 and S_2. In both cases diffuse bands are observed. Their number only varies. Contrary to what is observed in sulphur, selenium bands do not get sharp again when v' increases from the perturbation region, owing to the proximity of the dissociation limit. This results from the relative position of the potential curves of the stable excited state and the repulsive state, which cross each other near the dissociation limit of the stable molecule.

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High-current Cathode for Electron Linear Accelerator

For many years, the bombarded tantalum cathode gun has acted as a reliable source of electrons for injection into demountable linear accelerator systems[1,2]. An important advantage of the pure metal cathode, such as tantalum or tungsten, is its high resistance to poisoning under adverse vacuum conditions which exist in linear accelerators during multipactor resonances, during high energy beam collision and clipping on inner surfaces, and, in particular, when the microwave structure is initially out-gassed by application of the radio-frequency power. Furthermore, after the cathode has cooled, the accelerator may repeatedly be opened to atmosphere, re-evacuated and the cathode returned to operating temperature without loss of emission and without additional precautions or special restrictions. On several occasions, vacuum systems containing pure metal cathodes at elevated temperatures have been inadvertently opened to atmosphere, and it has been possible to re-operate these guns satisfactorily.

Even with the assurance of an extremely clean system and a higher order of vacuum (10^{-8}–10^{-6} torr) as offered by the application of sputter-ion pumps[3] to high-power linear accelerators (the first such combination was a machine constructed for the Danish Atomic Energy Commission and operated in December 1959), preference remained for the bombarded tantalum cathode because it did not require bake-out procedures, isolating valves, or activation schedules as is the case, for example, with coated emitters.

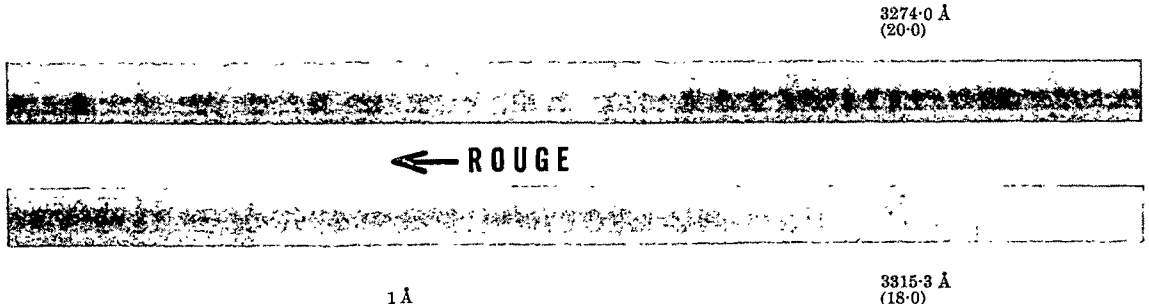


Fig. 1

Earlier accelerator applications required pulsed gun emission currents of the order of 1–2 amp, and the cathodes were operated under temperature-limited conditions which necessitated the use of sensitive control systems to maintain the injected pulse current at a constant level. An operating temperature of approximately 2,000° C (brightness) provided 250 m.amp/cm² and the larger sizes required 1,200–1,700 W of bombarder power at 3 kV plus 150 W of filament dissipation. Under these conditions tantalum cathodes are characterized by long life, and earlier installations have demonstrated in excess of 4,000 h.

Tantalum cathodes which have been operated at temperatures closer to the melting point have shown a tendency to exhibit large crystals which, becoming displaced with respect to each other, distort the original curved surface of the cathode and adversely affect the beam optics. Metallurgical examination of these cathodes indicates that the displacements are due to stresses set up by differential expansions between the cathode and its support structures, causing sliding of the crystal boundaries at the higher temperatures.

A further consideration in the application of a pure metal emitter is its relatively high evaporation rate at temperatures which are required for thermionic emission[4]. These high rates can result in appreciable loss of material over the working life of the cathode. The vapour pressure for tantalum at 2,200° C (about 2,000° brightness) is 10^{-6} torr corresponding to an evaporation rate of approximately 10^{-8} g/cm²/sec, and at 2,500° C these figures are about 40 times greater.

The vapour pressure for tungsten at a given temperature in the working range is an order lower than tantalum, but the emission per unit area is also lower (approximately one-third). Consequently, the required operating temperature to obtain the same total emission is higher, and the resulting vapour pressure is similar to that of a tantalum cathode. Also, additional heater power is needed to supply radiation losses.

Hence it became apparent that earlier established bombarded pure metal cathode designs would be impracticable for emission densities in excess of about 300 m.amp/cm² as occurs, for example, when the beam optics requirements of a high current injection system demands a cathode of relatively small diameter.

The suppression of pulse shortening[5,6] and the increasing popularity of short-pulse, high-current accelerator operation, that is, the stored energy régime, established a need for a reliable source which could: (1) provide emission densities of about 1 amp/cm²; (2) ensure a high degree of geometric stability; (3) operate under adverse vacuum conditions.

Earlier experiments (in 1960) in our laboratories using impregnated barium dispenser cathodes in a radiographic linear accelerator confirmed that very high emission densities could be obtained at moderate cathode temperatures (1,100° C), but considerable difficulty was encountered with residual gas poisoning. Failure of the impregnated cathodes could with some degree of certainty be ascribed to poor vacuum conditions. Nevertheless, it was considered that they were unnecessarily sensitive and impractical for this type of linear accelerator[7], which featured an X-ray target requiring a rotating high vacuum seal. A tungsten cathode was afterwards fitted and has since demonstrated life-times in excess of 3,000 h.

Storage of impregnated cathodes is also a problem as they tend to pick up water and carbon dioxide from the air which may cause them to swell and crack. Dispenser cathode studies are continuing and various activators are being investigated in an attempt to reduce residual gas poisoning sensitivity and field emission effects due to sputtered barium and barium compounds. In general, this type of emitter is seriously affected by oxygen and normally requires a guaranteed vacuum of better than 5×10^{-7} torr.

As a result of these experiences, we decided to investigate the possible application of thoriated tungsten type cathodes in linear accelerators. It was considered that this type of source would operate satisfactorily in relatively poor vacuum and that it would produce a space charge limited beam at considerably less temperature than the previously discussed bombarder designs.

According to Jenkins and Trodden[8], carburized thoriated tungsten thermionic emitters can operate satisfactorily in partial pressures of oxygen up to 10^{-5} torr, carbon dioxide up to $2{\cdot}5 \times 10^{-5}$ torr, and water vapour up to $0{\cdot}6 \times 10^{-5}$ torr. These gases are the most detrimental that are likely to be present in a demountable system. (This compares with a 10^{-7} torr upper limit for partial pressure of oxygen for operating impregnated barium dispenser cathodes[9].) The remaining residuals, hydrogen, nitrogen, carbon monoxide and organic vapours, have no harmful effects on these cathodes up to much higher pressures. Since the actual cathode region of the accelerator may reach total pressures estimated to be up to 5×10^{-5} torr and the emission capability, at comparatively low temperatures, of the thoriated tungsten cathode far exceeds the required 1 amp/cm², it will be appreciated that on the basis of this evidence the thoriated tungsten cathode appeared to offer a chance of satisfying the requirements of high current linear accelerators.

Cathodes were prepared by special machining techniques from solid tungsten which contained 2 per cent thoria. The emitting surface, usually in the form of a concave dish, was carburized by first uniformly spraying the surface with a suspension of pure graphite powder in a suitable binder solution and then vacuum furnacing the cathode to 2,000° C until all the carbon had reacted. This process was repeated until a carbide layer of about 0·010 in. was obtained. Carburized surfaces prepared in this manner had exceedingly uniform appearances. However, when layers thicker than 0·010 in. were attempted the cathode surface tended to blister and distort.

A number of cathode assemblies have been made with outside diameters of 0·75 in., 1·5 in., and 2·375 in. Each cathode was attached to a molybdenum ring and supported by tantalum strips welded between the molybdenum ring and the surrounding heat shields.

The 1·5-in. cathodes were assembled in 0·11 microperveance gun structures and produced space charge limited beams at a temperature of 1,580° C (brightness). These guns have been operated for several hundred hours at a pulse voltage of 105 kV and at total pressures of between 10^{-7} and 5×10^{-5} torr without sign of emission deterioration. The amount of carbon present should enable free thorium to be produced at an adequate rate for many thousands of hours. This type of cathode is not chemically active when brought out into the atmosphere and has no tendency to pick up water vapour and carbon dioxide from the air. No special storage or handling procedures are necessary, and geometrical changes during life have been avoided.

The high thermionic activity of these cathodes enabled the guns to be operated space charge limited with the attendant advantage of emission insensitivity to heater power fluctuations and the avoidance of a critical regulation control circuit. The lower temperature of operation required only 500 W of bombarder power for space charge operation compared with 1,800 W for a tantalum cathode of the same dimension (1·5 in. diameter).

In the event of cathode emission failure by loss of carbon or severe poisoning, it should be possible to raise the cathode temperature and recover the pure metal emission, thus permitting the completion of a run before replacing the source.

A recently constructed two-section, 45-Mc/s-band-width, *S*-band linear accelerator, model *V7709X*, specifically designed for high-beam current with a rating of 1 amp at 10 MeV and utilizing a 5-amp thoriated tungsten cathode electron gun has demonstrated a peak beam current of

2·5 amp at 8 μsec pulse-length without sign of pulse shortening. This cathode and wave-guide system had sufficient beam-loading capability not only to reduce the effective residual radio-frequency power to zero and readily achieve the maximum conversion efficiency, which was in excess of 85 per cent, but it also enabled a large proportion of the 8 MW input coupler radio-frequency power to be transferred back to the microwave circuit by beam induction and to appear in inverse phase at each wave-guide termination. This regenerated power condition was previously achieved in our laboratory, October 1962, also without pulse shortening, and using a bombarded tantalum cathode in a two-section, *S*-band high power accelerator, model *V*7709, of lower current rating and with a different wave-guide design concept than the *V*7709*X* machine. These higher beam currents and induced powers have encouraged further investigation of high-power radio-frequency generation by relativistic bunches of electrons in addition to offering alternate lay-out arrangements of multi-section linear accelerator systems.

A thoriated tungsten cathode of larger diameter and a higher perveance electron gun have been designed and constructed for attachment to the existing inflector type injection system on the *V*7709*X* machine. Replacement of the 5-amp gun with this higher current source will enable the ultimate beam loading condition to be attained. Additional high-current studies will be conducted for both the steady-state and the stored-energy (short-injection-pulse) modes of operation. It is also planned to increase the existing relatively low electric field of 9 MeV/m (loaded at 1 amp) by doubling the input peak power-level.

In conclusion, it should be reported that, on three separate occasions during the foregoing tests and while operating with a 2·5-amp beam at 6·0 μsec, a pin hole puncture developed in the accelerator output window causing a vacuum failure and closing down the system. The thoriated tungsten cathode was left *in situ* on each occasion and the system was re-evacuated. During out-gassing of the accelerator, the cathode was maintained at approximately 1,000° C for 15 min, and a space charge limited beam of the original magnitude reappeared when the cathode temperature was raised to its normal operating value.

The ability to produce beam pulses of greater than 100 joules in a 5-mm cross-section now poses a difficult beam extraction problem requiring an alternative approach to that of a simple transmission assembly.

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RADIOPHYSICS

Back-scatter of Radio Waves from the Equatorial *F* Region

The spread-*F* which is observed at equatorial latitudes varies in its appearance during the night[1], but may be divided into two basic types, which have been termed equatorial type[2] and temperate latitude type[3] respectively. As it is known that irregularities giving rise to equatorial type spread-*F* are capable of scattering radio waves with frequencies greater than the critical frequency of the *F* layer, it was decided to carry out, at Accra, an investigation of the irregularities, using a back-scatter radar operating on a frequency of 18 Mc/s. Such an equipment has a number of advantages over the normal ionosonde, including greater sensitivity, and the possibility of using directional aerials.

The equipment used for the experiment described in this communication consisted basically of an 18-Mc/s transmitter of 1-kW peak power, 300-μsec pulse duration, and 16·7-c/s repetition frequency, and a pulse receiver having a band-width of approximately 6 kc/s. The aerial used was a three-element Yagi, steerable both in elevation and azimuth; the same aerial was used both for transmitting and receiving.

Observation, on an *A* scan display, of the echoes obtained using the equipment already described, showed that during the period 1900–2200 G.M.T. there occurred echoes which could not have been caused by the mechanism of ionospheric reflexion and ground scattering which is responsible for the echoes normally observed on back-scatter radars. These echoes occurred at ranges shorter than the range of normal ground back-scatter, and at angles of elevation which on some occasions were approximately vertical; it was clear, therefore, that the echoes were due to direct back-scatter from irregularities in the *F*-region.

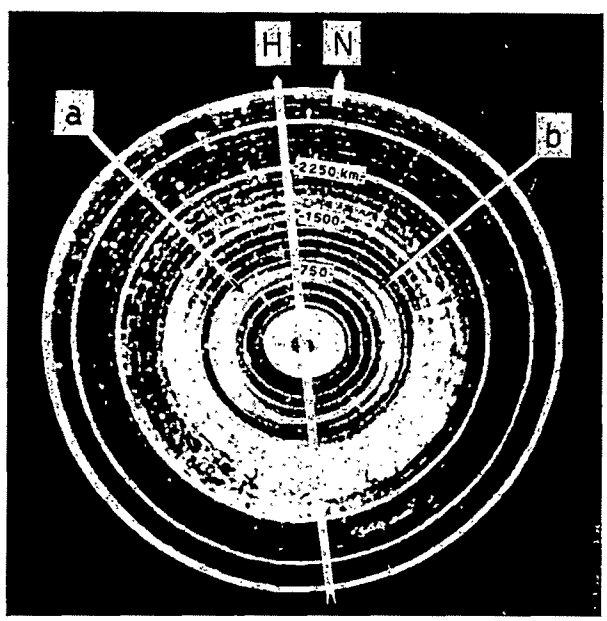


Fig. 1. Back-scatter, 2030 G.M.T., October 31, 1962

In order to record the occurrence of direct back-scatter echoes in a convenient form the equipment was arranged to produce plan position indicator photographs at pre-set intervals. A typical plan position indicator picture is shown in Fig. 1, where, in addition to the ground back-scatter echo at a range of 1,000–2,000 km, two direct back-scatter echoes (labelled *a* and *b* in Fig. 1) are also present, at ranges of 600–900 km in the west, and 750–950 km in the east (in Fig. 1 the range markers are at intervals of 150 km with every fifth marker accentuated; the directions of geographic and magnetic north are denoted by *N* and *H* respectively). It will be noted from Fig. 1 that the direct back-scatter echoes are present only to the west and to the east, and not to the north or south, and that the direction from which maximum scattering occurs is perpendicular to the magnetic meridian rather than the geographic meridian. The angular spread over which the echoes are observed approximates to the beam-width of the aerial and hence the actual angular

spectrum of the back-scattered signal in the horizontal plane is probably quite small. It will also be noted from Fig. 1 that the echoes have a fairly small spread in range. The most frequently observed range spread, allowing for the length of the transmitted pulse, is about 100 km, but occasional echoes occur showing a range spread as great as 400 km; on no occasion have echoes been observed simultaneously from all parts of the sky "visible" to the sounder. The aspect sensitivity, indicated by the observation of echoes to the east and west only, is undoubtedly due to field alignment of the elongated irregularities responsible for the echoes (the elongation and field alignment of irregularities in the equatorial F region has previously been noted by a number of workers[4,5]).

The range spread of echoes indicates that the irregularities occur in patches with east–west dimensions of approximately 100 km; this is in good agreement with the results of Kent[5], who found that the irregularities responsible for the scintillation of satellite signals at Ibadan occurred in patches approximately 100 km in horizontal extent; but is not in agreement with the findings of Cohen and Bowles[6], who observed that patches of irregularities, capable of supporting 50-Mc/s forward scatter near to the magnetic equator in South America, frequently extended over distances of up to 1,000 km in the east–west direction. This difference in the patch size may be due to the fact that the measurements of Cohen and Bowles were made at sunspot maximum, whereas the measurements reported here were made nearer to sunspot minimum; the measurements made by Kent[5], however, were carried out at the end of 1959, nearer to sunspot maximum than minimum.

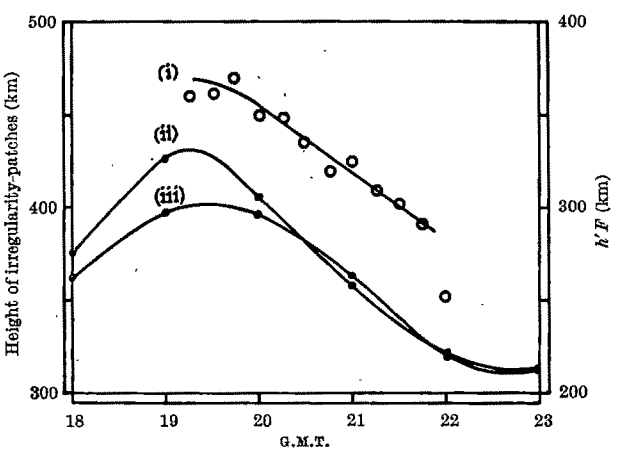


Fig. 2. Nocturnal variation in height of irregularity patches and $h'F$. (i) Back-scatter; (ii) $h'F$, back-scatter present; (iii) $h'F$, back-scatter absent

The plan position indicator records enable one to determine when a patch of irregularities is vertically overhead the sounder, and hence, from the minimum range of an echo produced by such a patch, to determine the height of the patch. The heights of some sixty patches determined in this manner were used to construct an average variation in height with time over the period during which echoes are observed. The variation in height is shown in Fig. 2 along with the variation in $h'F$ for the same period. It will be noted that the variation in height of irregularity patches is very similar to the variation in $h'F$, but the actual height of patches is approximately 150 km above that of $h'F$, which means that the irregularities occur in the region of h_m. It may also be seen from Fig. 2 that the post-sunset rise in $h'F$ is greater on days when scatter is present than on days when it is absent; a similar phenomenon has previously been reported by Lyon *et al.*[1] in connexion with equatorial type spread-F. The height of 150 km above $h'F$ may be compared with that of 50 to 100 km above $h'F$ observed by Kent and Koster[7] for the irregularities giving rise to scintillation of satellite signals observed at Accra during 1961.

A comparison between the occurrence of direct back-scatter at 18 Mc/s and the presence of equatorial type spread-F on ionograms taken at Accra has been made, and the result is given in the form of a contingency table (Table 1). It may be seen from Table 1 that although there is a strong correlation between the occurrence of direct back-scatter and equatorial type spread-F (the probability of independence is less than 0·01), and scatter is never observed in the absence of spread-F, scatter does not invariably occur when spread-F is present.

Table 1. Contingency Table for Occurrence of Direct Back-scatter and Equatorial Type Spread-F

		Spread-F Present	Spread-F Absent
Back-scatter	Present	26	0
	Absent	11	11

The period of time over which echoes are observed is 1900–2200 G.M.T., which is approximately the same as the period of occurrence of severe flutter fading[8], and abnormal Doppler shift of obliquely propagated signals[9]. Measurements made during 1962 indicate that the seasonal variation in the occurrence of direct back-scatter shows an equinoctial maximum similar to that reported by Koster[8] for flutter fading. It seems probable that the irregularities responsible for direct back-scatter at 18 Mc/s are similar to those which cause flutter fading and Doppler shift of obliquely propagated signals.

I thank Prof. R. W. H. Wright and Dr. J. R. Koster for many helpful discussions. This work was sponsored by the Cambridge Research Laboratories, OAR, through the European Office, Aerospace Research U.S. Air Force.

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CRYSTALLOGRAPHY

Whisker-like Growth of Polyoxymethylene from Solution

High-molecular-weight polyoxymethylene is known to crystallize very readily. Various forms of single crystals of this polymer have been described: crystals about 1 mm in size can be grown directly from monomeric formaldehyde[1–3], fibres are formed by polymerization of crystalline trioxane[4–6], while hexagonal lamellæ and thin star-shaped crystals have been grown from solution of the polymer[7,8].

We wish to report a new morphological variant of crystalline polyoxymethylene, which we obtained in copious yield from dilute solutions. As this type of crystal shares many characteristics with 'whiskers' grown from low-molecular-weight materials, we tentatively consider them as polymer 'whiskers'.

These characteristics are:

(*a*) Although the crystals possess a rather complex shape, they behave like single crystals, so far as can be

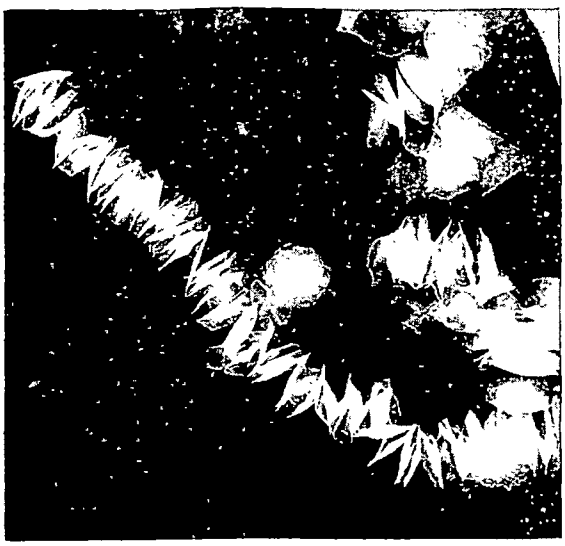


Fig. 1. Polyoxymethylene 'whisker' (× *c.* 3,200; platinum/carbon shadowed)



Fig. 2. Enlarged portion of Fig. 1 showing helicoidal structure of centra fibril

judged from microscopic observation between crossed polarizers.

(*b*) Growth in one direction, that of the crystallographic c-axis, is strongly preferred. The crystals may grow 10–100 times as long as they are thick.

(*c*) The crystals have a periodic shape, which microscopically gives at first sight the impression of a zig-zag chain of fibrils, or a string of beads.

Electron micrographs (Figs. 1 and 2) of some specimens show they actually consist of a central filament, from which hexagonal lamellæ grow at short intervals. It seems as though the central filament itself is helicoidal. The growth of helicoidal forms, and also of lamellæ on filamentous crystals has often been observed in the study of whiskers of low-molecular-weight materials[9]. Growth of (straight) whiskers on thin 'leaders' has been observed in the crystallization of hydroquinone[10].

(*d*) The crystals also show the normal phenomena of kinking and branching.

While in their extreme (but by no means exceptional) development these crystallites therefore exhibit an obvious analogy with low-molecular-weight 'whiskers', they do not appear different in their initial stages from the 'sheaves' observed so often in polymers crystallized from solution[11]. There is also a possible relationship to the 'axialites' described by Keller[12]. Very recently, Bassett and Keller[13] have reported on a chain-like structure apparently akin to those described here, although an order of magnitude smaller, which was formed by quenching heated polyethylene crystals.

I have obtained these 'whiskers' (besides the single hexagonal lamellæ) invariably when polyoxymethylene made by polymerization of irradiated trioxane in the crystalline state was recrystallized from dilute solutions (in phenol or in diethylene glycol dimethyl ether). From a commercial polyoxymethylene, 'Delrin', I have never obtained them, either under comparable or under different conditions. Even from mixed solutions of the two polymers each crystallizes in its own habit, which shows that an essential characteristic of the polymers themselves is involved, and not some extraneous influence like that of an additive. This characteristic might be the nature of the end groups.

I thank Miss G. Abels for obtaining the electron-micrograph.

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Regular Inclusion Pattern in Crystals

INCLUSIONS containing mother liquor are found quite commonly in many natural and manufactured crystals. Usually they seem to be at random positions within the body of the crystal. However, in the case of hexamine (hexamethylene tetramine) the inclusions often form a regular pattern which is related to the crystal symmetry. One of us has commented on this effect previously[1]; but we give below what we believe to be the first photograph.

Hexamine crystallizes from aqueous solution as regular dodecahedra although often with rounded faces. In the present instance the crystals were obtained by evaporating solution under vacuum at 40°–60° C with occasional stirring. Many crystals in the batch showed the same symmetrical pattern of inclusions, as in Fig. 1*a*, each pattern consisting of twelve members. These have spatial positions corresponding to the centres of the faces of a regular dodecahedron aligned with its faces parallel to the crystal

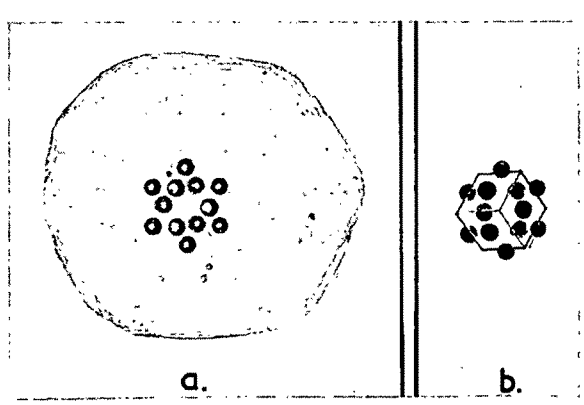


Fig. 1

faces. This may be seen by comparing Fig. 1*a* with Fig. 1*b*, which is a photograph of a 'Perspex' dodecahedron with spheres at the centres of each face.

The crystal photographed was about 315μ across and the inclusions were about 20μ across. The regularity of their pattern suggests that the mechanism of their formation is closely linked to the crystal symmetry and work is in progress to elucidate this mechanism.

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METALLURGY

Electrochemical Properties at the Curie Point

Some preliminary work with gadolinium (Johnson, Matthey and Co., Ltd.: Curie point, 16° C) has shown that this metal exhibits maximum electropositivity in the curie point region (Fig. 1).

The apparatus used consisted of an *H*-cell and each limb was connected by a closed and ungreased tap. The limbs and connexion were filled with the appropriate electrolyte. Each limb was provided with a similar electrode, thermometer and temperature control, one limb (the reference electrode) being kept at 21° C. The *H*-cell e.m.f. was measured directly with a 'Vibron' electrometer.

The selection of electrolyte, and preparation and ageing of electrodes, were governed by the requirement that a reproducible and steady cell e.m.f. should be quickly attained under standard conditions. It is emphasized that, in this type of comparative work, steady-state potentials (whether mixed or not) can be used without recourse to fulfilling stringent thermodynamic requirements for the sake of obtaining equilibrium or standard electrode potentials[1]. When a temperature difference exists between the two limbs, or half-cells, then it can be shown that the plot of *H*-cell e.m.f. versus temperature should be smooth. This smooth or normal behaviour was exhibited only by the copper, zinc and Monel electrodes.

Thus the electrolyte used for gadolinium was N potassium chloride + hydrochloric acid (*p*H 5) and a small amount of hydrogen evolution occurred in both half-cells.

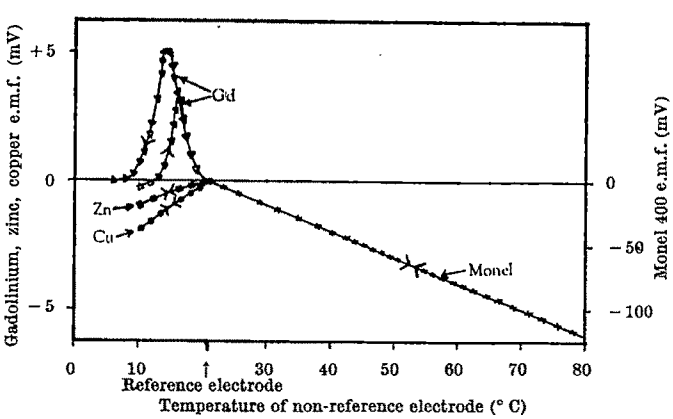


Fig. 1. Graph showing plot of *H*-cell e.m.f. versus temperature of non-reference half-cell for various metal-electrolyte systems. The sign of the e.m.f. is that of the non-reference electrode and, for the purpose of clarity, the plots have been confined to temperatures: (*a*) below 21° C for gadolinium, zinc and copper and (*b*) above 21° C for Monel 400

Fig. 1 also shows the results obtained using: (*a*) zinc, in N potassium chloride + hydrochloric acid (*p*H 5)—a system similar to gadolinium since very slow hydrogen evolution occurred; (*b*) copper, in N copper sulphate + N sulphuric acid—a system where no hydrogen evolution occurred; (*c*) Monel 400 (Henry Wiggin and Co., Ltd.) in N potassium chloride + hydrochloric acid (*p*H 1)—this alloy possesses a Curie point in the range 45°–60° C, but no 'gadolinium effect' was observed.

The gadolinium effect suggests that the rearrangement of 4*f* electron energy-levels at the onset of ferromagnetism affects also the valency electrons. The factors contributing to the e.m.f. of the type of cell used in these experiments which are affected by the ferromagnetism of one electrode are the work function φ and sublimation energy *S* (ref. 2). Changes in these quantities $\Delta\varphi_f$ and ΔS_f due solely to ferromagnetism can be estimated in the case of a copper–nickel alloy such as Monel, in which there is overlap between the nearly filled 3*d* band and the 4*s* band, on the basis of the collective electron theory of ferromagnetism[3,4]. The dominant term is $-\frac{1}{9}\int_0\sigma^2$ in $\Delta\varphi_f$ where σ is the temperature-dependent relative spontaneous magnetization and $\int_0$ the common Fermi energy of 3*d* and 4*s* electrons below the top of the 3*d* band at absolute zero (where ferromagnetism is absent). Using Wohlfarth's data[4] the net effect on the cell e.m.f. can be estimated to be for Monel 0·011 $(1 - T^2/\theta^2)$ V, where *T* is the absolute temperature and θ the Curie temperature, and in such a sense as to make the ferromagnetic electrode more electropositive. This effect is so small in Monel (due to a small $\int_0$) that no change in slope at θ is evident in the e.m.f. versus temperature graph.

The gadolinium effect, though complicated by hysteresis and possibly by a short-range order effect above θ, could perhaps be explained by a similar approach if more were known of the density of states function of the half-filled 4*f* band and the extent of overlap with the valency electron bands. However, in order to establish any mechanisms, further work is clearly necessary, but it is believed that the electrochemical approach may be useful in exploring the difficult subject of ferromagnetism.

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Epitaxial Oxide Growth on Thin Single-crystal Gold – Nickel Alloy Films

The oxidation characteristics of binary alloys and the growth and structure of oxidation products on such alloys have been examined by various investigators[1–3]. However, the only published evidence on the oriented growth of such oxidation products is that of Honjo[3]. He has reported on selective oxidation of beryllium in a beryllium-copper alloy and observed that the BeO phase grows with a preferred orientation in which the [00,1] axis is perpendicular to the free surface of the specimen. The origins of this orientation were not explained. This communication describes a novel orientation effect observed in the oxidation of thin epitaxed films of a noble metal alloy containing a small percentage of an easily oxidizable transition metal. The alloy examined was a gold–nickel alloy containing 5 wt. per cent of nickel. The growth and structure of the alloy and its oxide were investigated by transmission and reflexion electron diffraction techniques.

Gold–nickel alloy films were prepared by cathodic sputtering and also by vacuum evaporation. Cathodic sputtering has been shown by us to have advantages over vacuum evaporation as a preparative technique in that the source material is transferred to the film without change in composition[4]. The gold–nickel alloy films, usually 600–700 Å in thickness, were epitaxially grown on cleaved surfaces of rocksalt and mica maintained at 440° C, after preheating to 500° C. The orientations observed in the alloy films were:

(100) alloy‖(100)NaCl; [011] alloy‖[011] NaCl
(111) alloy‖(001)mica; [1$\bar{1}$0] alloy‖[100] mica

Fig. 1 shows an electron diffraction transmission pattern from a (100) gold–nickel alloy film, epitaxially grown on sodium chloride and then stripped from it for observation. The pattern shows that the film consists of a single phase with the face-centred cubic structure. The lattice constant of the alloy is found to be 4·02 Å using that of gold ($a_0 = 4{\cdot}078$ Å) as a reference. This is in good agreement with Fukano[5], who prepared gold–nickel alloy films by simultaneous deposition of the constituent metals of the alloy.

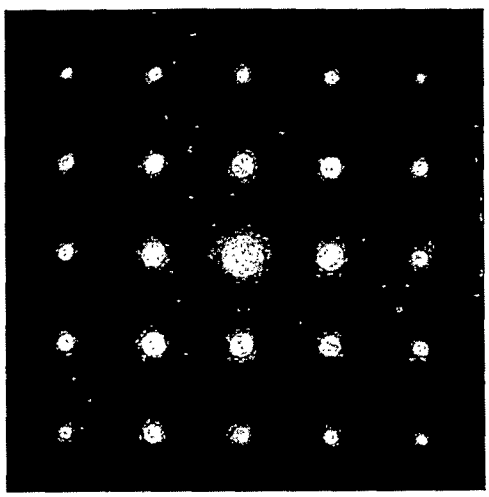


Fig. 1. Diffraction pattern from a (100) gold–nickel alloy film

The single crystal alloy film was first homogenized in argon–hydrogen at 390° C for several hours and then oxidized at about the same temperature either in air or in oxygen atmosphere for various lengths of time from a few minutes to several hours. The oxidation product, which could be identified as nickel monoxide, was observed to crystallize with the rocksalt structure. The lattice constant of NiO was calculated to be 4·17 Å, using that of gold ($a_0 = 4{\cdot}078$ Å) as a standard. The oxidation of the single crystal gold–nickel alloy films resulted in the formation of a double-layered structure: (100) nickel monoxide on (100) gold. The parallelism between the oxide and gold lattices is illustrated in Fig. 2, which is a transmission pattern obtained after oxidizing the alloy film in oxygen atmosphere for 45 min at 390° C. The photograph shows two spot patterns—one corresponding to gold and the other (inner spots, slightly arced) to nickel monoxide. The degree of orientation in the oxide layer was observed to depend on the atmosphere in which the oxidation occurred. The orientation was more pronounced in oxygen than in air. Similar orientation effects were obtained for gold–nickel films epitaxed on mica, and afterwards oxidized.

The oxidation of a one-phase binary alloy, of which one component is a noble metal, is the simplest example of alloy oxidation. In this case one is concerned presumably only with the oxidation of the base metal and the diffusion process by which it reaches the reaction site. It may be envisaged that as oxidation proceeds oxygen is dissolved at the surface of the gold–nickel alloy, and owing to concentration gradients, nickel from the alloy diffuses out and reacts with the adsorbed oxygen to form nickel monoxide, while gold remains inert. The orientation we have observed in the nickel oxide film might be explained on the basis of the diffusion of nickel atoms through the single-crystal gold–nickel alloy structure. Moreover, the parallel epitaxial relationship which develops between the oxide and the metal is plausible on considerations of misfit, which for (100) nickel monomide on (100) gold is very small ($\sim +2$ per cent).

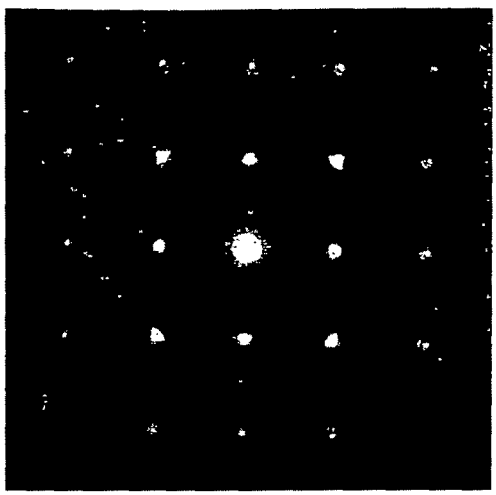


Fig. 2. Diffraction pattern from a (100) gold–nickel alloy film after oxidation

Further investigations on the thermodynamics of the oxidation process in these films are in progress and the results will be described in detail in a separate publication.

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GEOCHEMISTRY

Experimental Data bearing on the Role of Liquid Immiscibility in the Genesis of Carbonatites

The recognition of low-temperature melts in the system $CaO—CO_2—H_2O$[1] has opened a new field of heterogeneous phase equilibrium studies in systems containing both water and carbon dioxide, the volatiles most abundant in the Earth's crust and among the most important in igneous and metamorphic processes. Liquids in this system may be regarded as simplified carbonatite magmas. The petrogenetic relationships between carbonatite magmas and the associated alkaline rocks can be examined by determining the phase relationships in systems containing $CaO—CO_2—H_2O$ and silicate minerals. In a programme supported by the National Science Foundation, we have been investigating selected portions of complex systems in order to test the feasibility of the petrogenetic relationships which have been proposed on the basis of petrological investigations.

Two of the hypotheses which have been proposed for the origin of magmatic carbonatites are: (1) Carbonatite magmas are primary, possibly extremely rich in alkali carbonates[2], and the alkalis in the parent carbonatite magma are responsible for fenitization. Attention has been directed to this hypothesis by the recent volcanic

eruption in Tanganyika which produced a lava flow composed almost entirely of carbonates of sodium and calcium[3]. (2) Magmatic carbonatite is formed at a late stage in the crystallization of a peralkaline magma as a precipitation product of alkali–alkaline earth exchange reactions. Tomkeieff[4] postulated that sodium carbonate within the magma reacted with "shadow mineral molecules" to produce reactions of the type: anorthite + sodium carbonate ⇌ nepheline + calcium carbonate. Phase relationships determined in parts of the system CaO—Na_2O—Al_2O_3—SiO_2 in the presence of carbon dioxide and water under pressure have bearing on both these hypotheses.

The phase fields intersected by the join $NaAlSi_3O_8$(Ab)—Na_2CO_3 in the presence of 10 weight per cent of water have been delineated in the temperature-range 600°–950° C at a total pressure of 1 kilobar[5]. The results are illustrated in Fig. 1. In the presence of excess water at 1 kilobar pressure albite melts at 910° C. Addition of sodium carbonate lowers the saturated liquidus temperature to 730° C, with the solution of about 18 weight per cent sodium carbonate in the liquid. This liquid quenches to a glass. At temperatures above about 750° C (for this pressure and for this composition join) there exists a wide miscibility gap between the silicate liquid (L_1) and a liquid containing at least 90 weight per cent sodium carbonate (L_2). The second liquid does not quench to a glass. The solidus temperature is 690° C.

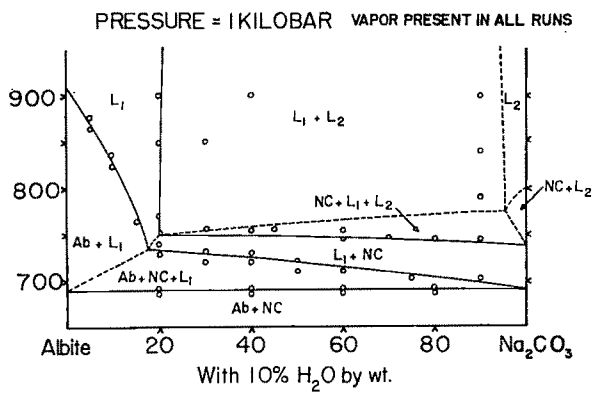


Fig. 1. Phase fields intersected by the composition join $NaAlSi_3O_8$—Na_2CO_3—H_2O (10 weight per cent) at 1 kilobar pressure. Albite, Ab; Na_2CO_3, NC; liquids, L_1 and L_2

The phase relationships in this composition 'join' form a necessary basis for interpretation of phase relationships in the more complex system involving anorthite ($CaAl_2Si_2O_8$, An) as well. We have recently determined the phase fields intersected by the silicate end of the join 80Ab20An—sodium carbonate in the presence of 10 weight per cent of water. The results obtained prove that the miscibility gap persists between the silicate and carbonate melts in the lime-bearing system, although it appears to be somewhat smaller. Several additional phase fields are intersected by this composition join, involving the phases plagioclase feldspar, nepheline, wollastonite, a cancrinite, a mineral with the sodalite structure, and calcite. Details of the experimental method, the phase relationships, and the evidence for the existence of two liquids will be published in due course. However, it is of interest at this stage to consider the bearing of these results on the petrological hypotheses outlined here.

In connexion with the first hypothesis, it is difficult to understand how a deep-seated primary magma composed largely of alkali carbonates could form in the silicate mantle of the Earth. Furthermore, if such a magma does develop, it is difficult to understand how it could persist until it reached a high level in the Earth's crust without the occurrence of almost complete reaction with the silicates of the crust. An immiscibility relationship between the alkali carbonatite magma and the silicates is a possible explanation. The lava flows from Oldoinyo Lengai in Tanganyika, whatever their origin, managed to reach the surface without becoming contaminated with silicates[3]. Again, it is difficult to imagine how such a reactive melt could persist unless there is a miscibility gap between the carbonate liquid and silicate liquids.

The appearance of calcite as a crystalline phase in equilibrium with liquids in the join 80Ab20An—sodium carbonate—water indicates that the exchange reaction postulated in the second hypothesis does occur in melts of appropriate compositions. The solution of sodium carbonate in the hydrated plagioclase feldspar melt leads to the development of several new mineral phases, and nepheline and calcite are among them, as predicted by Tomkeieff[4]. The nature of the exchange reactions occurring between alkalis and basic oxides is of fundamental importance not only for the origin of carbonatites and associated alkaline rocks, but also for many other petrogenetic problems.

It is worth emphasizing the fact that the two liquids illustrated in Fig. 1 are true melts. These liquids coexist with a third fluid phase (vapour), which is a dense aqueous solution containing a small proportion of dissolved silicate and an unknown proportion of sodium carbonate.

Of course, much experimental work remains to be completed before direct comparisons can be made between the synthetic systems and the natural rocks. However, the recognition of immiscibility between silicate melts and carbonate melts in a system with an alkali and a basic oxide present as components does direct attention to the possible significance of liquid immiscibility in the genesis of carbonatites and associated igneous rocks.

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CHEMISTRY

Ion Specific Polymer Membrane

Membranes, the permeability of which is based on complexing, are of interest because of their analogy to biological membranes. In the lipoid theory of permeability it is assumed that the permeating species dissolves in the cell membrane by complexing with it. Apart from their biological significance, synthetic membranes of this kind can be expected to be useful because their selectivity is far greater than that of any modified ion-exchange membrane. An example of a modified ion-exchange membrane, with an increased capacity for iron (III), has recently been described by Kargin[1]. His membrane consisted of a copolymer of an electrolyte, acrylic acid, and a complexing agent, the dibutyl ester of vinyl phosphonic acid. However, no uncharged membrane has yet been described the permeability of which is due solely to complexing.

We have recently prepared several membranes which, by virtue of selective complexing, were able to effect complete separation of a mixture of iron (III) and aluminium chlorides in strong hydrochloric acid solution. This was achieved either by dialysis or by electrodialysis. Under strongly acid conditions iron (III) is known to exist as a negatively charged chloro-complex which can be extracted by various organic solvents[2]. The extraction is explained by simultaneous solvation as well as hydration of the complex anion[3]. No similar complex of aluminium is formed under these conditions.

We tested several solvents as 'liquid membranes'. 'Liquid membranes' consisted of a porous film of polyvinyl chloride soaked with one of the solvents. All those tested turned out to be completely impermeable to aluminium chloride, but were permeable in varying degrees to iron chloride. The permeability properties of these membranes are best characterized by their resistance and transference number in an electrodialysis experiment. In the case of β,β′-dichlorodiethyl ether a resistance of 3×10^3 ohm cm² was measured in a solution which was 1·5 M in aluminium chloride, 0·3 M in ferric chloride and 1·5 M in hydrochloric acid. A resistance of 10^6 ohm cm² was measured in a solution containing the same concentration of aluminium chloride and hydrochloric acid, but without iron chloride. The average transference number of the $FeCl_4'$-ion in an electrodialytic separation of this mixture was 0·45; this means that, with the passage of 1 faraday, 0·45 mole of iron was transported through the membrane to the anode. The transference number of the $FeCl_4'$-ion in an experiment in which an ordinary ion-exchange membrane was used was 0·02, due to the large excess of chloride ion present in the system. In a diffusion experiment on the same system the transport of iron through the membrane was 10^{-6} mole/(h cm²). No transport of aluminium through the membrane occurred. The diffusion process was highly dependent on the concentration of hydrochloric acid. In the same mixture of iron (III) and aluminium chlorides but at a three times higher concentration of hydrochloric acid, values of the diffusion transport as much as 30 times larger were observed.

As analogues of these 'liquid' membranes we synthesized polymer membranes the monomer units of which showed the greatest possible similarity to the solvents previously tested. The analogue of β,β′-dichlorodiethyl ether synthesized was polyvinyl chloroethyl ether. A membrane of this polymer reinforced with glass fibres possessed considerable permeability for iron, but was impermeable to aluminium. Its resistance measured in the same mixture of iron and aluminium chloride was 10^4 ohm cm². Its transference number in an electrodialytical experiment was 0·34. The diffusion transport through the membrane was 5×10^{-7} mole/(h cm²).

The transport behaviour of ferric, aluminium and cupric ion across alginate membranes has been examined by Seno *et al.*[4]. Seno *et al.* expected an alginate membrane to show selective transport for ferric ion, since alginic acid resins preferentially adsorb ferric ion. However, they found the opposite, indicating, in our opinion, that the complex formed between the metal-ion and the membrane matrix was too strong.

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Binding of the Thiocyanate Ion in Complexes with Palladium (II)-containing Tertiary Phosphines

The infra-red spectra of several thiocyanate complexes of platinum and palladium suggest that these metals must be bound to nitrogen in compounds with the general formula $[M(PR_3)_2(CNS)_2]$ (R = aliphatic tertiary phosphine)[1]. Adams and Booth more recently reported that platinum must be co-ordinated to nitrogen in the complexes *trans*-$[Pt(CNS)CH_3(PEt_3)_2]$ and *trans*-$[Pt(CNS)(COCH_3)(PEt_3)_2]$ (ref. 2). On the other hand, palladium and platinum invariably appear to be bound through sulphur in a variety of complexes with the thiocyanate ion and other different ligands[3]. It thus appears that the binding of these metal ions with the thiocyanate group is peculiarly affected by the presence of phosphine ligands in the molecule of the complex. The occurrence of this unexpected nitrogen-metal binding may be of more general interest, being clearly related to important changes in the binding properties of the metal ions brought about by the presence of the phosphine ligands. However, all the previous evidence on which these conclusions were based came exclusively from infra-red spectra. A more direct demonstration of the nature of the metal-thiocyanate bond seemed desirable to us. Therefore, a determination by X-ray analysis of the structure of the compound $[Pd(PPr_3^n)_2(CNS)_2]$ was undertaken to resolve the question of the bonding of the thiocyanate group.

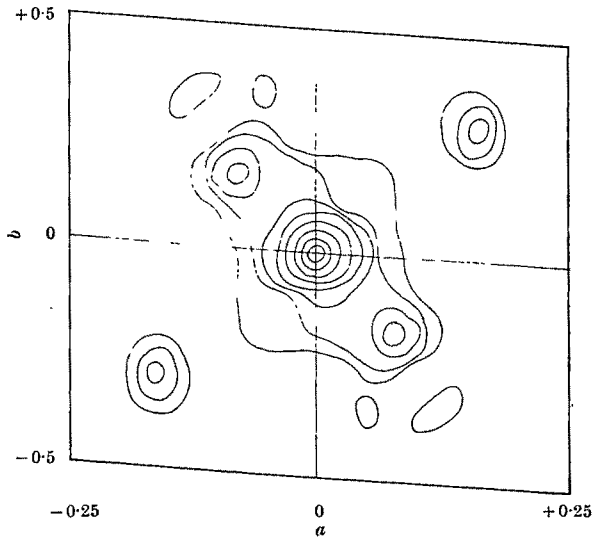


Fig. 1. Patterson projection on (001) for $[Pd(PPr_3^n)_2(NCS)_2]$. Contours at arbitrary but equal intervals

The compound crystallizes in the triclinic system. The space group may be either the $P1$ or $P\bar{1}$. The centrosymmetric space group has been chosen on the basis of intensity statistics. The unit cell has the following constants $a = 19{\cdot}53$ Å; $b = 8{\cdot}72$ Å; $c = 9{\cdot}36$ Å; $\alpha = 87°\ 58'$; $\beta = 119°\ 04'$; $\gamma = 94°\ 12'$ and contains two molecules of complex. The $hk0$ and $h0l$ reflexions were recorded on Weissenberg equatorial photographs. The intensities were determined by means of photometric integration. From the vector analysis of the Patterson projections on (001) and (010) it can be concluded that the molecule is square *trans*-planar and that the complex is actually an isothiocyanate with nitrogen atoms co-ordinated to palladium. The projection on (001) is shown in Fig. 1. There are four Patterson maxima arising from the relatively heavy phosphorus and sulphur atoms. Two of them are at 4·6 Å and therefore cannot represent the palladium-phosphorus distance. Since the Pd–NCS grouping must be approximately linear this must be the palladium-non-bonded sulphur distance expected for Pd–NCS units. It can be compared with the distance 4·8 Å between platinum and non-bonded sulphur in $[Pt_2(SCN)_2Cl_2(PPr_3)_2]$[4]. The other two maxima are at 2·2 Å in agreement with the expected palladium-phosphorus distance (2·16 Å in the platinum compound[4]).

These results show that the conclusions derived from the infra-red spectra were correct. The reasons for the change from the usual Pd–SCN co-ordination to the Pd–NCS co-ordination are not apparent at present. With metals of the first transition series, tertiary phosphines apparently affect the metal-thiocyanate bonding in a more complicated way. Cobalt (II), which is usually co-ordinated through nitrogen, is reported to be bound to sulphur in $[Co(PPh_3)_2(SCN)_2]$[5] and to be bound to nitrogen in $[Co(PCy_3)_2(NCS)_2]$ (Cy = *cyclo*hexyl)[3]. It is interesting to

note that in the complex $[Pd(PPh_3)_2(NCS)_2]$ the palladium atom must be bound to nitrogen as it is in the compounds with aliphatic phosphines. This is clearly shown by the value of the CS stretching frequency (850 cm^{-1})[6].

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Dissociation Energies of the Alkaline Earth Monofluorides

Although the gaseous alkaline monofluoride molecules have been known for many years[1], the stabilities of these molecules have not been accurately established. The Birge–Sponer extrapolations which Gaydon[2] used to arrive at dissociation energies are in doubt because of the limited number of vibrational levels used and the fact that the data were often based on head measurements of bands of a weakly degraded system. Furthermore, these extrapolations are not expected to be normal for such molecules in which ionic forces make an appreciable contribution to the binding. This communication presents new results and theoretical calculations which indicate consistently high dissociation energies for all the alkaline earth monofluorides.

Experimental results were obtained by utilizing a 12-in., 60° sector, single-focusing mass spectrometer to analyse the vapours effusing from a heated tantalum Knudsen cell containing an alkaline earth fluoride salt with aluminium present as a reducing agent. Ionization efficiency curves were obtained and temperature dependence investigations were made to determine the molecular precursor of the ions observed.

Fragmentation effects were minimized by working at low ionizing electron energies. In the case of magnesium fluoride, MgF^+ was found to be formed from both $MgF(g)$ and $MgF_2(g)$. The $MgF(g)$ contribution was determined from the ion intensity of MgF^+ at 5 V above the AP (MgF^+) from MgF, while the MgF_2 contribution was calculated from the MgF^+ intensity 5 V above the AP (MgF^+) from MgF_2 by subtracting the MgF^+ from MgF intensity extrapolated to this same ionizing energy, assuming the cross-section for ionization to be a linear function of the energy above onset. Pressures of the gaseous species used in the equilibrium constants for the various reactions were calculated by making use of the ion current-pressure relationship[3], $P = kI^+T$.

Free energy functions for $Ca(g)$, $Sr(g)$ and $Ba(g)$ were taken from Stull and Sinke[4]; for $Al(g)$, $AlF(g)$, $Mg(g)$, $MgF(g)$, $MgF_2(s)$ and $MgF_2(g)$ from the *JANAF Tables*[5]; for $CaF_2(s)$, $SrF_2(s)$ and $BaF_2(s)$ from Brewer *et al.*[6]; and for $CaF(g)$, $SrF(g)$ and $BaF(g)$ from Kelley[7] and Kelley and King[8]. The values of the equilibrium constants at different temperatures were then used together with the free-energy functions to calculate the third law heats of reaction at 298° K which are presented in Table 1. The uncertainties given are standard deviations, and the true uncertainties may be somewhat greater. Whenever possible, these heats were checked by the second law method, but the third law values are expected to be more reliable due to the small temperature range covered (about 100° K) and the low intensities of some of the ion currents measured.

From reaction (1), $D^0_{298}(MF)$ may be obtained by combining the observed ΔH^0_{rxn} with the known value (157·7 ± 2·0 kcal/mole) of $D^0_{298}(AlF)$ [5,9]. From reaction (2), $D^0_{298}(MF)$ may be obtained by combining the observed ΔH^0_{rxn} with $\Delta H^0_f(MF_2,s)$ [6,10], $\Delta H^0_{sub}(M)$ [4] and $D^0_{298}(F_2)$ [5]. From reaction (3), $D^0_{298}(MgF)$ may be obtained by combining ΔH^0_{rxn} with $\Delta H^0_f(MgF_2,s)$ [10], $\Delta H^0_{sub}(MgF)_2$ [11], $\Delta H^0_{sub}(Mg)$ [5] and $D^0_{298}(F_2)$ [5]. The results of these calculations are presented in the first two rows of Table 2.

When the difference, $IP(M) - EA(F)$, is small, the ground-state potential energy curve for the MF molecule will be primarily determined by the coulombic attraction of the M^+ and F^- ions. One useful rule[1] for deciding when ionic character will predominate is $R = r_s/r_e \geqslant 1{\cdot}5$, where r_s is the internuclear distance at which electrostatic attraction has just compensated for the energy $IP(M) - EA(F)$, and r_e is the equilibrium separation. Values of R for each of the MF molecules were calculated using experimentally measured values of r_e when known and addition of ionic radii for SrF and BaF [12]. The ratios obtained were $R(BeF) = 1{\cdot}82$, $R(MgF) = 1{\cdot}99$, $R(CaF) = 2{\cdot}73$, $R(SrF) = 3{\cdot}09$ and $R(BaF) = 3{\cdot}79$. Therefore, all the alkaline earth monofluoride molecules, with the possible exception of BeF, should be expected to have an ionic ground state. It should be noted here that the spectroscopically observed[1] ground states ($^2\Sigma^+$) of these molecules may be correlated with the states of the ions: $M^+(^2S) + F^-(^1S)$.

A calculation of $D(MF)$ was then made using Rittner's[13] ionic model. To do this, an estimate of the metal ion polarizability was made using the equation[14] $\alpha = 3\bar{r}^2/2I_2$, and $\bar{r}^2$ was obtained from the prescription of Hirschfelder *et al.*[15]. The polarizabilities used were $\alpha(Be^+) = 0{\cdot}84A^3$, $\alpha(Mg^+) = 2{\cdot}2A^3$, $\alpha(Ca^+) = 6{\cdot}0A^3$, $\alpha(Sr^+) = 8{\cdot}6A^3$ and $\alpha(Ba^+) = 11{\cdot}3A^3$. The resulting values of $D(MF)$ (Table 2) have the expected trend, and are in good agreement with the experimental results, especially when the heats of ionization, the actual quantities calculated, are compared.

Comparison of the atomization energies of the monofluorides and the difluorides[6] yields an average value of $D(MF)/D(MF_2) = 0{\cdot}46$, which does not agree with the

Table 1. Heats of Various Reactions for the Al–MF_2 Systems

Reaction	ΔH^0_{298} (kcal/mole) for M = Mg	Ca	Sr	Ba
(1) $M(g) + AlF(g) = Al(g) + MF(g)$	(⩾ 45·2)	32·7 ± 0·2	33·1 ± 0·3	23·9 ± 0·2
(2) $M(g) + MF_2(s) = 2MF(g)$	128·2 ± 0·8	120·7 ± 0·8	116·0 ± 0·8	97·9 ± 0·4
(3) $Mg(g) + MgF_2(g) = 2MgF(g)$	37·8 ± 1·0	—	—	—

Table 2. Dissociation Energies of MF Molecules by Various Methods

Method	D^0_{298} (MF) in electron volts for M = Be	Mg	Ca	Sr	Ba
Homogeneous reaction (1 or 3)	—	4·60 ± 0·06	5·44 ± 0·10	5·45 ± 0·10	5·80 ± 0·09
Heterogeneous reaction (2)	6·29*	4·50 ± 0·04	5·41 ± 0·12	5·45 ± 0·10	5·82 ± 0·10
Ionic model	5·10	4·21	5·14	5·36	5·74
Gaydon†	4·0 ± 1·0	3·2 ± 0·7	3·2	2·7 ± 1·0	3·0 ± 1·0

* Calculated from Be(*s*, 1) + $BeF_2(g)$ = 2BeF(*g*), M. Farber *et al.*, Rocket Power, Inc., Pasadena, California. Various reports under USAF Contract No. *AF*04(611)–7414, 1962–63.
† Ref. 2, p. 217.

valence state hypothesis of Skinner[16]. However, the suggestion of Büchler *et al.*[17] that a combination of $Ms^2Fp\sigma$ (non-bonding) and $[M(s+p) + Fp\sigma]^2$ $M(s-p)$ (bonding) structures are involved appears to be in harmony with the experimental D(MF) values and receives additional support from the observations that IP(MF) < IP(M) for M = Ca, Br, Sr, but not for M = Mg.

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Pre-ionization Phenomena in the Appearance of Some Polyatomic Molecular Ions

IT has been shown elsewhere[1-4] how the photo-ionization yield curves of ethylene and of some halogenated derivatives of the ethylene molecule can be interpreted by means of step functions resulting from vibrationally electronically excited states of the molecular ions.

As will be shown elsewhere[4], the photon energy-range used (between the first I.P. and 11·5 eV) enables us to detect in the same curves a number of sharp peaks in addition to the successive step functions characterizing vibronic states of the ions. The comparisons made with the same experimental device between the position in energy of these sharp peaks and the position of strong absorption bands in the C_2H_3Cl far ultra-violet spectrum above the first I.P., correlated with the existence of photographically recorded bands of absorption, in the same region, in the spectra published by A. D. Walsh[5], enabled me to conclude that the sharp peaks observed in photo-ionization yield curves are due to the so-called 'pre-ionization phenomenon'. In this phenomenon, it is possible to assess that the interaction of the light with the molecule results in an excitation to any electronically excited state of the molecule, the energy of which is greater than the value of the first I.P. This excitation is followed by a non-radiative transition to any ionization continuum covering the same energy-range. The characteristic function governing this phenomenon is the excitation function by light of the molecule for the vibronic states concerned. It is, therefore, not surprising that this function is a very sharp one. Similar conclusions have been drawn previously for a similar behaviour as observed in I_2 and CH_3I molecules by J. D. Morrison *et al.*[6]. They have never been generalized and analysed as I try to do here.

Three main causes are to be postulated in order to explain such a behaviour in more complicated molecules; they are: (*a*) excitation of one electron more strongly bonded in the molecule than the electron removed by the lowest energy ionization process; (*b*) simultaneous excitation of two electrons resulting in any excited state of the molecule situated well above the first I.P.; (*c*) excitation of the electron in Rydberg states leading to the appearance of the ion in an electronically excited state.

In order to test hypotheses (*a*) and (*b*), enquiries have been made on the possibilities of exciting in the molecules studied any or several other electrons than the electron removed by the first ionization process. The results of these enquiries are shown in Table 1. Some of these results (the starred ones) have been obtained on the following experimental basis: a smooth curve can be drawn through the values of excitation potentials, namely, I.P. of the excited electron, and the starred values result from interpolations on this curve.

Table 1

Substance	Electron	First pot. (eV)	I.P. (eV)	Absorption	Ref. (Exc.)	Ref. (Ionized)
CH_3I	$d\ \pi$ (I)	6·15	9·50	Discrete	7	8
C_2HCl_3	π (C—C)	6·32	9·47	Discrete	5	9
$I\text{-}2C_2H_2Cl_2$	π (C—C)	6·52	9·66	Discrete	5	1–4
C_2H_3Cl	π (C—C)	6·70	10·00	Discrete	5	4
CH_3Br	$p\ \pi$ (Br)	(7·75)*	10·53	?		8
C_2H_3Cl	$p\ \pi$ (Cl)	8·46	10·89	Discrete	10	9
CH_3Cl	$p\ \pi$ (Cl)	8·85	11·47	Discrete	7	8
C_2H_4	σ (C—C)	(9·00)*	11·65	Continuous		9
CH_3Br	σ (C—Br)	(9·00)*	11·62	?		8
CH_3Cl	σ (C—Cl)	(9·35)*	12·07	Discrete in CH_3Cl_2	11	9
CH_3F	σ (C—H)	9·75	12·85	Continuous	12	9
CH_4	σ (C—H)	9·90	13·16	Continuous	13	9
CH_3F	σ (C—F)	10·58	15·27	Discrete	12	9

Owing to the results presented in Table 1 we are in a position to regard the occurrence of mechanisms like those postulated in (*b*). The simultaneous excitation of two electrons of the types described in Table 1 would require energies well above the 11·5 V characterizing the ultra-violet radiation cut-off in the device used for our experiments. In addition, mechanisms like (*c*) have not been taken into account owing to the distribution of intensities in the pre-ionized peaks, mainly irregular, opposed to the normal distribution of intensity in Rydberg series which is well known to diminish with the convergence of the series.

It would be impossible to give an extensive discussion of all the results in such a short article. We should point out two main results: (*a*) For ethylene itself, as for the numerous hydrocarbon molecules studied by B. Steiner *et al.*[14], no pre-ionization structure is present. This is easily understood on the basis of Table 1. The possible excitations in such molecules result only in continuous absorption; that is to say, if such absorption occurs joining any ionization continuum, it is not able to give discrete peaks in the photo-ionization yield curve but only a continuous variation of the photon yield. (*b*) For all the halogen substituted ethylenes studied here, numerous peaks occur in photon-yield curves. For Table 1 and to the position in energy of the first of these peaks, it is possible to make some assumptions in each case about the excited electron and also to consider the successive peaks as forming the vibrational structure of the absorption band associated with the excited electron. The results obtained in this way are given in Table 2.

The values of the observed frequencies require comment. Frequencies lower than 1,000 cm^{-1} observed in all the molecules studied (except C_2H_3Cl) are probably to be associated with motions involving mainly the carbon and halogen nuclei. As to the frequencies around 1,000 cm^{-1}, the cases of C_2H_3Cl and C_2D_3Cl show a reduction in the frequency due to the deuteration which is only consistent

Table 2

Substance	Pre-ionization threshold (eV)	Excited electron	Associated frequencies (cm^{-1})
C_2H_4	None	—	—
C_2H_3F	10·645	σ (C—F)	1,136 – 327
1–1 $C_2H_2F_2$	10·510	σ (C—F)	1,008 – 484 – 404
C_2H_3Cl	10·050	σ (C—Cl)	1,150
C_2D_3Cl	10·050	σ (C—Cl)	1,030
$C_2H_2Cl_2$ *cis*	10·300	σ (C—Cl)	767 – 685 – 404
trans	10·015	σ (C—Cl)	756 – 620 – 363
C_2HF_3Cl *cis*	10·300	σ (C—Cl) σ (C—F)	1,104 – 722 – 675 – 379
trans	10·205	σ (C—Cl) σ (C—F)	1,073 – 806 – 721 – 556
$C_2H_2Br_2$ *cis*	9·520	σ (C—Br)	766 – 655 – 283
trans	10·037	σ (C—Br)	746 – 530 – 484 – 242

with the existence of the (C—C) valence vibration strongly reduced by the excitation from its value in the ground state (1,623 cm^{-1}), instead of the CHCl bending vibration which is equal to 1,279 cm^{-1} in the ground state[15]. This phenomenon occurs sometimes and I shall refer to the case of CH_3I in which the excitation of a $d\pi$(I) electron results in the apparition of vibrational frequencies related with C—H bond motions[16].

Owing to this discussion, it is difficult to decide for the fluorinated molecules if the presence of a frequency of about 1,000 cm^{-1} is due to the (C—F) valence bond frequency which is around the same value in the ground state or to the π (C—C) valence bond vibration as in the vinyl chloride molecule.

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Possible Use of Gallium as Negative Electrode in Galvanic Cells

From the electrochemical aspect gallium is notable for its relatively negative standard reference voltage, which for the equilibrium :

$$Ga + 4OH^- \rightleftarrows H_2GaO_3^- + H_2O + 3\ e^-$$

amounts to $-1{\cdot}22$ V (NHE)[1]. Apart from its low equivalent weight of 23 g/F, another remarkable feature is the high polarization with respect to hydrogen[2], which results in it having a high resistance to corrosion, in spite of its base character.

Also, the easy anodic dissolution and cathodic deposition of gallium in various electrolytes is well known, that is, it can act in a reversible manner. These properties and particularly the advantages for storage and transport arising out of its low melting point (29·8° C) and the ease with which gallium can be sub-cooled induced us to investigate closely the suitability of gallium as a negative electrode for galvanic primary or secondary cells.

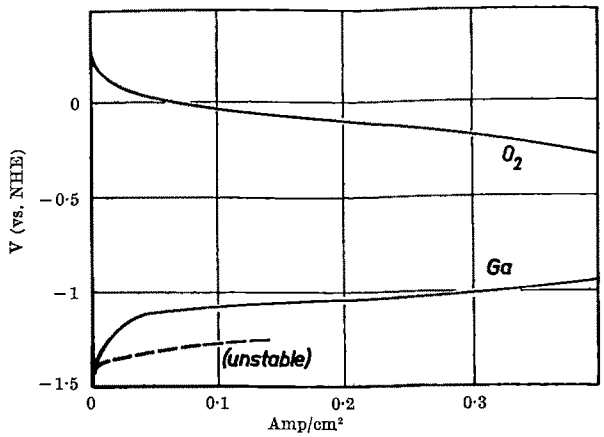


Fig. 1. Characteristic of an oxygen and a gallium electrode in 6 N potassium hydroxide at 80° C

Measurements of the rate of corrosion of pure gallium only yielded low values, even at high temperatures. In a 6 N solution of potassium hydroxide, without any flow of current, the amount of gallium that dissolves corresponds to a corrosion current of about 8 m.amp/cm^2.

Anode characteristics of gallium half-cells were plotted for different electrolytes in the temperature-range 20°–80° C. It was thereby established that in 2 N sulphuric acid the polarization is extraordinarily high (0·6 V at 50° C and 20 m.amp/cm^2), due to the formation of a surface layer of $Ga_2(OH)_4SO_4$. In 8 N hydrochloric acid the polarization was very low (0·1 V at 50° C and 200 m.amp/cm^2).

The anodic behaviour of gallium in 6 N potassium hydroxide was examined more closely, owing to the possibility of combining most cathode materials of conventional cells with the gallium anode in this electrolyte. Here the polarization amounted to 0·66 V at 20° C and 100 m.amp/cm^2 (with solid gallium). At 50° and 80° C and the same current, the respective figures were 0·45 and 0·37 V (Fig. 1). It was established that, in each case, about 0·2 V of the total polarization could be attributed to the drop in voltage across a suddenly formed passive layer on the surface of the gallium[3]. The steady-state potential of the gallium in 6 N potassium hydroxide amounted to about $-1{\cdot}42$ V (NHE). With increasing enrichment of the alkali with gallate, the polarization remained almost constant up to a content of roughly 300 g gallium per litre, after which it began to rise. With anode current densities of 100 m.amp/cm^2 the current yield at 80° C was 100 ± 5 per cent for all gallate concentrations.

The deposition again of the gallium from 6 N potassium hydroxide containing a high proportion of gallate occurred at 100 m.amp/cm^2 and 80° C with a polarization of only 0·05 V and a current yield of about 90 per cent. Only

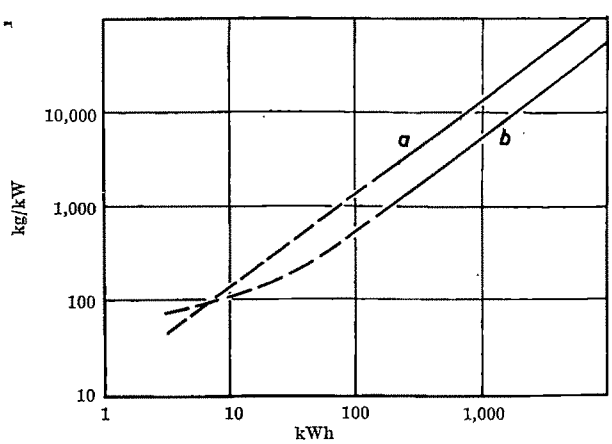


Fig. 2. Gross weight per unit output (kg/kW) of gallium cells as a function of their capacity (kWh)

when the gallate concentration dropped below 50 g gallium per litre did the polarization rise to 0·3 V and the current yield about 50 per cent.

Fig. 1 shows the anode characteristic of a gallium electrode together with the cathode characteristic of porous silver operating as oxygen electrode[4] (electrolyte 6 N potassium hydroxide at 80° C) from which the current-voltage characteristic of the primary cell Ga|6 N KOH|O_2, Ag can be deduced.

Based on rough estimates the gross weight per unit output (kg/kW) of 1-kW units of gallium-consuming galvanic cells is plotted against the visualized capacity (kWh) in Fig. 2. Curve *a* applies to the primary cell Ga |6 N KOH|O_2 (stored under pressure), silver with a cell voltage of 1·0 V at 100 m.amp/cm². Curve *b* refers to the secondary cell Ga|6 N KOH|NiO, Ni. The cell voltage was assumed to be 1·4 V at 100 m.amp/cm².

As the shape of the curves shows, the primary cell is superior to the secondary at higher capacities. The curves are both somewhat uncertain in the region of small capacities. The reason for this is that, so far, no suitable technical solution has been found to the question of the design of the cells, especially for the anodes of liquid gallium. Technical difficulties of this kind and especially the high price of the gallium therefore tend to stand in the way of the use of gallium for the electrochemical generation and storage of electricity at present.

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BIOCHEMISTRY

Alteration of the Biochemical Effects of Ethidium Bromide by Exposure of Cells to Hypotonic Media

Ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide) has been shown to inhibit completely the incorporation of preformed purines (adenine, guanine and hypoxanthine) into nucleic acids in Ehrlich ascites tumour cells *in vitro*, but does not appreciably impair the incorporation into nucleic acids of purines synthesized *de novo*[1]. This specific effect on preformed purine metabolism is not due to inhibition of their entry into these cells, conversion to nucleotides, or phosphorylation to nucleoside di- and tri-phosphates. Thus the purine nucleotides derived from exogenously supplied preformed purines are differentiated from those synthesized via the *de novo* pathway of purine nucleotide biosynthesis by this action of ethidium bromide. This suggests that these two types of purine nucleotides—chemically identical but derived from different sources—are separated within the cell. The nucleotides synthesized from exogenously supplied purines must mix with those made *de novo* at some point prior to their polymerization into nucleic acids, and it has been suggested that ethidium bromide interferes with this mixing process, which may be considered to be a movement of nucleotides from one compartment into another[1].

These results are most easily understood if ethidium bromide also is 'compartmented' within the cell and has access to only one compartment. The alternative to the postulated mixing of purine nucleotides prior to polymerization into nucleic acid would seem to be the existence of duplicate sites of nucleic acid polymerizing systems which could distinguish their chemically identical substrates by their source. Consequently, the synthesis of nucleic acid molecules by these enzymes would be dependent on the nutrition of the cells. This seems unlikely.

This intracellular compartmentation of purine nucleotides is most easily understood if there be some structural unit within the cell which separates the compartments. It was reasoned that if this barrier could be distorted or destroyed, ethidium bromide might either lose its inhibitory effects on preformed purine metabolism, or might now also inhibit the metabolism of purine nucleotides synthesized *de novo*. In order to distort cell structure in as gentle a manner as possible, cells were incubated in media of varying osmotic pressure. Using isotonic calcium-free Krebs–Ringer phosphate medium as a standard, hypotonic media having 90, 75 and 50 per cent the osmolarity of the standard medium were prepared by dilution of the standard medium with distilled water. The effect of 0·25 mM ethidium bromide on the incorporation of radioactive adenine into nucleic acids and of radioactive glycine into nucleic acids and proteins in Ehrlich ascites tumour cell was then tested when both drug and substrate were added simultaneously and the cells incubated for 1 h. Incorporation of radioactivity into macromolecules was measured by the membrane filtration technique, as previously described[1].

Control flasks without drug were incubated in media which were 100, 90, 75 and 50 per cent of the normal (isotonic) osmotic pressure. Adenine incorporation into nucleic acids at 90 and 75 per cent of normal osmotic pressure was slightly and progressively decreased, while that at 50 per cent was markedly depressed (Fig. 1). The slight diminution in the incorporation of glycine at 90 and 75 per cent was similar to that observed with adenine (Figs. 2 and 3). Although 50 per cent of normal osmolarity reduced incorporation of glycine markedly, this inhibition was not quite so profound as that of adenine incorporation.

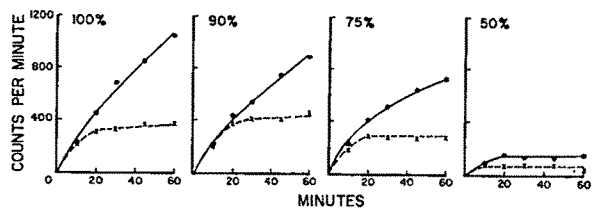


Fig. 1. Effect of hypotonic media on the inhibition of adenine incorporation into nucleic acid by ethidium bromide. Tumour cells, 24 mg wet weight, were incubated in calcium-free Krebs–Ringer phosphate media, *p*H 7·4, which were adjusted to 100, 90, 75 or 50 per cent the normal osmolarity of the media, with 5·5 mM glucose, 0·1 mM adenine-^{14}C, and 0·25 mM ethidium bromide

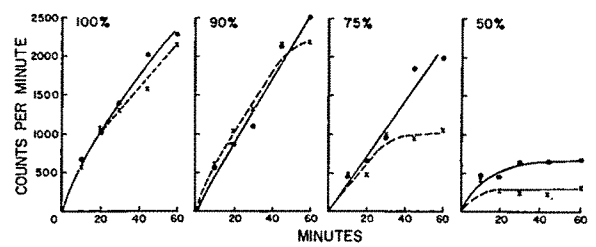


Fig. 2. Effect of hypotonic media on the inhibition of glycine incorporation into nucleic acids by ethidium bromide. Tumour cells were incubated as described in Fig. 1, but with 2 mM glycine-^{14}C

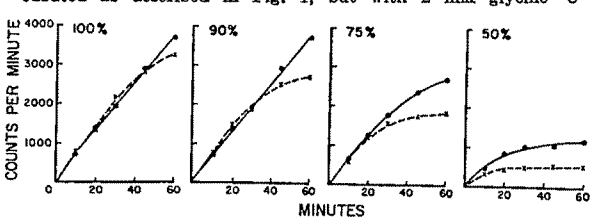


Fig. 3. Effect of hypotonic media on the inhibition of glycine incorporation into proteins by ethidium bromide. Tumour cells were incubated as described in Fig. 2

The usual inhibition by ethidium bromide of adenine incorporation into nucleic acids was not appreciably altered by hypotonic media, when due allowance was made for the effects of hypotonicity on control rates (Fig. 1). Even the drug's effect in 50 per cent hypotonicity was almost as great as that of cells in isotonic media.

In contrast to these results, however, hypotonicity markedly increased the inhibitory effect of ethidium bromide on glycine incorporation both into nucleic acids and into proteins (Figs. 2 and 3). The inhibitory effect increased with increasing hypotonicity, and the plots of these data became similar in shape to those seen with the radioactive adenine. These results appear to indicate that the swelling of the cells in hypotonic media destroyed or diminished the basis for the compartmentation of purine nucleotides and of drug postulated here.

Unsuccessful attempts were then made to localize and identify the specific basis for compartmentation. Attempts to localize the drug within the cells by conventional sub-cellular fractionation indicated that the drug was found more or less uniformly in all fractions. Fluorescence microscopy of whole cells incubated with ethidium bromide under different osmotic pressures was also attempted, but, under all conditions tried, fluorescence attributable to the drug was found uniformly in all parts of the cell.

Incubation of ascites tumour cells in hypotonic media has thus markedly altered the biochemical effects of ethidium bromide, which under these conditions does not distinguish between the two types of purine nucleotides. These data may be interpreted in the following ways: that the lowered ionic strength allowed some new action of ethidium bromide; that the target of its attack was now susceptible to inhibition; or that the drug was now able to be present at a locus which it previously had not reached in sufficient concentration. The latter interpretation seems most likely, and rupture of an intracellular barrier during the swelling of the cells would allow the drug to attain inhibitory concentrations at new loci. Such an occurrence is probably accompanied by a loss of compartmentation of purine nucleotides; but no direct evidence on this point is available. These observations cannot be due to an increased ethidium bromide concentration within the cells as a whole, as unpublished experiments have shown that incubation in hypotonic media does not change the total amount of drug entering the cells or the rate at which it enters.

The observations made following sub-cellular fractionations and fluorescent microscopy that ethidium bromide is apparently uniformly distributed within the cells do not necessarily dispute these conclusions. Newton[2] has found that resting cells of the trypanosomid flagellate, *Strigomonas oncopelti*, take up appreciable amounts of this drug and bind it irreversibly, although inhibition of growth is not exerted even when these cells are transferred to fresh medium and grown. Only drug which is taken up while these cells are actually growing produces growth inhibition. Non-specific binding of ethidium bromide within tumour cells could effectively mask any intracellular redistribution in hypotonic media.

These experiments have revealed new facets of the action of ethidium bromide which are consistent with the hypothesis that the drug interferes with a mixing of purine nucleotides from two intracellular compartments separated by a barrier which can be broken during the swelling of the cells in hypotonic media. These observations raise more questions than they answer, and such matters as the nature of the barrier, the size and even exact number of compartments involved, the detailed mode of inhibition by ethidium bromide, etc., must remain the subjects of future studies. The alteration of a drug's action on a non-excitable tissue in hypotonic media is unusual, and one may ask if this approach might shed light on the actions of other drugs as well.

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Influence of Testosterone and Polyuridylic Acid on the Incorporation of Phenylalanine into Peptide Linkage by Prostatic Ribosomes

Recent studies[1] on the transfer of radioactivity from soluble ribonucleic acid-(*s*RNA)-valine-^{14}C to protein-like material by isolated prostatic ribosomes suggested that the levels of template RNA associated with these particles are controlled by testicular hormones. In line with this conclusion was the observation that randomly ordered copolymers of uridylic and guanylic acids (poly UG) stimulated valine incorporation by prostatic ribosomes from orchiectomized animals to a much greater extent than was found with ribosomes prepared from castrates which were treated with testosterone. However, two considerations complicate interpretation of the response of cell-free valine incorporating systems to poly UG. First, poly UG (prepared by the action of polynucleotide phosphorylase on a mixture of uridine and guanosine diphosphates) represents a complex mixture of polyribonucleotides of varying size and base sequences, the exact composition of which is difficult to ascertain. Secondly, the effects of guanine-containing polyribonucleotides on ribosomal amino-acid incorporating systems seem to depend not only on the base compositions of the polymers, but also on the degree of ordered secondary structure which they may exhibit in solution[2]. It became of interest, therefore, to examine the response of prostatic ribosomes to polyribonucleotides containing only one base. It is well established that polyuridylic acid (poly U) directs the entry of phenylalanine into peptide linkage in the presence of ribosomes from a number of tissues[3–8]. The following experiments concern factors which influence the transfer of radioisotope from *s*RNA-phenylalanine-^{14}C to acid-insoluble products by prostatic ribosomes, incubated in the absence and presence of poly U.

Charging of liver *s*RNA with L-phenylalanine-^{14}C and 19 other ^{12}C-amino-acids, the isolation of prostatic ribosomes by method 2, and most other methods are described elsewhere[1]. Poly U was prepared by the action of partially purified polynucleotide phosphorylase of *Micrococcus lysodeikticus* on uridine diphosphate. The source of soluble protein factors needed for the incorporations was either a crude dialysed protein fraction obtained by ultracentrifugation of rat ventral prostate homogenates[1], or the proteins present in '*p*H 5 supernates' prepared from the latter fraction, and afterwards dialysed exhaustively against the medium *D* of Liao and Williams-Ashman[1].

Prostatic ribosomes catalysed a swift and extensive transfer of radioactivity from added *s*RNA-phenylalanine-^{14}C to products which were insoluble in hot 5 per cent trichloroacetic acid, and also in ethanol/ether (3 : 1, v/v)[1].

Table 1

System	Radioactivity transferred to protein fraction (c.p.m.) − Poly U	+ Poly U
Complete	258	1,326
Omit phosphopyruvate, pyruvic kinase and ATP	88	494
Omit GTP	203	1,169
Omit phosphopyruvate, pyruvic kinase, ATP and GTP	13	68
Omit supernatant protein fraction	24	36

Co-factor requirement for transfer of phenylalanine-^{14}C to protein-like material by prostatic ribosomes. Reactions carried out in a final volume of 1·0 ml. containing 51 μmoles *tris*-HCl buffer, *p*H 7·8; 11 μmoles $MgCl_2$; 51 μmoles KCl; 11 μmoles β-mercaptoethanol; 1 μmole ATP; 1 μmole GTP; 9 μmoles phosphopyruvate; 50 μg pyruvic kinase; 1·1 mg dialysed '*p*H 5 supernatant' proteins; 0·04 mg liver *s*RNA charged with L-phenylalanine-^{14}C and 19 other ^{12}C-L-amino-acids (3,600 c.p.m.) and prostatic ribosomes from adult Sprague–Dawley rats equivalent to 54 μg total ribosomal RNA. If added: poly U, 0·1 mg. Incubated for 50 min at 37°.

With prostatic ribosomes isolated from adult albino rats with every evidence of normal testicular function, the incorporation of phenylalanine was stimulated from 4- to 10-fold by saturating levels of poly U (approximately 0·1 mg per 1 ml. of reaction mixture). In the presence of an excess of poly U, as much as 40 per cent of the radioactivity initially added as *s*RNA-phenylalanine-^{14}C entered the acid-insoluble fraction. The phenylalanyl transfers were unaffected by a large excess (3 mM) of cold free L-phenylalanine, and were depressed more than 80 per cent by 'Puromycin' (0·2 mM), when tested in the presence of '*p*H 5 supernate' proteins. Neither polyadenylic acid nor polycytidylic acid increased the incorporation of phenylalanine into the protein fraction. However, addition of a two-fold molar excess of polyadenylic acid (but not of polycytidylic acid) completely nullified the stimulatory effect of poly U. Table 1 shows that in the absence or presence of poly U the amino-acyl transfers were enhanced to only a small extent by guanosine triphosphate. However, the incorporations were markedly increased by addition of a high-energy phosphate generating system (phosphopyruvate and pyruvic kinase), and also by the soluble protein fraction. The requirement for the energy-generating system is probably related to maintenance of adequate levels of guanosine triphosphate during the course of the reaction. No incorporation of phenylalanine into the protein fraction was observed in the absence of the prostatic ribosomes. If the soluble proteins were added in excess, the incorporations were proportional to the quantity of ribosomal material added to the reaction mixtures (Fig. 1).

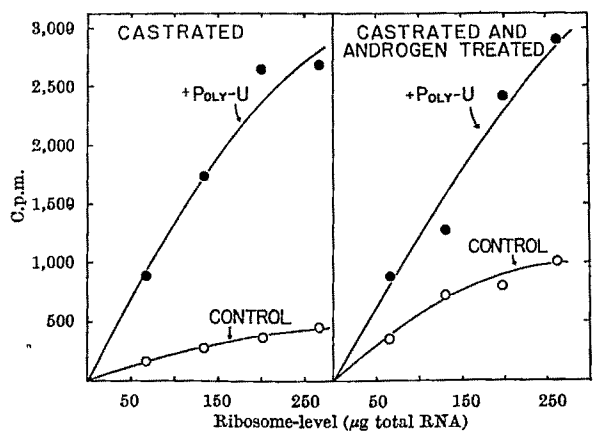


Fig. 1. Effect of polyuridylic acid on transfer of phenylalanine-^{14}C to acid-insoluble material by prostatic ribosomes from orchiectomized rats, with or without treatment with testosterone. Adult Sprague–Dawley rats castrated and killed 72 h after surgery. Testosterone propionate (2 mg) injected into each animal of one group just prior to orchiectomy, and also at 27 and 54 h thereafter. Controls injected with the same volume (1 ml.) of sesame oil solvent. Ventral prostate ribosomes isolated from pooled tissue derived from 60 castrates, and also from 30 androgen-treated castrates. Reaction mixtures as in Table 1. To all tubes was added: 1·1 mg dialysed '*p*H 5 supernate' proteins from testosterone-treated animals and 0·22 mg rat liver *s*RNA charged with L-phenylalanine-^{14}C and 19 other ^{12}C-L-amino-acids (16,800 c.p.m.). Polyuridylic acid (if added): 34 μg. Incubated for 60 min at 37°. Ordinate: radioactivity transferred to protein fraction (ref. 1). Abscissa: amount of ribosomal material (μg total ribosomal RNA) added to each tube

The ability of prostatic ribosomes isolated from recently orchiectomized rats to incorporate phenylalanine from *s*RNA-phenylalanine-^{14}C into the protein fraction was compared with that of similar particles prepared from castrates which were injected with testosterone propionate. In these experiments, the soluble prostatic proteins mandatory for the incorporations added to all vessels were isolated from the androgen-treated castrates, and were present in excess. The experiment depicted in Fig. 1 shows that, in the absence of poly U, the extent of phenylalanine incorporation by prostatic ribosomes isolated from orchiectomized rats was less than a half that found with ribosomes prepared from testosterone-treated castrates. Poly U markedly increased the aminoacyl transfers by ribosomes derived from both groups of creatures. But the stimulatory effect of poly U was greater with ribosomes isolated from the untreated castrates, so that in the presence of poly U the extent of the phenylalanyl transfers by both types of ribosomal preparation was nearly the same. These experiments are in accord with previous investigations[1] on the incorporation of valine into proteins by prostatic ribosomes, and are consistent with the view that testosterone governs the levels of template RNA associated with prostatic ribonucleoprotein particles.

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Stimulation of Amino-acid Incorporation by Nuclear Ribonucleic Acid from Normal and Methylcholanthrene-treated Rats

A SINGLE *in vivo* administration of 3-methylcholanthrene (MC) into rats increases the activity of a number of liver microsomal enzyme systems[1–4], and the *in vitro* rate of microsomal amino-acid incorporation into protein when either free or soluble RNA bound amino-acids are used as precursors[4,5]. The stimulatory effect of MC on the microsomal enzymes, benzpyrene hydroxylase[6] and amino-azo dye demethylase[7] is prevented by puromycin, an inhibitor of protein synthesis at the microsomal level. These findings suggest that the MC-induced changes in enzyme activity are due to enzyme synthesis. The *in vivo* administration of actinomycin *D*, an inhibitor of DNA directed RNA synthesis[8], prevents the MC-stimulation of microsomal amino-acid incorporation and inhibits the MC-induced increases in benzpyrene hydroxylase activity[9]. These results indicate that the MC-induced changes in both amino-acid incorporation and benzpyrene hydroxylase activity are dependent on DNA directed RNA synthesis. Since 'messenger RNA' is synthesized by a DNA directed polymerase, these results suggested the possibility that MC may induce alterations in the production of 'messenger RNA'.

Barondes[10] *et al.* have shown that RNA extracted from rat liver nuclei stimulates amino-acid incorporation into protein in an *E. coli* system. They have suggested that the amount of stimulation may represent an assay of the amount of 'messenger RNA' present. We have examined the effect of MC pretreatment on nuclear RNA content and measured the ability of the isolated nuclear RNA to stimulate the incorporation of phenylalanine into protein in an *E. coli* system.

Table 1. Effect of 3-Methylcholanthrene on Nuclear DNA and RNA Content

	DNA (mg) / Wet liver (g)			Nuclear RNA-P / DNA-P		
	Normal	MC	Diff.	Normal	MC	Diff.
Exp. I	2·45	2·54	+ 4%	0·24	0·33	+ 37%
Exp. II	2·52	2·36	− 6%	0·20	0·30	+ 50%
Exp. III	2·48	2·49	< 1%	0·20	0·23	+ 15%

RNA and DNA were extracted from isolated nuclei by the procedure of Fleck and Munro (ref. 15). Homogenates and nuclei were assayed for DNA by the diphenylamine procedure of Dische (ref. 16). RNA was assayed by the orcinol procedure of Albaum and Umbreit (ref. 17).

Groups of eight Sprague–Dawley male rats weighing 35–45 g were injected with 1 mg of MC in 0·25 ml. of corn oil or corn oil only. Sixteen h later the rats were decapitated, the pooled livers homogenized, and the nuclei isolated and purified by centrifugation through hypertonic sucrose[10]. Table 1 shows the results from three experiments. MC pretreatment did not significantly alter the amount of DNA/g rat liver. However, the amount of nuclear RNA was significantly increased by treatment with MC. In three experiments the nuclei from MC-treated rats contained 37, 50 and 15 per cent more RNA than did the nuclei from control rats.

Table 2. Stimulation of ^{14}C-Phenylalanine Incorporation into Protein by Nuclear RNA From Normal and MC-treated Rats

	Nuclear RNA (μg)	Specific stimulatory activity: μμmoles of phenylalanine incorporated per 100 μg of nuclear RNA		
		Normal	MC	Difference
Exp. I	8	11·3	16·2	+ 43%
Exp. II	11	14·8	23·2	+ 57%
Exp. III	25	12·3	15·9	+ 29%

The reaction mixture contained the following in a total volume of 0·33 ml.: 25 μmoles *tris*-hydrochloric acid *p*H 7·8, 3·5 μmoles magnesium acetate, 12·5 μmoles potassium chloride, 1·4 μmoles β-mercaptoethanol, 0·25 μmoles adenosine triphosphate (sodium salt), 0·0075 μmoles guanosine triphosphate (sodium salt), 1·87 μmoles sodium phosphoenolpyruvate, 4 μg crystalline phosphoenolpyruvate kinase (Cal.Biochem.), 0·66 μmoles of potassium acetate, 0·05 μmoles each of 19 L-amino acids, 0·05 μmoles of L-phenylalanine-(U)-^{14}C (S.A. 61·7 mc./μmole in Exp. I and 19·8 mc./μmole in Exps. II and III), 0·050 ml. of dialysed and pre-incubated *E. coli* extract (ref. 11) containing 1·6 mg of protein and 0·1 μg of crystalline DNase. The added nuclear RNA contained less than 2 per cent DNA and less than 2·5 per cent protein. The tubes were incubated for 30 min at 37° C., the reaction stopped with 2·5 ml. of 10 per cent trichloroacetic acid and the proteins isolated by a modification of the method of Siekevitz (ref. 18). Radioactivity was determined by suspension counting in a liquid scintillation counter (ref. 19). The values shown represent the difference between incorporation with added nuclear RNA and the control values without added RNA. The control tubes incorporated 1·6, 5·7 and 2·6 μμmoles of phenylalanine in Exps. I, II and III, respectively. The values represent the averages of duplicate determinations.

A comparison was made of the 'messenger activity' present in the nuclear RNA derived from normal and from MC-treated rats. Nuclear RNA from normal and MC-treated rats was isolated by phenol extraction[10] and tested for its ability to stimulate the incorporation of L-phenylalanine-(U)-^{14}C in the *E. coli* amino-acid-incorporating system of Matthaei and Nirenberg[11]. Table 2 shows that nuclear RNA derived from MC-treated rats stimulates phenylalanine incorporation to a greater extent than equivalent amounts of nuclear RNA from normal rats. In three experiments pre-treatment with MC increased the specific stimulatory activity of nuclear RNA by 43, 57 and 29 per cent. In this incorporating system, microsomal RNA had one-tenth the activity of nuclear RNA and soluble RNA had a slight inhibitory effect.

Methylcholanthrene has been shown to induce several microsomal enzymes in rat liver[1–4] and to increase benzpyrene hydroxylase in a number of other rat tissues[12]. The observed changes in the liver are paralleled by alterations in the protein synthetic apparatus[5,9] and by changes in both the amount and 'messenger activity' of the nuclear RNA. The relationship, if any, between those early MC-induced changes and chemical carcinogenesis remains to be elucidated. Our findings are consistent with the suggestion[13,14] that an early event in carcinogenesis may involve an alteration in the expression of genetic information which would be reflected in an altered metabolism of 'messenger RNA'.

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Action of 5-Bromo-3-*sec*-Butyl-6-Methyluracil as Regards Replacement of Thymine in Mouse DNA

McGahen and Hoffmann[1] were unable to demonstrate thymine replacement by 5-bromo-3-*sec*-butyl-methyluracil (I) in a thymineless *Escherichia coli* while 5-bromouracil (II) replaced 21 per cent of the thymine under the same test conditions. It was concluded that 5-bromo-3-*sec*-butyl-6-methyluracil is not, therefore, a biological analogue of thymine. The results of an investigation with juvenile mice are presented here.

Weanling Swiss-Webster white mice weighing 7–8 g, 15 days old, were obtained from Pied Piper Farms, Newark, Delaware. Five mice each were injected by oral intubation with (I) (2^{14}C) and with (II) (2^{14}C). The specific activities were 1·13 mc./mmole and 6·05 mc./mmole respectively. Dose schedules were 100 mg/kg twice daily for two days and 50 mg/kg twice daily for the subsequent 8 days. Weights were followed and compared with mice which did not receive compound. Sixteen hours after the final injection, the mice were killed and the livers and spleens were removed. DNA was isolated by the procedure of Marmur[2] and assayed by the diphenylamine method of Burton[3]. Radioactivity was determined by proportional counting.

Table 1. Incorporation of Labelled 5-Bromo-3-*sec*-Butyl-6-Methyluracil (I) and Labelled 5-Bromouracil (II) in Liver and Spleen DNA of Juvenile Mice

Compound	Tissue	Total DNA (μg)	Total compound (μg)
(I)	Liver	1,188	< 0·009
	Spleen	335	< 0·009
(II)	Liver	964	0·243 ± 0·057
	Spleen	232	0·023 ± 0·002

Dosage schedules of 100 mg/kg twice daily for 2 days and 50 mg/kg for the subsequent 8 days by intubation were followed for both compounds. Tissues were taken 16 h after final injection.

Both compounds at 100 mg/kg depressed gain in weight but, at 50 mg/kg, gain in weight was comparable with that of control mice. Livers and spleens appeared normal at death.

As is shown in Table 1, labelled (I) was not detectable in either liver or spleen DNA. Labelled (II), in contrast, was incorporated into the DNA of both liver and spleen.

Thus, it appears that 5-bromo-3-*sec*-butyl-6-methyluracil is not recognizable as a thymine analogue by the mouse within the limits of the test systems utilized in this experiment.

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An Antibiotic-like Effect of *Lactobacillus acidophilus*

Until a few years ago, the beneficial effects resulting from the ingestion of the *Lactobacillus acidophilus* in cases of gastro-intestinal disturbances were attributed to the simple overgrowth of the offending pathogen by the *Lactobacilli*. Recently a number of workers have indicated that there was more to the reaction than just competition between strains. The substance produced by *L. acidophilus* has been called variously antagonist[1], lactobacillin[2], lactocidin[3], and antibiotic[4]. All these workers found that the substance was extremely labile, and attempts to isolate it met with failure. A successful method for the visual demonstration of the phenomenon is described here but isolation has not yet been successfully accomplished.

The technique used was similar to the cup-plate assay for antibiotics. Skim milk–digest–tomato juice agar heavily inoculated with a *Staphylococcus* isolated from fæces (Fig. 1) or nutrient agar inoculated with *E. coli* (Fig. 2) was poured in a Petri dish, and allowed to harden. Cups were cut with a sterile, stainless steel ring and the depression so formed filled with agar seeded with *L. acidophilus* and incubated at 37° for 24 h. The plates were then photographed and zones of inhibition stand out clearly. The suppression of growth by production of acid was ruled out because there was no difference in the *p*H of the agar in the cup, in the inhibition zone or in the outer zone. A logical explanation of the therapeutic value of *L. acidophilus* can be predicted on the excretion of an antibiotic-like substance which, although weak and labile, is being continuously produced by the rapidly growing

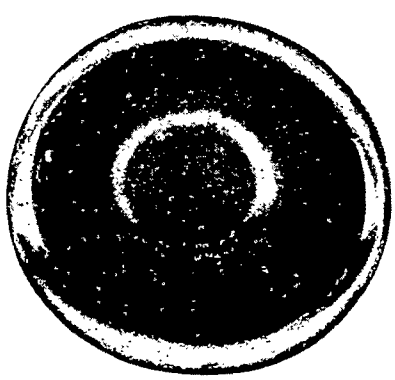


Fig. 1. The plate was seeded with a culture of *Staphylococci* isolated from human fæces and the inner cup seeded with *L. acidophilus*. After 24 h incubation at 37° C, the zone of inhibition is clearly visible

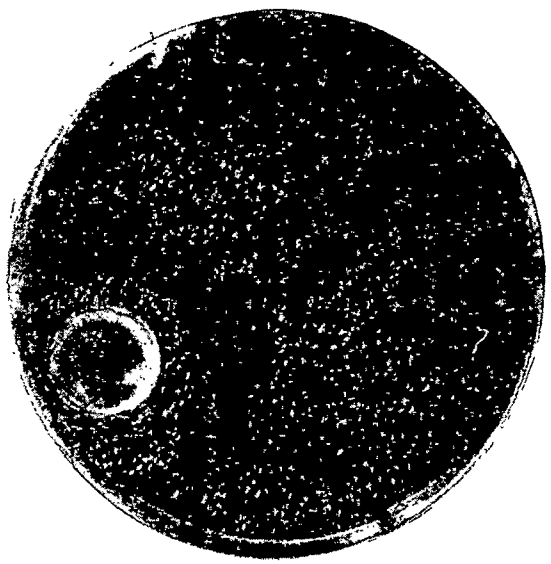


Fig. 2. The plate was seeded with *E. coli* and the cup on the left seeded with *L. acidophilus*. A pronounced zone of inhibition was produced after 24 h at 37° C. The 'ghost' cup on the right contained un-inoculated agar as a control, and has been overgrown by the *E. coli*

lactobacilli and therefore may, in part, account for the *in vivo* as well as the *in vitro* effect.

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Reaction of Hydrogen Chloride Gas on RNA and its Component Nucleotides

The release of purines and characteristic sequences of pyrimidine oligonucleotides from DNA has been achieved by the use of diphenylamine and formic acid[1]. In RNA the phosphodiester bonds are extremely acid and alkali-sensitive due to the ease with which cyclic phosphate intermediates are formed involving the 2′ hydroxyl group. In order to limit the possibility of cyclization an attempt was made to carry out a depurination reaction with RNA in the solid phase.

Preliminary investigations have revealed that the degradation of RNA by boron trichloride (in which the RNA is insoluble) shows a measure of specificity and yields purines together with some pyrimidine oligonucleotides[2]. The mechanism of this reaction is thought to involve the production of hydrogen chloride by hydrolysis of the boron trichloride with the water present in intimate association with the RNA.

To test this hypothesis, hydrogen chloride gas has been reacted with RNA in the solid phase and found to be much more efficient than boron trichloride in releasing purines and pyrimidine oligonucleotides. Although water was again found to be necessary for complete reaction, there was no loss of ultra-violet absorbing material as found with boron trichloride.

The experiments were carried out on yeast microsomal RNA[3] which had been freeze-dried at a concentration of 5–10 mg/ml. The water content varied from 12–15 per cent, but could be reduced to 2 per cent by standing over phosphorus pentoxide *in vacuo* for several days. The reactions occurred in tubes into which hydrogen chloride gas was introduced at room temperature and pressure. The tubes were then sealed off and heated in a water bath for a range of times and temperatures. The reaction

product was taken up in buffer at *p*H 7, and estimations made of the adenine, the inorganic phosphorus and the end-group phosphorus liberated.

It was found that 100 per cent release of adenine could be achieved at a temperature of 85° C. The rate of the reaction was found to be dependent on the water content, but below 5 per cent, that is one molecule of water/nucleotide, the reaction only goes to completion when very long reaction times were used. It seems likely the role of water in the reaction is to allow the hydrogen chloride gas to ionize *in situ*. Since the RNA is in the solid phase the possibility of 2′(3′) cyclization and consequent phosphodiester breakage is eliminated. However, the hydrogen ions seem able to split the acid-sensitive purine-glycosidic bonds.

The phosphorus analyses show that after an initial lag, during which some 30 per cent of the purines are liberated, the release of inorganic phosphorus bears a linear relationship to that of the purines and reaches a maximum value of 25–30 per cent of the total phosphorus. The end-group phosphorus reaches a value of 70–75 per cent of the total phosphorus, which is close to the theoretical for a random sequence of bases, on the assumption that the remaining phosphorus lies between the pyrimidine nucleotides. The lag in the appearance of the inorganic phosphorus and its maximum value of 30 per cent support the view that only interpurine phosphorus is released and that two adjacent purines must be liberated before this can occur.

The reaction between hydrogen chloride gas and the component nucleotides of RNA has been studied. The following facts emerge:

(1) The rate of release of adenine and inorganic phosphorus was the same for the 5′ and 2′(3′) adenylic acids, indicating that degradation of the ribose was responsible for the release of the phosphate. This may be compared with the effect of aqueous mineral acids on the adenylic acids in which the 5′ compound is much more resistant to hydrolysis than the 2′(3′) compound[4].

(2) In the case of the pyrimidine mononucleotides two extra zones were found on chromatography in an isopropanol – ammonia – water system[5]. For the cytosine mononucleotide, the R_P value was 0·28 for 2′(3′) cytidine monophosphate and 0·1 and 0·44 for the two unknowns. The faster one was identified as the 2′ : 3′ cyclic phosphate, by its base : phosphorus ratio (1 : 1) and its sensitivity to 0·2 N hydrochloric acid. After 4 h at 37° C in 0·1 N hydrochloric acid, this compound reverted to 2′(3′) CMP. The slower zone gave a base/phosphorus ratio of 1 : 1 but treatment with phosphomonoesterase released only 50 per cent of the phosphorus. If this was followed by treatment with alkali, chromatography revealed zones corresponding to cytidine and cytidylic acid. This confirmed that the slower moving zone was a dimer of cytidylic acid. The faster zone amounted to only about 3 per cent of the total ultra-violet absorbing material but the slower one accounted for as much as 20 per cent.

The formation of dimers from the mononucleotides probably occurs as a result of dehydration of the phosphate group, followed by condensation preferentially with a primary hydroxyl group, that is, the 5′ hydroxyl group of an adjacent nucleotide. The cyclic 2′ : 3′ compound probably arises from condensation with a secondary hydroxyl group, that is, the 2′(3′) hydroxyl group of the same molecule.

The fact that pyrimidine dinucleotides are produced points to the stability of the interpyrimidine nucleotide phosphodiester bonds to hydrogen chloride gas, but invalidates the use of this reaction for quantitative studies.

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PHYSIOLOGY

Sex Differences in the Serum Cholesterol Response to Stress in Monkeys

The investigation reported here was undertaken as an initial step in the assessment of central nervous system influences on the transport of serum lipids, with particular reference to their possible role in the pathogenesis of atherosclerosis. The experimental situation dictated that monkeys be moderately restrained for extended periods of time in order that the neural variables could be manipulated under controlled conditions. It became apparent, however, that such restraint itself was a significant variable affecting the level of cholesterol and other lipids in the serum, hence this study was designed to investigate this aspect of the problem in a detailed fashion.

Six male and 6 female rhesus monkeys weighing between 4 and 5 kg were examined. Initially the range of variation of cholesterol in the serum was determined under cage conditions. Blood samples (4 c.c.) were taken at varying intervals ranging from 1 day to 4 weeks. Aliquots from these samples were analysed in duplicate for total, free and esterified cholesterol using the method reported by Vahouney, Borja, Mayer and Treadwell[1] and modified by Goodman, Jarnigen, Meier and Shonley[2]. At least two such determinations were made during the control period before restraint was begun. When the level of serum cholesterol under cage conditions had been established, animals were stressed by confinement in a pillory-type primate chair for periods varying from 10 to 45 days. During this time blood samples were taken on an average of twice weekly. Following the period of restraint, animals were returned to their cages and the level of serum cholesterol determined for several weeks.

During the entire experimental period animals were kept on their normal diet of 3–4 'chimp crackers' (K. F. S. biscuits : protein, 21 per cent; fat, 4 per cent), plus one piece of fruit (apple, orange or banana) daily. Water was offered as desired. The weight of each animal was checked frequently before and after the period of restraint, and determinations of hæmoglobin were carried out at intervals to be certain that changes in these parameters were not significant.

The normal level of total cholesterol in the monkey under cage conditions was found to vary between 125 and 180 mg per cent. The mean value for 6 male monkeys was 159·6 mg per cent and in females was found to be 162·3 mg per cent. The difference between these two values was not statistically significant. Similar comparisons for free and esterified cholesterol fractions are shown in Table 1. These figures are similar to those obtained by other authors[3,4].

The serum-level of total, free and esterified cholesterol decreased under the stress of restraint in both sexes. Although there is considerable variation in the length of time for the maximal response to become manifest, initial indication of the decrease was usually apparent by the third day after the beginning of restraint. The maximum response was evident 5–10 days following the initiation of stress. In all 12 animals the serum-levels remained at this decreased value throughout the period of confinement. In Table 1 it can be seen that in male monkeys the mean total cholesterol value dropped from a level of 159·6 mg per cent to a mean value of 103 mg per cent. This amounted to a decrease of 35·5 per cent from the control period. Also in Table 1 it can be seen that the values for free and esterified fractions showed a proportionate decrease. The decrease in serum cholesterol in the 6 female monkeys was not so great (Table 1). The total cholesterol value for this group dropped from 162·3 to 132·0 mg per cent, which represented a 19·0 per cent fall in the cholesterol value during the period of restraint.

Table 1. Comparative Circulating Cholesterol Levels during Cage and Stress

		Cholesterol					
		Male monkeys			Female monkeys		
		I Total	II Free	III Ester	IV Total	V Free	VI Ester
A	No. of animals	6	6	6	6	6	6
	No. of test – pre-stress	11	11	11	9	9	9
	Mean	159·6 mg %	48 mg %	104 mg %	162.3 mg %	44 mg %	114 mg %
	S.D.	± 14·2	± 7·7	± 12·9	± 17·1	± 8·0	± 13·6
B	No. of animals	6	6	6	6	6	6
	No. of test – stress	68	68	68	67	66	67
	Mean	103 mg %	32 mg %	65 mg %	132 mg %	40 mg %	87 mg %
	S.D.	± 15·0	± 5·3	± 12·0	± 29·0	± 8·7	± 19·2
C	No. of animals	6	6	6	6	6	6
	No. of test – post-stress	20	20	18	20	20	19
	Mean	149·7 mg %	44 mg %	98 mg %	175 mg %	50 mg %	120 mg %
	S.D.	± 18·0	± 12·0	± 15·0	± 26·0	± 7·6	± 21·0

The statistical significance of these changes are summarized in Table 2. It can be seen that the decreases in the cholesterol-levels noted during stress are significant (P value less than 0·001) with the exception of the decrease in free cholesterol for the female group (P value 0·2). The differences in the total and esterified cholesterol response between the male and the female monkey also proved to be significant (P value less than 0·001) although the difference in the responses for free cholesterol was less significant (P value 0·05).

Table 2. Statistical Significance of Changes in Circulating Cholesterol

	t test	P
A I – B I	11·4	< 0·001
A II – B II	8 8	< 0·001
A III – B III	20·5	< 0·001
A I – C I	1·5	N.S.
A II – C II	0·9	N.S.
A III – C III	1·07	N.S.
A IV – B IV	3·0	0·01
A V – B V	1·29	N.S.
A VI – B VI	4·0	< 0·001
A IV – C IV	4·1	< 0·001
A V – C V	1·8	N.S.
A VI – C VI	0·07	N.S.
B I – B IV	7·2	< 0·001
B II – B V	2·0	< 0·05
B III – B VI	8·2	< 0·001

After animals were removed from the restraining chairs and returned to the cage environment their cholesterol-levels promptly increased. Four weeks after stress the male total cholesterol value was not significantly different from the pre-stressed level. In contrast, however, the mean total cholesterol value for female monkeys exceeded the control value by 13 mg per cent, which proved to be statistically significant.

This consistent response in the serum cholesterol-level to restraint is consistent with the findings of Mann and White, who applied other stressing agents to dogs and rats and found a similar decrease in the cholesterol-level, particularly in the esterified fraction[5]. Other investigators have noted a decrease in the serum cholesterol in human beings which could be correlated with the stress of surgery[2,6,7] and with myocardial infarction[8,9], while still others[10] have noted increases in serum cholesterol-levels in association with occupational stresses.

It is difficult as yet to delineate the intermediary mechanisms responsible for changes in the serum cholesterol fractions, or the sex differences noted in response to stress. Presumably such factors as altered rates of synthesis, utilization, excretion or deposition into bodily tissues are involved which must be identified in subsequent radioactively labelled tracer studies. Since in our experiments such factors as diet, weight and circulating hæmoglobin were kept constant, attention must be more sharply directed to the possibility that the psychological factors accompanying restraint played a major part in triggering the metabolic factors which led to alterations in the cholesterol-level. Although the neural and endocrine mechanisms which might subserve such function remain obscure, recent experimental evidence presented by Gunn, Friedman and Byers clearly indicates that the hypothalamic region of the brain may influence the level of circulating lipids and even the induction of arterial atherogenesis[11]. It seems likely that additional experimental evidence will rapidly accumulate to more clearly define the role of cerebral factors in the control of lipid metabolism and its possible relationship to the pathogenesis of atherosclerosis.

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Blood Rheology in Cardio-vascular Diseases

The detection of impending thrombosis and initial atherosclerosis is one of the unsolved problems in medicine. In many cases[1,2] diagnosis is made *postmortem* because of lack of methods for the detection and assessment of the pathological states. However, thoughts were expressed recently that one of the factors involved in these states is blood rheology.

Wasilewski suggested[3] that blood has high viscosity in the cases of cardiac decompensation. Aksyantsev observed[4] an increase of blood viscosity in 33 per cent cases exhibiting transient interruption of the cerebral blood circulation. Guyton and Richardson believed[5] that it is logical to accept that viscous changes in blood could greatly influence the venous return to the heart and in this way also affect the cardiac output. Marin and Stefanini postulated[6] that a sudden coagulation of the blood within an apparently intact vascular channel may result from the

combined effect of stasis or slowing of the blood flow, and of a transient 'hypercoagulable' state.

I showed recently[7,8] that an examination of viscosity of blood, if carried out at very low rates of shear (0·001–1 sec^{-1}), renders it easily and readily to distinguish between the normal and abnormal blood. The effect of hæmatocrit value is minimal at low rates of shear, the main contributing phase to the blood viscosity being thixotropy caused by a reversible aggregation of red cells. I have observed that, in few cases examined, the thixotropy and viscosity of blood are up to ten-fold larger in patients suffering from thrombosis and coronary occlusion. These increases in the thixotropy and viscosity at low rates of shear are due to an enhancement of aggregation (flocculation) of the red cells. It appeared that an increased thixotropy and viscosity might indicate a precursor state of thrombosis.

A possible relation between the aggregation of red cells and thrombus formation was envisaged as early as in 1921 by Fahraeus[9].

While it is not the subject of this communication to discuss a relation between thrombosis and atherosclerosis, or the dilemma which of these is the primary and which the secondary event[10–13], it must be stated that if the viscosity of blood is to play a part in these phenomena, it should be capable of changing its value in the various areas or sites of circulation, and should permit the variability of rheological parameters to be quite pronounced at these various sites of circulation.

As the circulation time is very short, being of the order of 1 min or less[14], it might be difficult to see how any pronounced changes of blood viscosity may occur within such short time periods. Indeed, were blood a Newtonian fluid, only slight changes could have been expected in viscosity and attributed to variations in oxygen tension, concentration of the metabolic products, etc.

However, blood happens to be a thixotropic fluid[15]. Its viscosity is greatly affected by time and rate of shear. The complexity of blood rheology is due to the fact that the final viscosity is influenced by three thixotropic mechanisms: (*a*) reversible aggregation of red cells, which is responsible for the greater part of thixotropic properties; (*b*) thixotropy of the red cell (the effects of the interior and of the membrane are grouped together); (*c*) thixotropy of the plasma (most likely due to the hydrogen bondings).

This triple-thixotropic mechanism, each of which components shows a different response to the velocity gradient, is superimposed on the intrinsic properties of flow in circular vessels in which the profile of velocity gradient requires a zero rate of shear in the axis of the vessel. The profile is a function, also, of the visco-elasticity of the vessel wall, of the pressure gradient, of the pulse, of the smoothness of the endothelial lining, etc. Consequently, a great variability of the levels of thixotropy and viscosity can exist at various sites of circulation.

When blood is examined under equilibrium conditions, that is in a homogeneous field of velocity gradient, available in a cone-plate or a cone-cone rotational viscometer[7,16], the fundamental flow curve of the particular sample of blood can be plotted. The flow curve, if measured at low rates of shear and in the absence of anticoagulants, permits an appreciation of the degree of aggregation of red cells under any conditions which might occur in the living body.

The data obtained in an examination of a series of coronary occlusion, venous thrombosis and polycythæmia cases are plotted on a log–log scale as viscosity, in poises, against the rate of shear, in reciprocal seconds (Fig. 1). These blood samples have hæmatocrit of 30–77 per cent, the age of patients being 35–70 years. The control series (marked by open circles) contains hæmatocrits of 36–46 per cent, the age of donors varying from 19 to 55 years. The control series was made up of 10 persons, while the former series was composed of 12 cases. Blood viscosity was tested at 36° C, for a period of up to 4 min after the

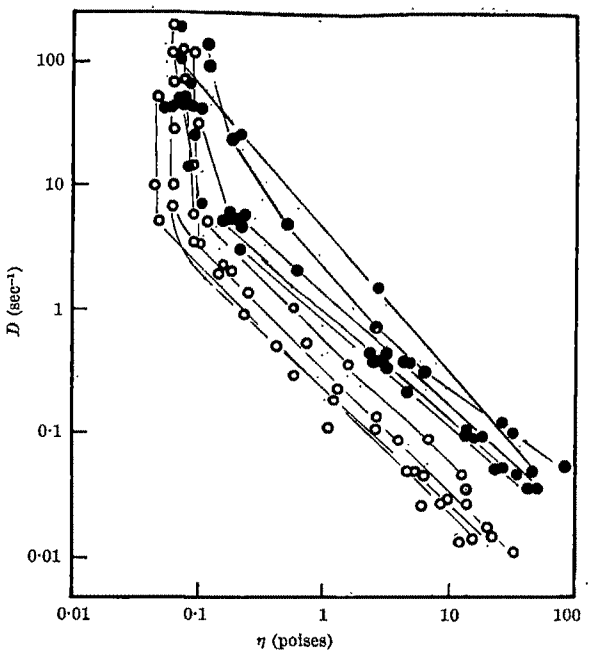


Fig. 1. Viscosity of blood obtained from 10 normal persons (circles) and 12 patients suffering from venous thrombosis, coronary occlusion, and polycythæmia (dots). The faint lines indicate the preponderant direction of the flow curves. Viscosity, in poises, is plotted against the rate of shear, in reciprocal seconds on a log–log scale. The viscosity determinations have been carried out in the absence of anticoagulants, at 36° C, by means of a rotational cone-in-cone viscometer (ref. 7). The clotting of blood occurred in about 4–6 min, and hence two or three samples of blood were required from each person in order to determine the viscosity of unclotted blood over the whole range of rates of shear. Viscosity changes due to clotting are not shown in this figure

extraction of blood. During this period no changes in viscosity of blood can be observed. Clotting starts after various time-periods, depending not only on the intrinsic properties of the particular blood sample, but also on the velocity gradient at which such clotting takes place. The micro-rheology of the clot formed depends on the velocity gradient.

It may be evident from Fig. 1 that a great difference exists between blood of healthy persons and blood of patients suffering from cardio-vascular disorders. It can be seen that an up to 10-fold difference exists in the viscosity—if compared at some constant rate of shear—and in the critical rate of shear. A statistical analysis indicates that the probability is less than 1 in 1,000,000 that such results are due to chance only.

It is feasible that red cells might contribute to thrombus formation through the two mechanisms: (*a*) At low rates of shear, the aggregation of red cells (as indicated by thixotropy) will lead to an intermittent and reversible intravascular sedimentation; this, in its turn, may lead to a formation of irreversible agglutinates and to sludging observed by Knisely[17]. An agglutinate may also plug an entrance to an arteriole or capillary, causing a more or less prolonged stasis. Stasis may follow also an abnormal plasma skimming[18], the degree of separation of plasma flow and red cell flow increasing with the increasing degree of aggregation of red cells. While the fluidity of the red cell interior[19] does not permit local hæmatocrit fluctuations to interfere greatly with circulation in the absence of a pronounced aggregation of red cells, that is not the case when the latter is enhanced by different causes (viruses, high level of fibrinogen, etc.). The degeneration of the red cell surfaces during sedimentation or stasis, due to hypoxia or anoxia, may induce contact activation of plasma clotting, or, alternatively, the red cells themselves might release factors initiating clotting[20]. It is relevant to note that Heilbrunn stated[21] that a clot can be formed by the aggregation of red cells solely.

(*b*) At high rates of shear, or under turbulent conditions, the rupture of red cells (such as mechanically-induced hæmolysis or breakage) may lead to a clot formation of a distinctly different type. This mechanism is now under consideration.

The present series of tests confirms the results obtained in the pilot series[7], and appears to confirm the hypothesis that the increased thixotropy and viscosity of blood (at low rates of shear) are symptomatic of the thrombotic condition. The work continues now on a larger scale in order to substantiate further all deductions and to test the applications of micro-rheological theories and techniques to some cardio-vascular problems.

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Absorption of Chymotrypsin from the Intestinal Tract

THE primary objective of work reported here was to determine whether an intact enzyme can be absorbed from the gastro-intestinal tract of adult animals retaining its enzymatic activity.

Plasma enzyme-levels obtained after rectal and intestinal administration of chymotrypsin to healthy, adult, New Zealand White rabbits were compared to those obtained after intramuscular administration. The enzymatic activity appearing in the circulation following the administration of chymotrypsin was measured by the hydrolysis of *N*-acetyl-L-tyrosine ethyl ester (ATEE)[1]. Quantitation was accomplished by comparing the plasma activity with chymotrypsin standards dissolved in the plasma of the animal being studied. The plasma enzyme-levels shown in Fig. 1 represent mean values obtained from five rabbits receiving 10 mg/kg intramuscularly; five rabbits, 20 mg/kg intra-intestinally; and seven rabbits, 20 mg/kg rectally. In all cases the endogenous enzyme-level determined just prior to administration of chymotrypsin was less than 1·0 μg/10 ml. in terms of chymotrypsin activity, and in most cases it was less than 0·5 μg/10 ml.

It can be seen that a significant increase in enzymatic activity results after administration by all three routes. It remained to be shown, however, that this activity was that of chymotrypsin. When chymotrypsin is added to plasma or administered to rabbits, esterase activity against ATEE and benzoyl phenylalanine naphthol ester (BPNE) was obtained, but no activity against tosyl arginine methyl ester (TAME) or benzoyl arginine ethyl ester (BAEE) could be detected. The analytical procedure used for BPNE was a modification of that described by Ravin[2]. The procedures for TAME and BAEE were modifications of that used for ATEE[1]. The specificity obtained eliminates the possibility that the esterase found was plasmin, thrombin, urokinase, or the esterase derived from the first component of complement[3].

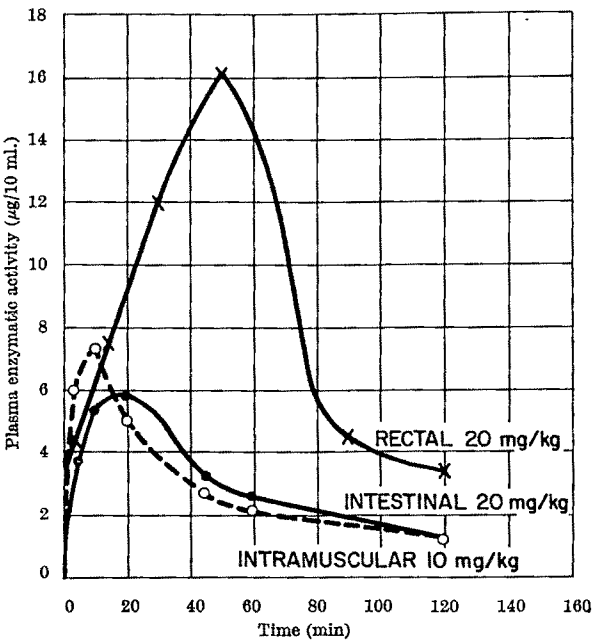


Fig. 1. Mean plasma levels after administration to rabbits

In order to characterize further the esterase appearing in the blood after chymotrypsin administration, we determined the hydrolytic activity of the plasma against two substrates, ATEE and BPNE, after rectal administration of the enzyme at several dosage-levels. The activity of aqueous solutions of chymotrypsin at several concentrations against the same two substrates was also determined. The *in vitro* activity ratio (ATEE/BPNE) was found to be $1{\cdot}82 \pm 0{\cdot}16$. After rectal administration the ratio was $1{\cdot}78 \pm 0{\cdot}49$. It appears, then, that chymotrypsin has been absorbed from the gastro-intestinal tract in an enzymatically active form.

Preliminary results with adult human subjects indicate that here, too, chymotrypsin is being absorbed from the gastro-intestinal tract.

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Cæsium–Choline Interaction on Muscle Membrane

IT has been observed that the depolarizing effect of cæsium ions on membrane potential of frog muscle fibres increases when external sodium concentration is replaced by a so-called inert osmotic substitute, such as choline chloride[1]. This dependence on sodium choline of the depolarization of cæsium was in contrast to the independence of the sodium–choline observed on the potassium depolarization.

The purpose of the work recorded here is to determine whether variations in external sodium concentration produce this specific change in the depolarization of cæsium, or whether a cæsium–choline interaction on the muscle membrane mechanisms exists independent of the external sodium concentration. Sartorius muscles of frog were carefully dissected, so as to leave the lower side as free as possible from connective tissue. Muscles were mounted in a special holder to obtain 120 per cent of their resting length by means of a micrometer system. Forty pairs of muscles were used in each experimental solution. The average of membrane potential measurements on 220 muscle cells is represented in each point shown in Fig. 1.

Bathing solutions were made up by adding 10 mM cæsium or 10 mM potassium to standard Ringer's formula (112 mM sodium; 1·89 mM calcium; 2·5 mM disodium hydrogen phosphate; 0·5 mM sodium dihydrogen phosphate). Choline chloride or sucrose was substituted for part of the 112 mM sodium chloride to obtain osmotic equivalence, as indicated in Fig. 1.

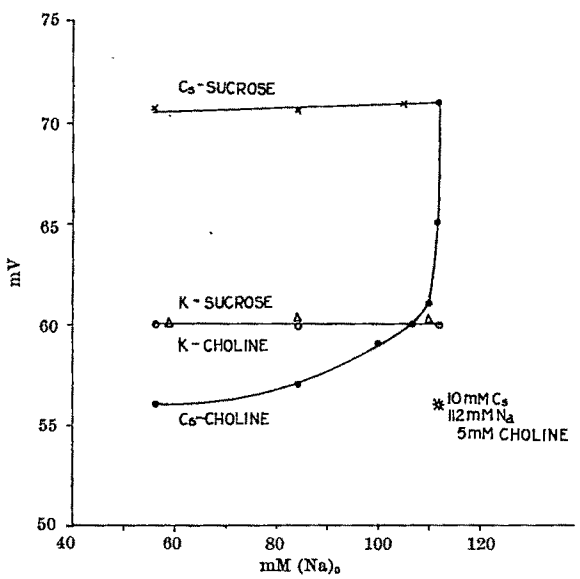


Fig. 1. Membrane potential obtained in 10 mM cæsium or 10 mM potassium at several external sodium concentrations. Osmolarity is adjusted with choline chloride or sucrose. ×, Cæsium sucrose; Δ, potassium sucrose: sodium is replaced by sucrose in order to maintain the osmolarity of the Ringer solution; ●, cæsium–choline; ○, potassium–choline: sodium is replaced by choline to maintain the osmolarity of the Ringer solution. 5 mM choline chloride was added to 10 mM cæsium–Ringer solution

All experiments were carried out at 25 ± 0·25° C and *p*H 7·2–7·3. Muscles were equilibrated for 60 min in 500 ml. of the experimental solution prior to microelectrode penetration. Membrane potentials were measured with glass micropipette electrodes filled with 3 M potassium chloride having a resistance between 10 and 12 megohms. Electrical measurements were made with conventional equipment for this work. Only potentials instantaneously developed with microelectrode penetration in the muscle cell were recorded and used in the data. The standard error of the mean is less than 1 mV.

The magnitude of the depolarization produced by 10 mM cæsium or 10 mM potassium as a function of the external sodium concentration when osmolarity was adjusted with choline chloride or sucrose is shown in Fig. 1. These results show that:

(1) Potassium depolarization is independent of external sodium concentration when osmolarity is adjusted with either choline chloride or sucrose.

(2) Cæsium depolarization is independent of external sodium concentration when osmolarity is adjusted with sucrose, but it is dependent on the external choline concentration.

(3) Cæsium depolarization at constant sodium concentration (112 mM sodium) is further increased when choline is added to the Ringer solution.

The specific change in the cæsium depolarization is produced by choline ions, and it is independent of the external sodium concentration.

The cæsium–choline effect is significantly dependent on choline from 1 to 15 mM and gradually decreases at higher concentrations of choline. The increase in depolarization of cæsium by choline is interpreted as due to a complex choline–cæsium interaction on muscle membrane mechanisms. It indicates that cæsium ions provoke 'activation' of specific membrane 'sites' to choline and, that the number of 'sites' activated must be related to the external concentration of choline ions.

Experiments in progress indicate that:

(*a*) The further increase of cæsium depolarization produced by addition of choline is not due to an increase of cæsium influx. Cæsium-137 was used to measure the uptake of cæsium by the muscle.

(*b*) The rate of penetration of choline labelled with carbon-14 (for example, rate of choline metabolism) in sartorius muscle increases with the presence of cæsium ions.

(*c*) The rate of oxygen consumption is 20–30 per cent higher in 10 mM cæsium than in 10 mM potassium.

These observations show that cæsium ions have far been implicated in enzyme activation in a pathway in which choline may be metabolized. The sequence of transformations associated with cæsium–choline depolarization and 'integration' of choline or acetylcholine system into the metabolic pathways of muscle cells is under investigation at present in this laboratory.

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Facilitated Diffusion of Oxygen through Hæmerythrin Solutions

Hæmoglobin present in water solution facilitates the steady-state diffusion of oxygen through that solution[1,2]; the total oxygen flux is greater than the flux through an equivalent protein solution, for example, a solution of methæmoglobin. The hæmoglobin-mediated oxygen flux may be considered to be additive to the simple diffusion flux of oxygen. Fig. 1, which although obtained with the protein hæmerythrin is entirely typical of results obtained with hæmoglobin, reveals that the flux mediated by an oxygen-binding protein is constant in amount for a particular protein concentration and independent of the oxygen partial pressure difference across the solution at oxygen partial pressures sufficient to saturate largely the carrier. This property has the interesting consequence that at the low oxygen partial pressure presumably existing in tissue the hæmoglobin mediated flux would be expected to make up the largest part of the total oxygen flux. Quite possibly the facilitation of oxygen diffusion in the interior of muscle cells is a major function of myoglobin.

The mechanism of facilitated oxygen diffusion is not immediately apparent. The question arises whether some particular property of hæmoglobin is required or whether other substances that reversibly bind oxygen will facilitate

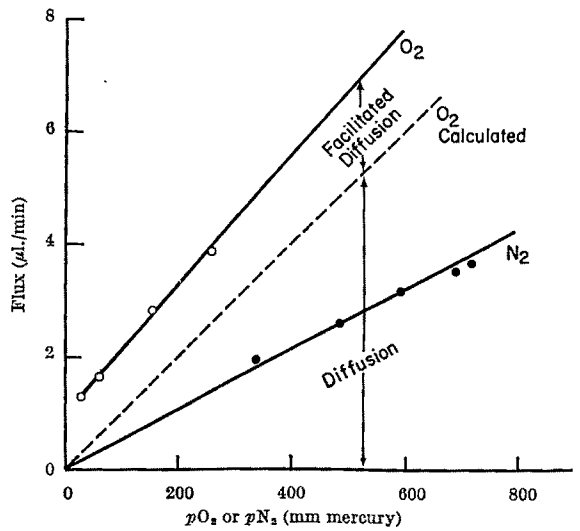


Fig. 1. Gas fluxes through a hæmerythrin solution. The solution is supported in a 'Millipore' filter (45 mm diameter) which separates two gas-filled chambers. The solid lines are the simultaneously measured fluxes of oxygen and nitrogen. The dashed line is the oxygen flux expected for simple diffusion

oxygen diffusion. The respiratory pigment hæmerythrin which occurs in the cœlomic corpuscles of certain marine worms offers an opportunity to test this question. Hæmerythrin (reviewed in refs. 3 and 4) is an iron-containing protein of molecular weight 105,000, which is thought to be made up of eight sub-units of molecular weight 14,000 (ref. 5). Each sub-unit contains two iron atoms and combines reversibly with one molecule of oxygen. No hæm group has been detected. The oxygen-combining capacity per weight of protein is approximately the same as in hæmoglobin.

A solution of hæmerythrin was prepared by lysis of washed cœlomic corpuscles from the sipunculid worm phascolosoma (*Golfingia gouldii*). The oxygen-combining capacity of this solution, estimated from the iron content ($2{\cdot}2 \times 10^{-2}$ g equiv. iron/l.), was 245 ml./l. The partial pressure of oxygen required for half-saturation of the hæmerythrin in this solution was estimated spectrophotometrically by the method of Wyman *et al.*[6] to be 2·2 mm mercury. The measurements described here were made at oxygen partial pressures greater than 25 mm mercury, which was sufficient to ensure full saturation of the hæmerythrin with oxygen.

A 'Millipore' filter (Type *HA*, Millipore Filter Corporation, Bedford, Mass.), wetted with the hæmerythrin solution, was clamped between two chambers, one of which contained thoroughly stirred mixtures of oxygen and nitrogen, saturated with water vapour. A rapid (80 ml./min) stream of water-saturated helium was passed through the second chamber. The helium stream was directed at the surface of the 'Millipore' filter to ensure rapid mixing with the oxygen and nitrogen passing through the hæmerythrin solution, and thus provide very low partial pressures of these gases at the 'Millipore' surface. The effluent helium stream passed to a gas sampling valve by means of which portions of the stream were introduced into a gas chromatograph for analysis. The flux of gases through the hæmerythrin solution is given by the product of the flow-rate and the fractions of oxygen and nitrogen in the effluent stream.

Fig. 1 presents the experimentally determined fluxes of oxygen and nitrogen as functions of the partial pressure of these gases in the mixtures exposed to the hæmerythrin-loaded membrane. The solid lines are drawn through the experimental points. The dashed line is the oxygen flux expected from simple diffusion. The latter can be established either by measurement in separate experiments or approximated from the determined nitrogen flux by the relation:

$$\text{Flux } O_2 = \text{Flux } N_2 \cdot \frac{\alpha O_2}{\alpha N_2} \times \frac{\sqrt{28}}{\sqrt{32}}$$

where α is the solubility coefficient in water, and 32 and 28 are the molecular weights of the gases. It will be noted that the determined oxygen flux is greater than the calculated diffusion flux by a constant amount. This difference is the hæmerythrin facilitated oxygen flux. It is of about the same magnitude as the oxygen flux facilitated by a hæmoglobin solution of equivalent oxygen-binding capacity.

It is concluded that the hæm group is not required for the facilitated diffusion of oxygen.

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Metabolism of Endogenous Substrate in the Retina

THE use of radioactively labelled material has made possible a direct appraisal of the proportions of metabolic products derived from a particular substrate in an isolated tissue. Such *in vitro* metabolic studies on the young retina have shown that the respiratory carbon dioxide produced by the tissue during the oxidation of labelled exogenous glucose substrate does not correspond to the measured oxygen uptake. Cohen and Noell[1] found in the young, non-functional rabbit retina that about 65 per cent of the respiration appeared to be due to the oxidation of endogenous non-carbohydrate material, even when adequate glucose was available to the respiring tissue, whereas in the adult rabbit retina glucose oxidation accounted for the major proportion of respiration.

In the developing rat retina, Reading and Sorsby[2], using universally labelled ^{14}C-glucose, found in retinæ from 10-day-old animals (that is, where the retina had not become functional) that the quantity of glucose converted to carbon dioxide corresponded to only 25 per cent of the total oxygen uptake. At 21 and 60 days after birth, glucose to carbon dioxide conversion corresponded to 55 and 67 per cent, respectively, of the oxygen uptake.

These findings suggest that the retina oxidizes an endogenous substrate material and the proportion of this substrate contributing to the respiration is greater during the early stages of development than after the tissue has undergone full differentiation and assumed its full functional capacity.

It was decided to investigate the characteristics of the respiration of the retina in the absence of added substrate, in comparison with retina in the presence of adequate glucose. In addition, determinations of respiratory quotient values for retina at different stages of development, in the absence of added substrate, were carried out in an attempt to throw some light on the nature of the endogenous substrate being utilized. Material for the experiments was obtained from black-hooded (*PVG*) rats which were originally obtained from Glaxo Laboratories, Ltd., and from Dutch rabbits. In all experiments, litter mates were used. Rats were killed by cervical fracture, rabbits by injection of air into an ear vein. The eyes were excised and retinæ dissected out as rapidly as possible, the tissue being kept under ice-cold oxygenated saline during these operations. Retinæ were incubated at 37° C for periods of 1 h in Krebs–Ringer phosphate medium[3] in an atmosphere of oxygen, using a conventional Warburg

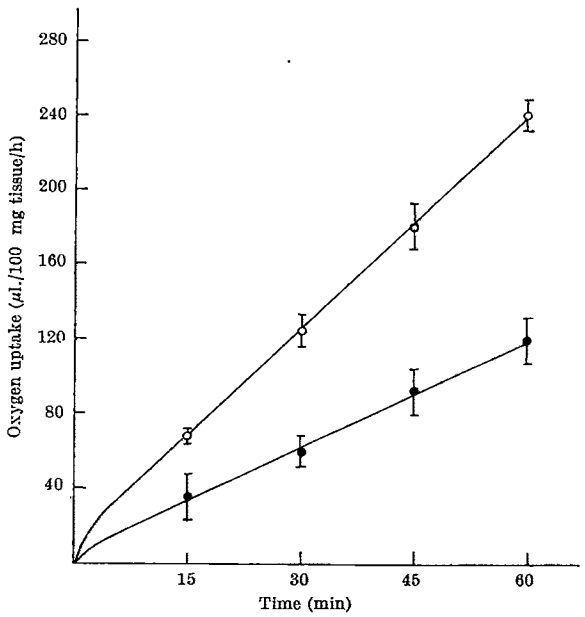


Fig. 1. Comparison of oxygen uptake by rat retina in the presence and absence of added glucose. Experimental details: Rats, black-hooded (*PVG*) litter mates, 40 days old. Four retinæ (wet weight approx. 40 mg) per flask, incubated in 1 ml. Krebs–Ringer phosphate medium at 37° C in an atmosphere of 100 per cent oxygen. ○, 0·1 per cent glucose in medium; ●, no glucose in medium. Horizontal lines show limits for each estimation

apparatus. Respiratory quotient determinations were made using the second method of Dickens and Simer[4]. This method requires two aliquots of relatively large weight of the tissue under examination and, in order to maintain homogeneity in the samples, rabbit rather than rat retina was chosen for respiratory quotient determinations. The time required for dissection and preparation of rabbit retina was much less than that for the rat, where a greater number of animals would have been required to produce a similar weight of tissue.

In the adult rat retina, without added carbohydrate substrate, oxygen uptake was linear over a period of at least 1 h. The rate of oxygen uptake was half that when glucose was available to the tissue, all other conditions being identical (Fig. 1). It would appear therefore, in the adult rat retina, that oxidation of endogenous material accounts for about 50 per cent of the measured oxygen uptake.

Table 1. Respiratory Quotients (R.Q.) and Qo_2 Values for Rabbit Retina in the Absence of Added Substrate

Age	R.Q.	Qo_2
15 days	0·75	−88·0
26 ,,	0·89	−94·0
70 ,,	1·02	−137·0

Qo_2 values expressed as mm³/100 mg wet weight tissue/h.

Respiratory quotient determinations carried out on retinæ taken from rabbits at ages 15, 27 and 70 days gave values of 0·75, 0·89 and 1·02 respectively (Table 1). These results are suggestive of a gradual change in the character of the endogenous substrate being oxidized, concomitant with development and functional maturation of the retina. In the young retina, where endogenous respiration is relatively high, the respiratory quotient value indicates an oxidation of material of a lipid nature. As development proceeds, the value of the respiratory quotient indicates a mixed carbohydrate–fat oxidation, and an entirely carbohydrate oxidation in adult tissue.

Futterman and Kinoshita[5] suggested lactate as the major endogenous substrate for retina, since aerobic lactic acid utilization by homogenates of cattle retina was unimpaired by the presence of sufficient iodoacetic acid to block glycolysis. This possibility seems to have been ruled out by the work of Cohen and Noell[1], who demonstrated, by the use of radioactive material, that glucose was the sole source of lactate carbon in the intact rabbit retina. This implies, of course, that in the absence of a source of carbohydrate lactate would not be available to the retina. In addition, there is the possibility that results obtained with retinal homogenates cannot be interpreted for the intact tissue, since it is well known that the metabolic characteristics of the retina are markedly changed by loss of integrity of the cells[6].

The classical researches of Warburg *et al.*[7] established the high rates of aerobic and anaerobic glycolysis in the retina, and, in consequence it has long been accepted that the mammalian retina depends almost entirely on glucose for its energy requirements. It seems likely, however, that the young developing retina to a large extent utilizes endogenous fatty material for oxidative purposes. The character of this substrate changes with increasing age, until at adult stages it is entirely of a carbohydrate nature. In addition, the capacity of the retina to utilize endogenous substrate of any nature decreases with increase in age.

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Secretion of Magnesium and Calcium in Parotid Saliva of Sheep

With a conscious sheep, Kay[1] confirmed the findings of Coats and Wright[2], obtained with anæsthetized sheep, that the concentration of Na^+ and K^+ in parotid saliva showed a strong inverse correlation. However, neither author reported the saliva concentrations of Mg^{2+} and Ca^{2+}. McDougall[3] estimated the average magnesium concentration in parotid saliva of sheep as 0·6 m.equiv./l. and that of calcium as 0·4 m.equiv./l. Average values of 0·36 and 0·33 m.equiv./l. for saliva magnesium and calcium concentrations, respectively, were obtained here.

Saliva samples were obtained from a conscious Merino × Welsh Mountain ram after the left parotid duct had been cannulated some days earlier by the method described by Ash and Kay[4]. Secretion rates were measured for each sample and the saliva concentrations of magnesium estimated by atomic absorption flame photometry, those of calcium by high-temperature emission flame photometry and those of sodium and potassium by low-temperature emission flame photometry. The sheep was maintained sodium replete by the daily intraruminal administration of 50 g sodium bicarbonate.

Fig. 1 shows the relationship obtained between saliva magnesium concentration, x, and saliva secretion rate, y. This could be expressed in the form:

$$y = 8{\cdot}0\ e^{-5{\cdot}3x}$$

A similar exponential relationship was obtained between saliva potassium concentration and saliva secretion rate by Coats and Wright[2].

The saliva Ca^{++} concentration showed no significant correlation with changes in saliva secretion rate within the range studied, 0·11–2·39 g saliva/min. In accordance with the results of Kay[1] and Coats and Wright[2], an inverse correlation was noted between the concentration of Na^+ and K^+.

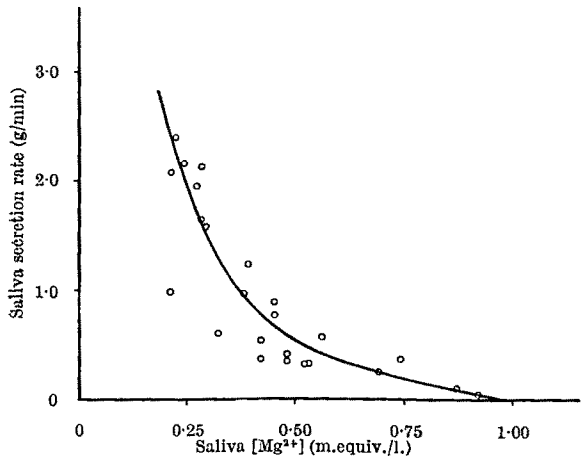


Fig. 1. Relationship between saliva magnesium concentration and saliva secretion-rate

The intravenous administration of 500 μg D-aldosterone was followed by a small fall in saliva Na^+ concentration, a marked rise in K^+ concentration and a small rise in Mg^{2+} concentration, after correction for the effect of variation in salivary secretion rate. There was no significant change in the saliva Ca^{2+} concentration. It thus seems likely that the re-absorption of Mg^{2+} from the salivary ducts is influenced in a manner similar to that of K^+ by the plasma concentration of aldosterone. This extends the similarity between the effects of mineralocorticoids on potassium and magnesium homeostasis already observed in the sheep by Care and Ross[5].

An increase in the plasma magnesium concentration to twice its normal value was associated with an increase in the saliva Mg^{2+} concentration, after correction for the effect of secretion rate, but there was no effect observed on the concentrations of Ca^{2+}, Na^+ or K^+.

No sudden increase was noted in the secretion rate of parotid saliva Mg^{2+} by a sheep, the diet of which was changed sharply from hay to heavily fertilized grass fed in the frozen state. A decrease in the efficiency of magnesium absorption is associated with the hypomagnesæmic response to such a dietary change[6]. Thus it is unlikely that an increase in the salivary secretion of Mg^{2+} is a contributory factor in the ætiology of this type of ruminant hypomagnesæmia.

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HÆMATOLOGY

Demonstration of a Hæmolytically Active 11 *S* Component of Rabbit, Guinea Pig and Human Serum by means of Antigen – Antibody Precipitates

Taranta, Weiss and Franklin[1] and Müller-Eberhard and Kunkel[2] independently described a heat-labile substance in normal human serum which was capable of precipitating soluble aggregates of γ-globulin. The latter authors[2] isolated the factor from aggregated γ-globulin precipitated with normal human serum in the presence of ethylenediamine tetraacetic acid (EDTA). They showed that it was a euglobulin with an S^0_{20w} of 11 and further demonstrated that it constituted a component of hæmolytic complement distinct from the known components of the human system. They termed it the "11 *S* component". It will be shown that this same component is also present in guinea pig and rabbit serum and that it can be removed from these sera with immune precipitates in the presence of EDTA.

Pools of rabbit, guinea pig and human serum were adsorbed with preformed, washed egg albumin–rabbit anti-egg albumin precipitates prepared at equivalence. The adsorption of each serum was carried out at 4° C in the presence of 0·01 M EDTA using 1·0 mg of precipitate nitrogen per 1 ml. of serum. Guinea pig and rabbit serum required only 15 min incubation time with precipitate although on occasion it was necessary to incubate rabbit serum for as long as 30 min in order to remove the hæmolytic activity. With human serum, however, the time of incubation had to be extended to 24 h and even then an additional adsorption with 0·5 mg preformed precipitate nitrogen per ml. of serum for 3 h was needed before a suitable non-hæmolytic serum was obtained.

The supernatant serum after removal of the precipitate and the addition of 0·01 M Ca^{++} and Mg^{++} was termed the R_{11S} (ref. 2); that is, a serum deficient in the 11 *S* component. The 11 *S* component used to restore hæmolytic activity to the R_{11S} was prepared from normal human serum using soluble aggregates of human γ-globulin exactly as described[2].

Table 1. Restoration of Hæmolytic Activity of Rabbit, Human and Guinea Pig R_{11S} by Isolated Human 11 *S*

Species	Dilution	CH_{50}/ml.			Percentage restored
		Original serum	R_{11S}	R_{11S} + 11 *S*	
Rabbit	1/10	46	0	57	124
Human	1/50	166	0	122	75
Guinea pig	1/300	900	0	563	63

Fig. 1 shows the percentage of lysis obtained when untreated rabbit serum, R_{11S}, and R_{11S} with 11 *S* were added to sensitized sheep red blood cells using the complement assay system described by Kent *et al.*[3]. The 11 *S* component used was in a volume of 0·1 ml. containing 7 μg of total protein. Essentially the same results were obtained using the R_{11S} prepared from human and guinea pig serum. However, as Table 1 shows, the restoration of hæmolytic activity of human or guinea pig R_{11S} by the

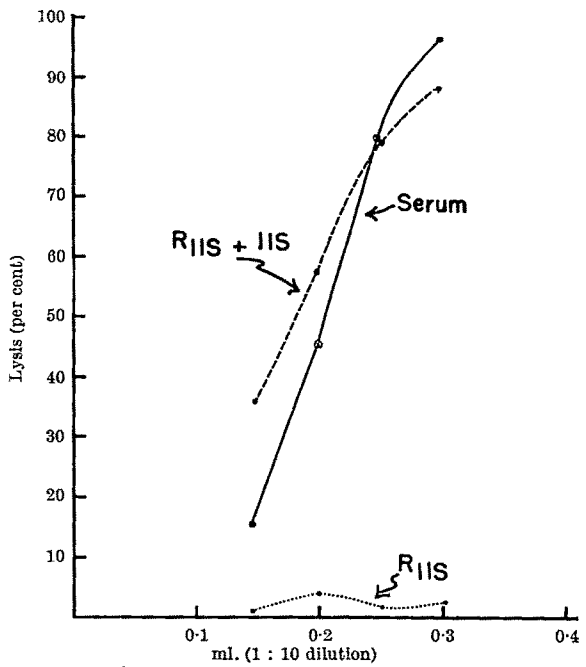


Fig. 1. Hæmolysis curves demonstrating loss of hæmolytic activity of rabbit serum by depletion of 11 *S* component (R_{11S}) and regaining of activity on addition of a preparation of isolated 11 *S*

human 11 S was not complete. In these experiments, there was 75 per cent restoration of the human and 63 per cent restoration of the guinea pig R_{11S}.

The 11 S component did not contain the first component of complement (C'1) and was inactivated by heating at 56° C for 30 min as described by Müller-Eberhard. It was also not inhibited by pre-incubation with 50 μg of soy bean trypsin inhibitor at room temperature for 15 min.

The lack of hæmolytic activity of the rabbit, human and guinea pig R_{11S} was not due to a lack of C'1. When tested with an $R1$ alone, hæmolysis was not obtained with any of the R_{11S}, but when tested with an $R1$ fortified with 11S the rabbit R_{11S} showed full C'1 activity, the human contained 77 per cent and the guinea pig showed 50 per cent of the C'1 activity of the original serum.

Whether there was an actual loss of C'1 activity in the preparation of the human and guinea pig R_{11S} or whether the foregoing results reflect the well-known inadequacy of the classical component reagents is not known.

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HISTOCHEMISTRY

Thalamic Microneurones

In view of the recent deduction by experimental neurophysiologists[1] of the presence of 'interneurones' in the region of the somato-sensory relay (ventralis posterior) nucleus of the thalamus, it would seem timely to recollect that such neurones are almost certainly demonstrable under the light microscope (and generous to recall that the presence of such *Schaltzellen* was deduced sixty-seven years earlier, from brilliant experimental and pathoanatomical observations, by von Monakow[2]). It is rather surprising that the first histological report of thalamic 'microneurones' (to the 1949 International Neurological Congress[3]) has not been confirmed by anatomists outside Germany[4,5]. The explanation probably rests largely in the fact that in most of the commoner mammals used in neuro-anatomical research the somata of the microneurones are too similar in size and shape to those of astrocytes to be distinguished with confidence. From extensive surveys in the macaque (in celloidin-embedded, well-differentiated cresyl-violet preparations) I have ascertained that the microneurones tend to be proportional in size to the macroneurones in most of the sub-nuclei of the thalamus, and hence largest in nucleus ventralis posterior pars gigantocellularis. Unfortunately, again, the most commonly studied sub-primates do not possess a conspicuous pars gigantocellularis. In the dog, however, it is well developed, and its microneurones are as prominent as in the macaque. In both the dog and the rabbit, but not so far in the macaque, I have obtained occasional thalamic sections, impregnated by the silver sulphide method and counterstained by methylene blue[6], in which the display of heavy-metal granules is distinctly more condensed, and hence blacker, in the cytoplasm of the astroglia than in both orders of neurones.

In man the somata of the thalamic microneurones are, in a majority of the sub-nuclei, larger and less rounded than those of the astroglia, and hence can be confidently counted here, especially in paraffin-embedded cresyl-violet-stained sections. Their incidence ranges from about 1 microneurone to 3 macroneurones in nuclei ventralis posterolateralis, anterior and dorsomedialis, through about 1 microneurone to 6 macroneurones in the other main sub-nuclei, to about 1 microneurone to 12 or more macroneurones in the intralaminar group and reticularis. (They occur also in, for example, habenular and mammillary nuclei.) They seem as evenly distributed throughout their respective sub-nuclei as are the macroneurones, and in Nissl stains do not give the impression of being specially related topologically to particular macroneurones or groups of these. Golgi preparations show no obvious inter-relationship of processes, either, between the two neurone types; but, of course, the one-in-forty selectivity of Golgi impregnation provides an unsatisfactory tool for testing visually the synaptic validity of von Monakow's *Schaltzellentheorie*[2] and the closely similar schema of the Canberra workers[1].

The most clear-cut picture of microneurones is obtained, in my experience, in combined 'Luxol' fast blue and cresyl-violet preparations from multiple sclerosis brains with plaques affecting the thalamus: topology is optimal, any lipofuscin is conspicuous, background myelin is minimal, and astroglial nuclei are characteristically 'activated'. Next in clarity come those of the normal anterior nucleus with its richly staining neuronal cytoplasm and its paucity of myelinated fascicles. In diabetics the particularly deep-purple character of the lipofuscin in 'Luxol' preparations helps appreciably in distinguishing microneurone features from astroglial ones; and Lemieux[7] has described how clearly the still more conspicuous cytoplasmic lipoids in amaurotic family idiocy show up "small type thalamic neurones". If Turner's[8] "big spider cells" in the human thalamus stained by methylene blue and peroxide were in fact microneurones, then I can confirm his observation of their pathology in paretic dementia, in so far as the microneurones appear to be preferentially neuronophaged in the thalamic sections preserved in this laboratory from a series of congenital G.P.I. brains. I have been unable to discover any consistent microneurone pathology in any category of organic brain condition now commonly seen in a hospital for mental disorders; have satisfied myself that these small neuronal entities offer nothing obvious towards elucidation of the pathogenesis of the schizophrenic or the affective psychoses; but have not investigated them in mental defectives where the brain is otherwise microscopically normal. In an isolated case of ether-anæsthesia catastrophe with 50-h survival[9], I had the impression that the thalamic microneurones had been earlier overwhelmed by hypoxic ischæmia than the macroneurones. On that occasion I speculated on their conceivable role as step-function 'switches' (in ignorance of von Monakow's term), analogous to those in Ashby's cybernetic homeostats, concerned in an attention-focusing mechanism.

In my original report[3], based mainly on brains from leucotomized patients with up to 5 years' survival of the operation, I said that thalamic microneurones "do not appear to degenerate after cortical damage". I have since confirmed this in the anterior nuclei of macaques with 8–15 months' survival of cingulectomy, and in the dorsomedial nuclei of brains from further leucotomy patients with less than 10 years' survival. Some of the most impressive microscopic fields displaying only microneurones, apart from proliferated gliocytes, are to be seen in these preparations. In patients with 9, 9½, 11, 12, 14 and 16½ years' survival of the operation, the centres of the largest gliosed zones in dorsomedialis of the last four lack microneurones as well as macroneurones, and there is then an intermediate halo in which normal numbers of microneurones become visible before normal numbers of macroneurones. (The one short-survival case which showed neurone-free haloed zones similar to this was the only one where the patient had been given a course of insulin-coma therapy subsequent to the operation.)

My interpretation of all these degeneration observations is that microneurones do not suffer significant retrograde degeneration, but do suffer substantial disuse atrophy within about ten years if widely isolated from surviving

macroneurones. Hassler[4] judged that his "thalamic internuncial cells" in the human dorsomedial nucleus, indistinguishable in his Fig. 14 from my "microneurones", suffer equal retrograde degeneration with the "specific" neurones after substantial cortical removals, but relatively less after very restricted lesions; and surmised that they project 'unspecifically' to the cortex. It may be that the tempo of degeneration is higher after wide cortical damage than after standard leucotomy; but in my judgment at least the majority of microneurones are wholly intrathalamic 'interneurones', susceptible to disuse atrophy rather than to retrograde degeneration, and I would still predict that they will be found to constitute structural essentials to the brain's attention-focusing mechanism, as well as to its electroencephalographic alpha-rhythm[1].

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Reversal of Solubility Characteristics of 'Luxol' Dye-phospholipid Complexes

'LUXOL' fast blue *G* (Du Pont), the diarylguanidine salt of a sulphonated azo dye, forms complexes stoichiometrically with phospholipids[1]. These complexes have been found to be insoluble in a varying pattern in the lower alcohols. For this study phospholipid-dye complexes were prepared with the same dye and phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositol, or sphingomyelin. The complexes were produced by bringing together the dye and phospholipid in pyridine solution. After evaporation of the solvent, excess uncomplexed dye or phospholipid could be removed by the alcohol in which the complex was insoluble. The solubility of the complexes in methanol, ethanol, isopropanol and isobutanol was determined. The results are summarized in Table 1. It was found that the complexes were soluble to the extent of at least 0·1 per cent or else were completely insoluble in the alcohols used. When alcoholic solutions of dye and phospholipid were mixed, precipitates were formed in those alcohols in which the particular complex had been found to be insoluble. The absorption maximum for the dye alone in the alcohols used is 597 mμ. When alcoholic solutions of the dye and phospholipid were mixed, the absorption maximum dropped to 570 mμ before precipitation took place. If the precipitate was then centrifuged and the deposit redissolved in methanol or an alcohol in which it is soluble the absorption maximum was found to return to 597 mμ. This can be taken as evidence of dissociation where complexes are marked soluble in Table 1.

With the foregoing *in vitro* data as a guide, we tried to devise a method for the differentiation of phospholipids in tissue sections. Only partial success was achieved. In sections of brain tissue some differential staining of the phospholipids present was accomplished when solutions of the dye in the various alcohols were used. However, in other tissues some non-phospholipid material was stained. Tryptophan, present in the necrotic areas in livers of experimental animals, stained intensely with the dye in isopropanol solution. In methanol the dye stained neither phospholipid nor tryptophan but collagen and elastin were stained selectively. Frozen unfixed tissues were employed with similar results.

The dye 'Luxol' fast blue *G* is not a specific histochemical reagent for phospholipids, but its staining affinities for certain tissue components vary with the solvent used. This may explain some of the anomalous staining of phospholipids found in the literature. Recently, Wolman[2] found that the affected neurones in three cases of Neimann–Pick's disease were not stained by 'Luxol' blue; whereas Baker's procedure for phospholipids was positive. Current 'Luxol' blue techniques utilize ethanol as the dye solvent, and from reference to Table 1, it will be seen that sphingomyelin does not form insoluble complexes with 'Luxol' fast blue *G* in this alcohol. As an increase in sphingomyelin storage can be expected in Neimann–Pick's disease, the *in vitro* data furnish a possible explanation for the non-staining of the affected neurones by 'Luxol' blue dissolved in ethanol.

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RADIOBIOLOGY

Effect of Multiple Injections of Calcium Compounds on the Survival of X-irradiated Rats

A MODERATE increase in the survival of X-irradiated rats has been observed when either parathyroid extract or ethylenediamine tetraacetate (EDTA) was injected before or after mid-lethal doses of X-rays[1–3]. The action of these substances has been attributed to bone resorption, which was thought to protect the adjacent radiosensitive bone marrow by exposing it to an increased concentration of newly-released calcium. In the work recorded here, we have tested this view directly by the injection of calcium compounds.

Adult males of a hooded strain of laboratory rat, weighing between 270–340 g, were used. Calcium was administered intraperitoneally as the acetate (0·12 M), lactate (0·12 M), or gluconate (0·067 M). The sodium salt of each of these compounds was also tested and administered in twice the molar concentration so that an equivalent amount of anion could be given in the same volume of fluid. All compounds were dissolved in a Krebs–Ringer salt solution[4] without phosphate buffer. The radiation source was a Philips constant-potential X-ray machine operated at 300 kV and 10 m.amp (half-value layer, 1·14 mm copper), with a target-object distance of 80 cm and a dose-rate in air of approximately 34 r./min. All rats received a whole body dose of 740 r. The animals were exposed 12 at a time in a revolving, partitioned, 'Lucite' cage. Each group of 12 consisted of 4 control animals (injected with Krebs–Ringer solution only), 4 animals injected with the sodium salt, and 4 animals with the calcium salt of the compound under investigation.

Table 1. SOLUBILITY OF 'LUXOL' DYE-PHOSPHOLIPID COMPLEXES

Dye or dye complex	Water*	Methanol	Ethanol	Isopropanol	Isobutanol	Pyridine
'Luxol' fast blue G	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
Phosphatidyl choline—dye complex	Soluble	Soluble	Insoluble	Insoluble	Insoluble	Soluble
Phosphatidyl serine—dye complex						
Phosphatidyl inositol—dye complex						
Phosphatidyl ethanolamine—dye complex	Soluble	Soluble	Soluble	Insoluble	Insoluble	Soluble
Sphingomyelin—dye complex	Soluble	Soluble	Soluble	Insoluble	Soluble	Soluble

* *p*H 7·0

Multiple injections were used to prolong the circulating concentration of the injected materials. The toxicity of the calcium compounds was a limiting factor, but 3 injections spaced 30 min apart were found to be non-toxic in the concentrations used (exception to this was afterwards found with post-irradiation treatment (see Table 1)). Consequently, in pre-irradiation treatment the solutions were injected 60, 30 and 5 min before X-irradiation, and in post-irradiation treatment injections were given 5, 30 and 60 min after X-irradiation.

The three calcium compounds tested, either before or after X-irradiation, were seen to produce a moderate increase in survival (Table 1). In two cases, that is, calcium lactate and calcium gluconate injected after X-irradiation, the increase in survival was apparently not specific for the calcium moiety, since injection of sodium lactate and gluconate also produced a similar increase in survival. In three cases, however, that is, pre- and post-irradiation treatment with calcium acetate and pre-irradiation treatment with calcium lactate, calcium appeared to be responsible for the enhanced survival. This effect of calcium is quantitatively comparable with the effects previously observed with parathyroid extract and EDTA, and supports the possibility that calcium is involved in the protective activity of these substances.

Table 1. Effect of Multiple Injections of Calcium Compounds on the Survival of Rats irradiated with 740 r. of X-rays

Test compound	Dose* (m.moles/kg/injection)	Time of injections† (before or after irradiation)	No. of 30-day survivors / No. used: Control (salt solution)	Sodium compound	Calcium compound
Calcium acetate	0·40	B	12/48	13/48	22/48‡
Calcium acetate	0·40	A	15/48	13/48	25/48‡ (7)§
Calcium lactate	0·40	B	15/48	13/48	25/48‡
Calcium lactate	0·40	A	9/48	19/48‡	19/48‡ (4)§
Calcium gluconate	0·67	A	10/48	19/48‡	24/48‡

* Twice as much of the sodium salt of each compound was given I.P. as that indicated for the calcium salt (see text).
† Each animal received three injections either at 60, 30 and 5 min before (B) or 5, 30 and 60 min after (A) irradiation.
‡ Significant ($P < 0.05$; chi-square) using only internal controls.
§ Indicates No. of deaths from calcium injection within 2 days after X-irradiation, apparently from potentiation of toxicity by X-rays.

Two other substances which may involve calcium in their protective action and which have not previously been mentioned[2] are inorganic condensed phosphates and magnesium sulphate. Condensed phosphates, which have recently been demonstrated as protective agents in mice[5], are known to form stable complexes with calcium and in some cases to produce hypocalcemic effects[6,7]. Magnesium sulphate, which has been reported to protect X-irradiated mice and rats[8,9], is known to cause bone resorption[10]. It is possible, therefore, that condensed phosphates could function as chelating agents similar to EDTA and that magnesium sulphate could act through its osteolytic action.

An effect of calcium on cell membrane permeability and the stability of chromatin material has been considered as an explanation of its action against radiation[2,3]. Recently, calcium has been found to reduce the erythrosin staining of X-irradiated thymocytes *in vitro*[11]; this might be interpreted as a prevention of change in membrane permeability. On the other hand, the role of calcium in chromatin stability may be supported by the recent finding that X-rays break the bonds between deoxyribonucleic acid and its protein and that this damage to the deoxyribonucleoprotein complex can be reversed by the addition of calcium ions[12]. This latter may be related to the chromatin condensing action of calcium and explain in part the reason that calcium prevents post-irradiation mitotic delay in cultures of *L* mouse fibroblast[13].

Since the action of parathyroid extract and EDTA, and possibly other substances, may function in animals through bone resorption, the release of organic constituents from bone should be considered as well as a release of calcium. In this respect, chondroitin sulphate, a possible product of bone resorption[14], has been reported as a radioprotective agent[15]. Although the release of organic constituents may be involved, the evidence at present would suggest an action of calcium as the most likely possibility.

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Induction of Mutations in Barley with Ultra-violet Radiation

Utilization of ultra-violet radiation (wave-length 2600 Å) for induction of mutations in higher plants has been limited to irradiation of pollen[1–3], in view of the low penetrating ability of this radiation. Though seed treatment with ultra-violet radiation had not been found effective, pre- or post-treatments with X-rays have been found to decrease or increase X-ray-induced mutation rate (depending on the X-ray dose) in hexaploid wheat[4,5] and oats[6]. From these consistent results, it was obvious that some effects of ultra-violet radiation were recoverable in spite of its low penetrability in seeds. Hence, it was decided to examine whether mutagenic response to ultra-violet alone could be enhanced by appropriate pre- and post-treatments.

To facilitate maximum penetration, naked barley seeds (var. *CN*. 292), in which the embryos are situated in the periphery, and thus easily accessible to treatments, were used. An ultra-violet germicidal lamp, with a maximum output (86 per cent) at wave-length 2537 Å, was used. The seeds were irradiated in monolayer arranged with the embryos facing the source at a distance of 15 cm. The maximum increase in temperature due to treatment was found to be 2° C. Soaking of the seeds in water for 4 h in room temperature (22° C) and oxygen saturation of seeds by subjecting them to high oxygen pressure of 70 atmospheres for 4 h were given as pre- or post-treatments. Ultra-violet treatment inhibited growth of seedlings to an extent of 27 per cent of the control at 7-day-old stage, which, however, recovered to normal condition later. No chromosomal aberrations were recorded in any of the treatments except for stickiness. The X_1 plants were gathered individually and the chlorophyll mutations were scored in the X_2 generation. The results are presented in Table 1. *albina*, *tigrina*, *xantha* and *viridis* were the types of chlorophyll mutations recorded.

Pre-treatment of oxygen is effective in increasing the mutation rate of ultra-violet, whereas post-treatment with oxygen has no effect. This implies that oxygen during treatment is essential for the enhanced effect. With our method of oxygen saturation, the oxygen becomes bound to the seeds in a way that it is very difficult to remove by evacuation[7]. The oxygen effect during ultra-violet irradiation has been demonstrated in several aqueous systems like DNA in solution[8]. It is known that the action

Table 1. Frequency of Chlorophyll Mutations in X_2 Progenies of Barley Seeds, Irradiated with Ultra-violet Radiation

Treatment	No. of X_1 families studied	% of families segregating for mutations	No. of X_2 plants scored	% of X_2 plants mutated
Control	135	0	9,722	0
Oxygen pressure (4 h)	50	0	3,450	0
Ultra-violet (4 h)	80	2·5	6,042	0·07
Oxygen—4 h + ultra-violet 4 h	65	6·2	4,262	0·14
Ultra-violet—4 h + immediate oxygen saturation	60	0	4,560	0
Soaking in water—4 h + ultra-violet (4 h)	127	7·1	7,877	0·35

of ultra-violet light is mainly through the excitation of the molecules and this may set in a prolonged free-radical reaction in the presence of oxygen[9]. This may be the probable explanation for the enhanced effect of ultra-violet light in the present case. Such reactions are short-lived and hence are not available for post-irradiation oxygen effect, unlike X- or γ-irradiated barley seeds, which respond to post-irradiation oxygen treatment[10]. The increased effect of ultra-violet light on soaked seeds appears to be due to one or more of the following three causes: (1) a better penetration of ultra-violet light in hydrated systems[9]; (2) the initial metabolism set in the seeds due to soaking, making them a more susceptible system; (3) the effects of ultra-violet light on water molecules which are in excess in soaked seeds and which can act indirectly on the genetic material. The failure to induce mutations with ultra-violet irradiation alone in hexaploid wheat[4,5] may be attributed to its high polyploid nature as well as the chromosomal origin of most of the induced mutations in this crop.

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In vivo Investigation of the Removal of Trace Elements from Nucleic Acids of Yeast by Ionizing Radiation

Investigations by several authors[1,2] have demonstrated that paramagnetic metal ions produce a certain amount of resistance to radiation in cells. This fact is due partly to the redox effect of these ions[3], and partly to the facilitation of forbidden transitions of excited electron states[4]. Since different nucleic acids contain trace elements in significant amounts[5], and since the biological activity of nucleic acids is dependent to a large extent on their trace-element content[6–8], it seemed essential to us to investigate the trace-element content of those most important macro-molecules of the cell before and after irradiation. It was also shown that by roentgen irradiation removal of Ca^{++} and Mg^{++} from nucleohistone is possible, whereby at the same time reduction in DNA occurs[9].

Determinations of the trace-element content were carried out by means of neutron activation analysis[10]. The removal of trace elements from nucleic acids was determined between 0 and 24,000 rads of cobalt-60 irradiation. Part of the trace elements was detected in the low molecular cell fraction after irradiation in this dose-range. Another part might be bound by metal binding molecules, or else might be re-bound at ionizing points caused by the irradiation. In this way the decrease of the trace element content at doses above 8,000 rads could be explained. Yet it seems that in the investigation range of dose no diffusion of trace elements occurs through the cell wall owing to changes in permeability.

Yeast was suspended in aqua bidest., and air was bubbled through for 5 h. For the irradiation carried out at 23° C the centrifugated yeast was re-suspended in aqua bidest. (20 ml./g yeast). Thon the yeast was centrifugated (distribution of trace elements in this centrifugate is given in Table 3), the low molecular compounds were extracted with 60 per cent ethanol and the nucleic acids with 1·0 M ammonium formate as recently shown[11].

Table 1. Trace Element Content (μg Ions per Sample) of Nucleic Acid of Yeast after Different Irradiation Doses (Mean Value of Six Different Cultures)

Dose (rad)	Cu	Mn	Ni	Zn	p
0	30·1 ± 3·0	5·9 ± 1·2	32·3 ± 6·0	130 ± 27	3,870 ± 190
3,000	27·7 ± 2·7	7·3 ± 1·3	30·0 ± 5·9	87 ± 18	3,720 ± 180
8,000	13·1 ± 1·4	3·7 ± 0·8	14·9 ± 3·1	66 ± 14	3,950 ± 200
24,000	15·9 ± 2·0	1·7 ± 0·7	10·1 ± 2·2	27 ± 9	3,637 ± 180

Table 2. Trace Elements (μg Ions per Sample) in the Alcohol Soluble 'Low Molecular' Fraction of the Yeast after Different Irradiation Doses

Dose (rad)	Cu	Mn	Ni	Zn	p
0	4·4 ± 0·4	1·6 ± 0·3	< 2·0	5·2 ± 1·0	581 ± 30
3,000	7·5 ± 0·7	1·2 ± 0·2	< 2·0	6·2 ± 1·1	530 ± 25
8,000	9·8 ± 1·0	2·2 ± 0·5	6·8 ± 1·5	8·8 ± 1·7	517 ± 26
24,000	8·4 ± 0·9	1·1 ± 0·2	15·6 ± 3·0	5·4 ± 1·1	528 ± 24

Table 3. Release of Trace Elements from the Cell caused by Irradiation in μg per Sample

Dose	Cu	Mn	Ni	Zn	p
0	0·34 ± 0·04	0·14 ± 0·03	2·4 ± 0·5	0·4 ± 0·1	15·4 ± 1·6
3,000	0·36 ± 0·05	0·15 ± 0·04	2·0 ± 0·4	0·5 ± 0·1	17·2 ± 1·8
8,000	0·38 ± 0·05	0·18 ± 0·04	2·2 ± 0·5	0·5 ± 0·1	19·1 ± 2·0
24,000	0·36 ± 0·05	0·16 ± 0·03	2·3 ± 0·5	0·4 ± 0·1	16·2 ± 1·7

The metal ions liberated from nucleic acids may cause a diminution of the biological activity of the macromolecules (eventual change of the tertiary structure). Moreover, they may cause slight damage (early stages) to the cell by operating as inhibitors or stimulators of enzyme systems[12].

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BIOLOGY

A Barley Endosperm Bioassay for Gibberellins

Since the review of bioassay techniques for gibberellins by Phinney and West[1], several new techniques have been proposed[2–4]. These are based on the increased elongation of plant tissues induced by gibberellins. Recent work has defined other biological characteristics of gibberellic acid (GA_3), notably a stimulation of the hydrolytic enzymes in germinating cereal grains[5]. Paleg[6–8] demonstrated that this response resides in the cereal endosperm, and the bioassay reported here was developed from procedures used in those studies.

A naked barley variety, Triple Awned Lemma, was used to obviate dehusking the grain prior to sterilization. Seeds were soaked in a freshly prepared sterilizing solution of bleaching powder (4 per cent w/v) for 24 h and rinsed ten times in 100-ml. lots of distilled water. Seeds were then cut transversely 3 mm from the distal end with a razor blade and the embryo-containing fragment discarded. The remaining endosperm portions were weighed in groups of 4, each group being placed in a 2 in. × 1 in. specimen vial containing 1·0 ml. of the test solution. Each vial contained 0·05 ml. streptomycin (10 mg/ml.), either 0·5 ml. GA_3 solution or 0·7 ml. plant extract and water to a total of 1·0 ml. The stoppered vials were placed in an oven maintained at 30° C for 48 h and samples of the ambient media were then assayed for reducing sugars by the procedure previously described[6]. A typical dosage-response curve is shown in Fig. 1. In all experiments duplicate observations were made at each standard GA_3 concentration and triplicate observations on each plant extract.

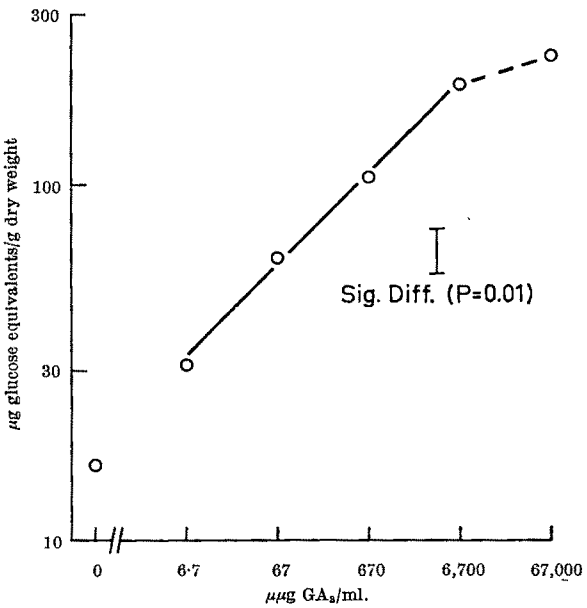


Fig. 1. Response curve of amounts of reducing sugars (glucose equivalents) released from barley endosperm treated with various doses of GA_3. The concentrations of the GA_3 solutions ranged from 2×10^{-11} M to 2×10^{-7} M. The curve represents the mean of three experiments

It has been found that 6·7 μμg (2×10^{-11} M) GA_3 gives a response which is generally significantly greater than water control and that the response at 67 μμg is invariably significant. In the dwarf pea and dwarf corn tests a significant response may be obtained from 1,000 μμg GA_3 applied to each of 10 plants, and Naylor and Simpson[4] have demonstrated a response to 250 μμg GA_3 in the bioassay based on germination of excised *Avena fatua* embryos. It is suggested that the smallest detectable amount in the barley endosperm bioassay is of the order of 10 μμg GA_3, a feature which enables the use of extracts of smaller quantities of plant material than has been examined hitherto[9]. For example, an ethyl acetate extract of 2 mg of freeze-dried barley inflorescences (3–4 inflorescences) was estimated, after evaporation and solution of the residue in water, to contain 2·4 μg gibberellic acid equivalents per gram dry weight. There was sufficient activity in this extract to obtain significant responses from 10- and 100-fold serial dilutions; the dosage-response curve was parallel to the standard.

Neither indolyl-3-acetic acid nor 6-furfuryl-aminopurine has been found to cause a response in the bioassay (in contrast to the recent report of Boothby and Wright[10]); but inhibitors of sulphydryl-containing enzymes do inhibit the sugar release. Gibberellins other than GA_3 also induce the release of reducing sugars from barley endosperm and hence the bioassay would appear to be a valid and sensitive bioassay for endogenous gibberellin-like substances.

One of us (P. B. N.) has held a C.S.I.R.O. senior postgraduate studentship during this work.

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A Periclinal Chimæra in Tea

Since the production of new morphological types in the course of grafting experiments of tomato on *Solanum nigrum* in 1910 by Winkler[1] and the description of the difference between the white-edged and solid-green leaf varieties of *Pelargonium zonale* by Baur[2], the spontaneous occurrence of chimæras, particularly in fruits and flowers, has been reported in many crops and ornamental plants. More recently, Sativa and Blakeslee[3] have reported the induction of chimæras by treatment of germinating seed with colchicine. The occurrence of a chimæra in tea of the type described, however, has not been previously reported. Gadd[4], in a discussion of non-infectious chlorosis in tea, says: "The yellow colour is due to lack of chlorophyll, though why it should not develop normally in particular zones is not known".

Chimæras are plants having genotypically distinct tissues lying adjacent to one another. The periclinal chimæra, which is the most stable, has an entire layer of tissue, one, two or more cells in depth, surrounding a particular organ.

The periclinal chimæra of tea (*Thea sinensis*, L.) described was found at Mount Vernon Estate in the Dimbula (Patna) region of Ceylon at an elevation of 4,017 ft. The plant was 5 ft. high and was bifurcated at the base of the main stem. One of the two main branches and its laterals produced only normal, green-coloured leaves, while the other branch and its laterals had leaves with a white-edged margin (Fig. 1).

The laterals on the branch producing normal leaves were at an angle of 45° from its main axis, while the laterals on the branch with white-edged leaves were at an angle of 60° from its main axis, the latter giving the

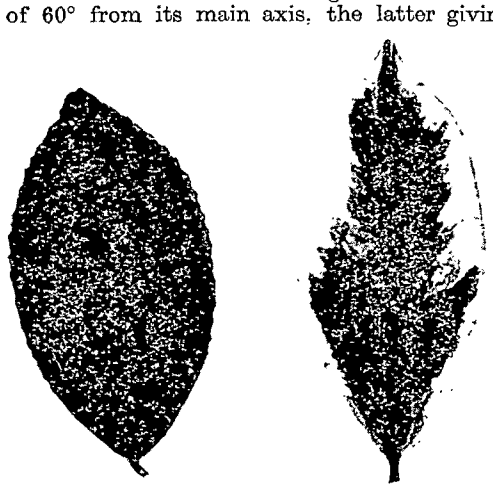


Fig. 1. Left, normal leaf; right, periclinal chimæra

appearance of a 'witches broom'. Cuttings propagated from the normal branch produced normal leaves only and cuttings propagated from the aberrant branch produced leaves with a white-edged margin only. This difference could be explained on the basis of a mutation in the bud primordium in the somatic tissue which produced the branch bearing leaves with a white-edged margin. Since the mutated, tissue surrounds the normal tissue, it is a periclinal chimæra.

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Maximum of Starch during Spring in the Woody Plants (*Vitis riparia* Michx.)

Investigation of carbohydrate metabolism, carried out with lignified grape-vine[1], proved the sugar⇌starch interconversion to be characteristic for this plant as in other woody plants[2]. The changes in the vines, both those which spend the winter on the rootstock, and the others cut off and stored in the pit, can be characterized by a sugar→starch conversion in the late summer, by a starch→sugar in autumn, respectively, early in the winter, and at the beginning of spring once more by a sugar→starch conversion[3].

It is the changes in the carbohydrate during the early spring period which interest us here.

In the course of our experiments the grape-vine (*R. portalis*) was brought, at the time of the sugar-maximum, in two different pits to a temperature of about +8°–10° C and +25°–30° C, respectively. The content of carbohydrate was determined monthly by means of anthron-reagent[4].

The beginning and the course of the synthesis of starch, just as the values of the starch-maximum, are dependent on the temperature. At a temperature of about +8°–10° C there is no decrease in the total values of the carbohydrates, until the development of the starch-maximum. This very fact indicates little respiration. It is remarkable how rapidly the decrease of the total carbohydrate—as a result of an intensified respiration[5]—begins immediately after the starch-maximum.

At +25°–30° C the carbohydrate reserve disappears quickly, and at the same time new organs are formed through the energy set free by it—callus-tissues, shoots and roots.

The obligate connexion of the respiratory oxidation with the phosphorylation is a well-known phenomenon[6].

$$\left.\begin{matrix} DPNH_2 + 1/2\ O_2 \\ +3\ ADP + 3\ P \end{matrix}\right\} \rightarrow \left\{\begin{matrix} DPN + H_2O \\ +3\ ATP + 3\ H_2O \end{matrix}\right. \quad (1)$$

It appears that the intensity of respiration is determined by the intracellular ADP : ATP quotient[7], and indirectly by the biosyntheses promoting ATP[8]. The intracellular localization of ADP and that of ATP must also be taken into account, since the diffusion through the membranes of the mitochondria is an easy matter for ADP, whereas ATP permeates it in a limited way, though it can be rapidly used by the different organs of the cell[9].

The changes of carbohydrate are the result of the amylophosphorylase, acting in both directions[10]:

$$\text{glucose-1-phosphate} \rightleftharpoons \text{starch} + PO_4^{3-} \quad (2)$$

The synthesis of starch begins with the phosphorylation of either glucose or fructose:

$$\text{Glucose (fructose)} + ATP \xrightarrow[\text{hexokinase}]{} \text{glucose (fructose)-6-P} + ADP \quad (3)$$

Hexokinase therefore requires ATP for the synthesis of starch. This enzyme is one of the most important precursors of the 'glycolysis'. The sudden beginning and the subsequent stagnation of the carbohydrate utilizing respiration (Fig. 1*a*), must be assigned to the change of the phosphate acceptor-concentration. On this spot ADP acts as a feedback mechanism[6]. The most probable explanation for this phenomenon lies in the supposition of Hess and Chance[9]. Accordingly, the hexokinase uses the ATP-quantities present in the cytoplasm:

$$ATP \rightarrow ADP + PO_4^{3-} \quad (4)$$

ADP permeates, as an acceptor for the oxidizing phosphorylation, from the cytoplasm into the mitochondria, inducing there the 'endogenous respiration'. At a low temperature, this process will take a time as long as the ADP—quantities of the mitochondria, for their greatest part, become phosphorylated into ATP. The respiration must then stop, because—as contrasted with Fig. 1*b*—there is no on-going process of organization utilizing energy. The permeation of the intramitochondrial ATP into the cytoplasm is, in fact, highly limited and the syntheses bound to the mitochondria are still inhibited at this low temperature. As a consequence of deficiency in ATP-quantities by the cytoplasm, the activity of the hexokinase (3), and thereby the amylophosphorylation (2) is limited. In case the syntheses are not inhibited by low temperature (Fig. 1*b*), the starch-maximum becomes lower and the spontaneous regulation of the respiration blends.

By reason of the considerations put forward in this communication, we continue to uphold our earlier hypothesis, according to which the starch may serve as a direct substrate for the respiration[3]. In this way the synthesis of starch before the sprouting is energetically established, because, under these conditions, the release of the glycolytic phase of the respiration from the 'pre-

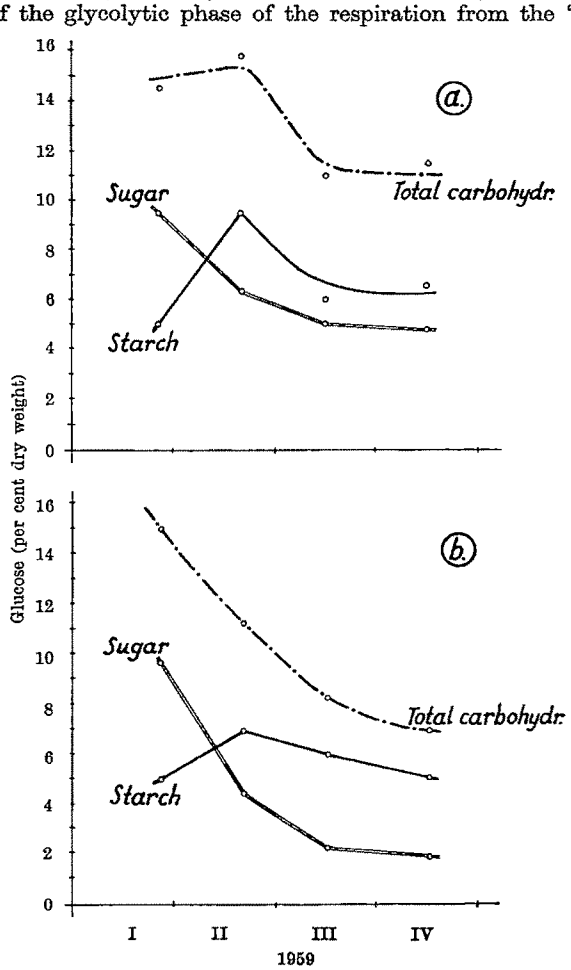


Fig. 1. Changes in the carbohydrate content of the lignified vines stored at different temperatures. Storage temperatures: *a*, 8°–10° C; *b*, 25°–30° C

cursory' control of the ATP-requiring hexokinase is just possible. The high ADP- and PO_4-concentration, at the same time, will ensure the possibilities of a quick oxidation for the substrates of respiration. The respiratory energy-production—the oxidizing phosphorylation—thus has a free path for the intensive biosyntheses related to sprouting.

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Heat-death Temperature in *Allolobophora terrestris* (Sav.) forma *longa* (Ude) and *Eisenia foetida* (Sav.)

An endeavour to deparasitize earthworms by heat in order to obtain infection-free hosts (for experimental purposes described elsewhere[1,2]) revealed an interesting difference of probable ecological significance between the two species *Eisenia foetida* (Sav.) and *Allolobophora terrestris* (Sav.) forma *longa* (Ude) (henceforth referred to here as *Allolobophora longa*).

The method of deparasitization by heat was used by Macdougall[3] to kill the gregarine parasites in the larvæ of *Tenebrio* sp. To make use of this method with earthworms it was first necessary to determine the heat-death temperature of the worms under specific conditions. Smith[4], using crude methods, observed that temperatures of 35°–40° C were fatal to *Allolobophora foetida* Sav. (= *Eisenia foetida* (Sav.)) but she did not specify the duration of exposure nor the pre-condition of the worms. Wolf[5,6], using very small numbers of specimens (six per trial), determined the heat-death temperature for *Lumbricus terrestris* (L.) as 28° C for an exposure of 400 min. In the experiments described here it was decided to determine the highest temperature which worms could survive after 12 h exposure (as long exposures were more likely to be effective in deparasitizing worms). The criterion of survival adopted was 80 per cent survival in soil at room temperature, 48 h after the end of an exposure of 12 h to the temperature under review. All worms used in the experiments were adult, and had been acclimatized in the same soil at a mean laboratory temperature of 15° C for a period of at least four weeks.

Worms were exposed to the differing temperatures by floating them individually in boiling tubes in thermostatically controlled water baths. Each tube was ballasted with clean moist gravel to make it float with the lip of the tube an inch above the water surface. Under these conditions the interior of a tube, at the surface of the gravel, attained the same temperature as the water bath in less than ten minutes after immersion. Worms were subjected to a particular temperature in batches of 20. They were taken from well-fed laboratory cultures, and washed in water at laboratory temperature before being placed in the boiling tubes floating in the water bath. 12 h later the worms were removed and placed individually in soil in lidded glass dishes and then examined after a further 48 h. Batches of 20 control worms were floated under similar conditions in water at room temperature.

In order to incorporate a confirmation procedure into the experiments (and also to shorten the total time taken) observations were made with two separate water baths—one to begin with a temperature near the lower limit for heat-death given by Wolf[6] and the other to begin with a temperature near the upper limit given by Smith[4]. Thus one bath was maintained at 20° C while the other was at 38° C for the first trial. Specimens of both species were placed in each bath. Successive approximations were then made, using fresh batches of worms for each water bath for each trial, making the 'cool' bath hotter and the 'hot' bath cooler. After the second trial the difference between the species had become sufficiently obvious to enable them to be segregated, *E. foetida* into the 'hot' bath and *A. longa* into the 'cool' bath. Trials were continued until the criterion of survival was satisfied in each case.

In the case of *E. foetida* the critical temperature was 33·3° C; in the case of *A. longa* it was 25·7° C. This very marked difference in heat-death temperature not only points to a difference in the powers of physiological adaptation of the two species but would also explain the ability of *E. foetida* to survive the relatively high temperatures in compost heaps. It may also be associated with the habit of æstivation in the deeper soil layers, seen in *A. longa* in spells of hot dry weather.

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ENTOMOLOGY

Effect of Low Temperature on the Induction of Imaginal Differentiation of Dauer Pupa of the Silkworm

In lepidopterous insects (in which diapause occurs in the pupal stage), the pupal brain is activated by treatment by low temperature[1–5]. It has not yet been established, however, whether artificially diapausing, brainless pupæ (dauer pupæ) are activated by low temperature. Therefore, this question has been investigated by chilling dauer pupæ of the silkworm, *Bombyx mori*.

Bombyx pupæ of F_1 hybrids between two strains, *J*.122 and *C*.115, from the Sericultural Experiment Station, Tokyo, were used as specimens. The brain was extirpated from each individual immediately following pupation[6], and the brainless pupæ were kept for 56 days at 25° C following ablation.

Three groups of dauer pupæ, 56 days old, were maintained for 20, 40, and 60 days at 5° C respectively. Afterward, animals in each group were kept for 30 days at 25° C. The control consisted of non-chilled dauer pupæ at the same age already mentioned which were observable for 46 days at 25° C following operation.

Moths did not appear in group 1 for 30 days after chilling (Table 1). However, two out of twenty-five dauer pupæ in both group 2 and 3 emerged as moths in 23–26 days following treatment, but no other animals emerged for 30 days. Moreover, of twenty-five dauer pupæ used as controls, four animals emerged as moths respectively on days 132 (♂), 138 (♀), 138 (♂), and 146 (♀) following removal of their brains. Other animals did not show any sign of imaginal differentiation for 146 days.

After treatment with either oxygen or carbon monoxide, more than half of the dauer pupæ tested emerged as moths in 30 days following treatments[7]. Also, when a fresh brain was implanted into each dauer pupa, the implanted animal became a moth in 18–25 days following implantation[8]. On the contrary, the effect of low temperature on induction of imaginal differentiation of each dauer pupa is very weak, if present at all, as demonstrated in Table 1. Moreover, as suggested in Kobayashi,

Table 1. Effect of Chilling on Imaginal Differentiation of Dauer Pupæ

Groups of exp.	Duration of chilling (days)	No. of specimens	No. of moths emerged* (days to emergence following chilling)	No. of undeveloped pupæ
1	20	25		25
2	40	25	1 (23), 1 (36)	23
3	60	25	2 (23)	23

* During 30 days at 25° C (following chilling).

Fukaya and Mitsuhashi's work[9], most dauer pupæ emerged as moths in 120–300 days following extirpation of brains under the same conditions.

Because of the reasons mentioned here, and the results presented, it is not likely that low temperature affects the induction of imaginal differentiation of dauer pupæ, that is, the prothoracic glands in dauer pupæ are not usually activated by treatment with low temperature, although a few animals did emerge as moths.

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Feulgen – Schiff's Technique for Examination of the Distribution of the Intracellular *Rickettsia*-like Micro-organisms in Whole Mounts of Ticks, or their Tissues

Intracellular *Rickettsia*-like micro-organisms are known to exist naturally in ticks, usually occurring in the Malpighian tubules and oocytes[1,2]. Their distribution in the organs of the ticks has been, in the past, studied by various investigators[3,4] from conventionally stained smears and sections of tick tissues.

Histochemical methods for nucleic acids, including the Feulgen technique, have recently been used in this laboratory with sectioned material of ticks[5,6] to demonstrate in virtue of the persisting DNA content of the micro-organisms, their occurrence and distribution in the tick tissues. This type of work, however, entails dissection and serial sectioning of a considerable number of ticks.

Recently, Newcomer[7] has introduced for serial-section studies a histochemical technique, in which tissues are fixed in isopropanol fixative[8], then treated with Feulgen–Schiff's reagent before embedding. However, by modifying Newcomer's technique, I was able to overcome this tedious matter of surveying ticks in serial section and to observe directly in whole dissected ticks under the binocular microscope the occurrence and distribution of the intracellular *Rickettsia*-like micro-organisms which are to be found in their Malpighian tubules and developing oocytes. The modified technique, depending on selective straining of the DNA of the micro-organisms, is as follows.

Ticks are dissected in 0·9 per cent saline. The dorsal integument is gently separated from underlying tissues, and the alimentary canal removed. The organs thus exposed are then covered *in situ* with the fixative, isopropanol mixture, and kept thus for 1–2 days. Hydrolysis of the dissected ticks follows in N hydrochloric acid at 60° C for 10–20 min. The ticks are then treated with Schiff's solution, prepared according to the method of Newcomer[7], for 20–30 min, and washed in sulphurous solution several times. After washing in running water, they are passed through graded alcohols up to 70 per cent. The treated ticks can then be fixed in a dissecting dish and examined in 70 per cent alcohol for the micro-organisms under the binocular microscope. Moreover, pieces of Malpighian tubules and oocytes could also be removed at this stage from the ticks, dehydrated, cleared in xylene and mounted for microscopic examination in D.P.X.

As would be expected with a DNA-selective stain, tissue nuclei persisted and were stained, in this technique, purplish-red. In addition the DNA-positive *Rickettsia*-like micro-organisms were also seen in the cytoplasm of Malpighian tubule cells and of oocytes as purplish-red aggregates. Their distribution was readily determined, and confirmed by microscopic examination of mounted pieces of those tissues.

Several species of argasid and ixodid ticks have been investigated in this way, and different patterns of distribution of the micro-organisms observed particularly in the Malpighian tubules. The results of these investigations using this and other techniques are in preparation for publication.

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MICROBIOLOGY

Host-range of *Proteus morganii* Bacteriophages

Colicine *H*, which kills many *Escherichia, Shigella, Salmonella* and a few *Proteus hauseri*[1] also attacks many strains of *P. morganii*[2]. The relationship between *P. morganii* and the other genera mentioned has recently been strengthened by the finding[3] that the guanidine + cytosine content of their deoxyribonucleic acids are very similar and differ from *P. hauseri, P. rettgeri* and *Providence* strains. On the other hand, it has been shown[4,5] that although *P. morganii* strains are not susceptible to *P. rettgeri* phages, many *P. morganii* organisms are attacked by *P. hauseri* and *Providence* phages. There is also a well-known antigenic relationship between *Proteus morganii* and *P. hauseri*. Work on *P. morganii* phages has been confined to temperate varieties, and host-range determinations were limited to the *Proteus* group of organisms[2,6]. The phages were found to be species specific. In view of the uncertainty[7] about the taxonomic position of *Proteus morganii*, it was decided to investigate the action of *P. morganii* phages derived from both lysogenic strains and sewage on members of the family Enterobacteriaceae.

The 17 strains of *Proteus morganii*, 28 strains of *P. hauseri*, 22 strains of *P. rettgeri* and the 24 *Providence* strains used have been described[5]. The other Enterobacteriaceae comprised 21 *Salmonella* serological varieties,

30 isolates of *Escherichia coli*, 4 serological varieties of *Shigella flexneri*, 3 varieties of *Sh. boydii*, 2 strains of *Sh. schmitzii*, 4 of *Sh. sonnei* and 30 strains of *Serratia marcescens*, of which 15 were from the National Collection of Type Cultures. Media and techniques were as previously described[4,5].

Phage was isolated from sewage against 16 of the 17 strains of *P. morganii*. In many instances a lysate produced more than one kind of plaque but further work was confined to one phage/organism. All 17 strains of *P. morganii* proved lysogenic by ultra-violet induction for one or more of the *P. morganii* or other strains tested. Because of the complete success of the ultra-violet method of induction no other methods were used to demonstrate lysogeny. All phages could be differentiated by their host-ranges.

Eight strains of *P. rettgeri* were lysed by 1 or more of 10 of the sewage phages, and 9 of the *Providence* cultures were attacked by 1 or more of 3 sewage and 4 temperate phages. Twenty-one of the *P. hauseri* strains were lysed by 1 or more of all 17 sewage and 6 of the phages derived from lysogenic strains. None of the 33 phages had any action on the *Salmonella* strains and only 1 temperate and 1 sewage phage productively lysed single strains each of *S. marcescens*. On the other hand, 10 strains of *E. coli* and single strains of *Sh. sonnei* and *Sh. boydii* were susceptible to one or more of 5 temperate and 14 of the phages derived from sewage. The efficiency of plating of all the phages on heterologous hosts approached unity and no abortive infections were noted.

Isolated instances of common antigens present in *Escherichia*, *Shigella* and *P. morganii* have been described[3], but these groups of organisms certainly do not possess the degree of susceptibility to individual phages which they exhibit to colicine *H*.

The *Proteus* group of organisms are regarded as less closely related to *Escherichia*, *Shigella* and *Salmonella* than the latter are related to each other[2]. The work recorded here has shown that *P. morganii* phages possess a degree of extra-genus activity far greater than other *Proteus phages*[4,6] and this may support (along with other evidence mentioned) an intermediate position for *P. morganii* linking the genera *Escherichia* and *Shigella* to the *Proteus* group of organisms.

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Overnight Incubation Technique for obtaining Transformable *Bacillus subtilis* Cells of Reproducible Competency

The use of *Bacillus subtilis* for DNA-mediated transformation investigations has found wide acceptance since the system was described by Spizizen[1] in 1958. In all bacterial transforming systems, technical difficulties arise in having available at the desired time a recipient bacterial population containing a high per cent of transformable (competent) cells. The usual preparation of *B. subtilis* recipient cells involves overnight incubation in broth, a 4-h period in growth medium followed by a 90-min period in transformation medium before maximum competence is obtained[2]. These freshly prepared cells are highly competent, although the competence-level will vary and cannot always be predicted.

In order to obtain reproducible results and to shorten the preparatory procedures for obtaining competent cultures, many investigators have utilized frozen cells. *B. subtilis* cells are rapidly frozen in a glycerol-containing medium either just before or just after the 90-min period in transformation medium. Cells frozen before the attainment of competence may be stored at $-20°$ C (ref. 3) while the already competent cells are stored at temperatures below $-40°$ C (ref. 4). In my experience, any slight deviation above $-40°$ C in the latter case resulted in a drastic loss of competency. In neither case was it possible to predict the competency from day to day over a long period of time. Recently a technique has been reported for freezing replicate competent cultures in liquid nitrogen and storing them at a temperature of $-196°$ C (ref. 5). Although cultures prepared by this method may give more precisely reproducible transformation frequencies, the practical advantages of the present procedure would seem to make it ideal for most experimental purposes.

The routine method reported here for obtaining transformable *B. subtilis* cultures produces cells the competency of which can be closely predicted over a long period of time, uses a convenient overnight incubation technique and has the added practical advantage that the frozen cell inoculum may be stored at $-20°$ C. A satisfactory uniform inoculum is obtained by the use of cells which have been propagated on synthetic growth medium for 4 h prior to freezing. These cells can be used for several months. For daily work a culture is incubated overnight at 30° C in synthetic growth medium rather than the usual 4-h period at 37° C. Cells usually develop the maximum potential for competence during the latter part of the logarithmic growth phase[2]. For overnight culture, the growth rate is further decreased by using a small inoculum and less vigorous aeration. Under these conditions, the maximum potential for competence is attained at 16 h and does not decrease until about 18 h.

B. subtilis strain *SB*-19 was used as the DNA donor and the indole-requiring strain 168 as the recipient. The synthetic growth medium and transformation medium utilized have been described[2]. A temperature-controlled water bath-reciprocal shaker (Research Specialties, Richmond, Calif.), stroke length 1·5 in., was used for aeration. Erlenmeyer flasks of 125-ml. capacity containing 20 ml. of medium were used for growing all cultures. Transformation was conducted in 18 mm × 150 mm culture tubes, with metal caps, containing a total volume of 1 ml. Transformant colonies, with no interfering background growth, were easily counted after overnight incubation on the following medium. Spizizen's minimal medium[2], solidified with Oxoid 'Ionagar No. 2' (1 per cent) or Difco 'Noble Agar' (1·3 per cent) was autoclaved at 15 lb. pressure for a time sufficient to cause the agar to become slightly amber. Glucose was added aseptically after autoclaving as also was vitamin-free casein hydrolysate-acid (Nutritional Biochemical Corp.) to a concentration of 0·02 per cent. With less autoclaving time, the agar was white and only pinpoint-sized transformant colonies developed after overnight incubation. Colonies on amber agar were much larger, but colony count was not affected. The nature of the growth-promoting response has not been investigated. Total viable counts were performed on the same medium supplemented with *l*-tryptophan, 5 μg per ml.

For preparation of the cells for freezing, strain 168 was grown overnight in 'Penassay Broth' (Bacto Antibiotic Medium 3) with vigorous aeration at 37° C. At the end of 16 h there were $3–5 \times 10^8$ colony formers per ml. The broth-grown cells were sedimented, washed once with growth medium and carefully resuspended in 10 volumes of growth medium to give a homogeneous suspension. The flasks were incubated with slow shaking, 50 c.p.m., for 4 h at 37° C. After centrifugation, the cells were resuspended in 0·75–1 volume of Spizizen minimal medium[2] containing 5 per cent glycerol and dispensed, 1 ml. per screw cap tube.

Table 1. Reproducibility of Results obtained with Frozen Cell Inoculum and Overnight Incubation in Growth Medium at 30° C

Exp.*	Transformants/ml.† ($\times 10^4$)	Total colony formers/ml. ($\times 10^8$)	Transforming activity (%)
1	2·8	2·2	0·013
2	2·3	2·2	0·011
3	2·6	2·5	0·010
4	2·7	2·3	0·012

* Experiments performed on different days over a period of two weeks.
† Used 0·02 μg DNA/ml. in transformation tube.

After standing about 20 min, the cells were quickly frozen in a dry ice–ethanol mixture and stored at −20° C. The final cell concentration was $1{\cdot}5–2 \times 10^8$ per ml. If greater aeration was provided during the 4-h growth period by increasing the shaker speed or altering the volume to surface ratio, the cultures were not as satisfactory for subsequent preparation of competent cells.

The transformation procedure finally adopted for daily use with the frozen cell inoculum is as follows: All incubations are carried out at 30° C. The cells are thawed, diluted to give $2–4 \times 10^7$ cells per ml., and 0·1 ml. is inoculated into 20 ml. of growth medium. The flask is incubated overnight with shaking, about 100 c.p.m. At 16–18 h, the cells are sedimented and resuspended carefully in transformation medium to give usually a 1 : 3 or 1 : 5 dilution The best dilution to use must be determined with each new batch of frozen cells. After dispensing 0·9 ml. per tube, the cultures are shaken at 125 c.p.m. (not critical) for the usual 90 min (ref. 2). After a 30-min contact with DNA and a 10-min reaction with deoxyribonuclease, the cells are ready to be diluted and plated.

From the results given in Table 1, it can be seen that consistent and predictable results are obtained from day to day. The number of viable centres in the transformation tube and the fraction which becomes competent remain quite constant. With different lots of frozen cells, the transformation efficiency with limiting concentrations of DNA (0·02 μg per ml.) varied from 0·01 to 0·07 per cent. Since about 2×10^8 cells/ml. were present in the transformation tubes, this frequency was very satisfactory for quantitative assays of transforming activity. Freshly prepared competent cells transform frequently at an efficiency of 1–2 per cent with saturating concentrations of DNA[2].

Several batches of frozen cells have been prepared and all have given satisfactory results over a period of several months. Since the procedure was developed empirically, the optimum conditions for transformation efficiency probably have not been achieved. It has been noted, however, that neither 27° C nor 37° C were satisfactory for overnight incubation in growth medium. At 30° C, raising the shaker speed from 100 to 120 c.p.m. or increasing the inoculum 2–3 fold caused the peak for maximum potential for competence to occur at 12 h, which was not suitable for overnight incubation. Varying the time in transformation medium gave no added advantage.

Saito *et al.*[6] reported overnight growth of a *B. subtilis* heat-shocked spore suspension at 27° C and subsequent transformation by a 2-h contact with 30 μg/ml. of DNA at 37° C. The long contact with DNA was necessary for greatest transformation efficiency. In the procedure reported here, both growth and transformation incubations were done at one temperature (30° C) and a 30-min contact with limiting concentrations of DNA gave high yields of transformants.

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VETERINARY SCIENCE

Formation of Histamine in Ruminal Fluid

Histidine and other amino-acids normally undergo deamination in ruminal fluid[1], and decarboxylation with the formation of histamine does not normally occur in the rumen to any great extent. When sheep or cattle are fed a diet containing excessive amounts of carbohydrate, however, the histamine content of ruminal fluid may increase considerably and concentrations >70 μg/ml. have been recorded[2]. Van der Horst[3] has shown that the concentration of amino-acids and amines in ruminal fluid can be increased by incubation with glucose.

In the investigation recorded here ruminal contents have been incubated with glucose under a variety of conditions and the concentrations of histamine formed have been assayed biologically on the guinea pig ileum preparation. Fluid was obtained from half-bred ewes fitted with permanent ruminal cannulæ. Glucose (20 mg/ml.) was added and samples were incubated for periods of 3, 18, 24, 48 or 72 h. In some experiments 1·5 mg/ml. L-histidine was also added. Histamine concentrations were compared with those obtained from identical samples containing boiled ruminal fluid. (The histamine concentration of the latter never exceeded 0·1 μg/ml.)

Results for 2 such experiments with fluid from the same sheep are shown in Fig. 1. Incubation for periods of more than 3 h caused a fall in *p*H, which thereafter remained at 4·0–4·5. Samples incubated for less than 24 h produced little histamine. The concentration of histamine did not increase much after 24 h, but in one experiment there was a further rise during the period 48–72 h. Incubation of samples from other sheep gave similar results.

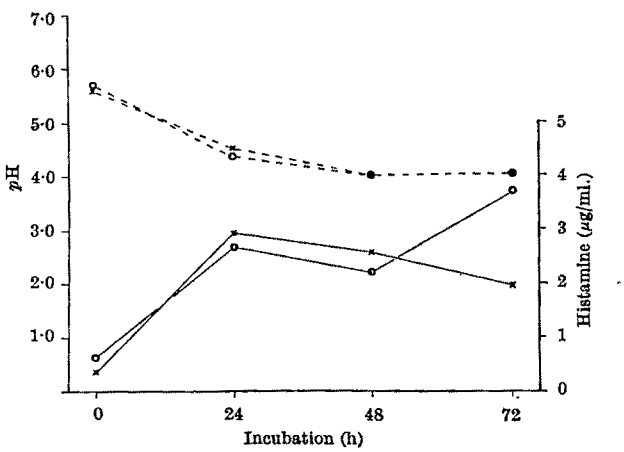


Fig. 1. Histamine formation and changes in *p*H of ruminal fluid incubated at 37° C with 2 per cent glucose. – – –, *p*H; ——, histamine content; O, Exp. 1; ×, Exp. 2

Histamine formation was not greatly increased by the addition of L-histidine to give a concentration of 1·5 mg/ml. Pyridoxal 5-phosphate, reported to be a co-enzyme of tissue histidine decarboxylase[4], was added to some samples without effect.

Excessive carbohydrate intake was attained experimentally by dosing sheep with glucose[5]. Sheep, fitted with permanent ruminal cannulæ, were given 3 successive doses of 300 g glucose dissolved in 500 ml. water in 6 h. Samples of fluid were removed at intervals during the following 5 days for determination of *p*H and histamine content. Results obtained with 3 sheep are shown in Fig. 2.

Acidity increased rapidly in the first 6 h and the *p*H had fallen to 4·0–4·5 at 24 h. After 24 h the *p*H returned slowly to normal in two sheep but remained low in the third sheep for a further 24 h. The histamine content of ruminal fluid showed some increase at 24 h, but there was a secondary peak at 48–52 h in all three sheep. In two

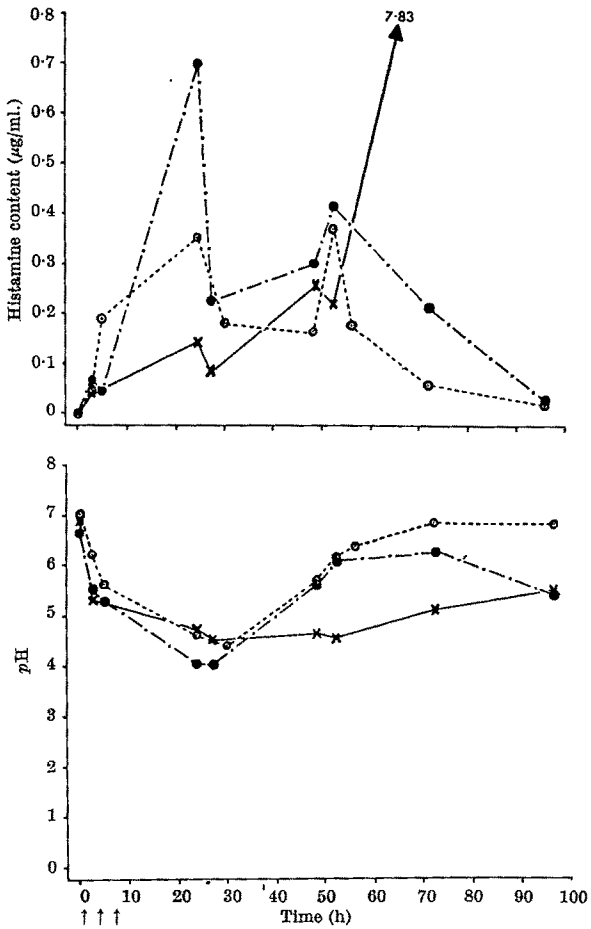


Fig. 2. The effect of *p*H and histamine content of adding glucose to ruminal contents of three cannulated sheep. ×, S_5; ●, S_8; ○, S_{16}; ↑, 300 g glucose

sheep the histamine content fell afterwards to reach normal limits by 96 h. In one sheep (*S5*), however, in which the *p*H of the ruminal contents remained low, histamine concentration increased to 7·8 μg/ml. at 72 h and was > 3·0 μg/ml. at 96 h.

In all three sheep, the increase in histamine content appeared to depend on a fall in *p*H and where high acidity persisted a marked increase in histamine content occurred. The delay of 24 h between the fall in *p*H and the rise in histamine content suggests that histamine formation might result from a change in ruminal flora brought about by the altered environment. This view is supported by the similar delay in incubation experiments and by the finding that incubation of normal ruminal fluid with histidine for periods of 3–4 h with or without the addition of glucose did not result in histamine formation.

Further investigations are in progress to obtain a more quantitative assessment of the rate of histamine formation in the rumen and to measure the histidine-decarboxylating activity of ruminal samples withdrawn at different stages in the experiment.

I thank Imperial Chemical Industries, Ltd., for a grant towards this investigation.

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GENETICS

Study of Several Gene Frequencies in Yugoslav Population

Recent years have witnessed a rather rapid development of the population genetics of certain hereditary characters in man. Its findings are based mostly on the analyses of material obtained from voluntary blood donors[1–3]. This method of collecting research material has numerous advantages, but also several marked disadvantages. For example, data on the frequency of hereditary characters are mostly limited to the antigen properties of red blood cells[2]. The value of work in population genetics increases with the number of analysed genes from a certain population sample. Some of the genes being factors of marked polymorphism in man, their frequency can lead us to remote ancestors, and genes can serve as markers in the study of linkage in the chromosomes in man, where the need for just such markers is felt most acutely[4].

For these reasons we have tested a group of healthy young persons in order to discover normal hereditary characters. Some of the tests used are rarely performed in the blood transfusion services.

The blood groups of ABO, MN, Rh(D) systems, saliva secretion status, PTC threshold, and serum haptoglobin (Hp) types of 459 medical students (male 198, female 195) between eighteen and twenty-two years were determined. In this way a total of 14 genes was registered for every person tested. Each person first gave several ml. of saliva, was then tested with PTC and finally gave several ml. of venous blood. The test lasted six days and took place in the beginning of May 1962.

Blood groups and factors were determined by standard methods[3], with the application of absorbed anti-A_1 serum and specially prepared extract of seeds of *Ulex europaeus* to detect a possible 'Bombay' type[5–7]. The secretion status, namely, specific groups of substances in saliva, were determined by McConnell's method[8] with the application of the *Ulex europaeus* (anti-H,O) extract. The method used in determining taste sensitivity for bitterness was that of Harris and Kalmus[9]. Three common Hp-types from serum were determined by our modification of Smithies's method of vertical electrophoresis in starch gel[10,11].

The results obtained are shown in Table 1. A statistical elaboration of results shows that the sample is representative of the tested population, because with corresponding degrees of freedom χ^2 values indicate a high

Table 1. Phenotype- and Gene-frequencies of Tested Traits among 459 Students

No. = 459	A_1	A_2	B	O	A_1B	A_2B	M	MN	N	Rh+	Rh−	Hp types 1—1	Hp types 2—1	Hp types 2—2	Secretor status: Secretor	Secretor status: Non-secretor	PTC: Tasters	PTC: Non-tasters
Abs.	148	30	88	162	24	7	148	218	93	373	86	66	210	183	375	84	317	142
Percentage	32·24	6·54	19·17	35·29	5·23	1·53	32·25	47·49	20·26	81·26	18·74	14·38	47·75	39·87	81·70	18·30	69·06	30·94
Gene frequencies	A_{1c} = 0·21291		B_c = 0·14326		A_2c = 0·05247		O_c = 0·59136		M = 0·55995 N = 0·44005		D = 0·56711 d = 0·43289		Hp^1 = 0·37255 Hp^2 = 0·62745		Se = 0·57222 se = 0·42778		T = 0·44376 t = 0·55624	
	χ^2 = 0·58434 *D.F.* 2 *P* > 0·70						χ^2 = 0·43297 *D.F.* 1 *P* > 0·50					χ^2 = 0·21092 *D.F.* 1 *P* > 0·50						

probability. No tested character shows any difference in values between males and females.

As for the ABO system frequency, our results are very similar to those already published[12,13] for the territory of Yugoslavia. The same holds good for MN and Rh(D) factors, except that the frequencies of Rh negative phenotypes are somewhat lower[13] in our material. According to the published[11,14] and unpublished observations of the distribution of Hp-types in Yugoslavia, the results shown match those from certain areas, bearing in mind the fact that the Hp^1 frequency of our sample is slightly lower than the average frequency found in Yugoslavia until now. We have no data concerning the ABH secretion status of our healthy population[15], but a comparison with some sources[3] shows that the frequency of non-secretors in our sample is lower than the European average (about 23 per cent). The frequency of non-tasters in this test corroborates the existing data[16,17] regarding the distribution of this hereditary character in our populations. Laboratory analyses did not yield a single 'Bombay' type. Generally speaking, frequencies of all tested characters agree with the distribution of the same characters in European populations[2,3,18–20].

This work reports an effort to answer the first of basic questions of human population genetics, propounded so brilliantly by Penrose[21]. We shall attempt to answer other questions in our further work.

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Transferrin Variants in Finland

THE gene responsible for transferrin *C* seems to be very common in all human populations. Of the rare alleles, those producing the faster-moving variants have mainly been found in Caucasoids. The most common of them is B_2, which has been observed in 1 per cent of Canadian Whites, Englishmen and Swedes[1–3]. *Tf* B_1 has been reported in 1 of 139 British Whites[2] and 2 of 1,173 Swedes[3].

The more slowly migrating transferrins (*D* type) are rare in the white populations studied so far, but common in some other races. The phenotype CD_1 is found in about 10 per cent of the New York Negroes and Australian Aborigines[4]. In a Chinese population about 6 per cent of individuals were of the phenotype CD_{Chl}[5]. Reports on the occurrence of the *D*-alleles in Whites have been published by Harris *et al.*[6], who found 2 cases of CD_4 (CD_{0-1}) among 1,000 English and Italian samples; and by Beckman *et al.*, who discovered 6 CD_1 phenotypes among 329 Swedish Lapps[7] and 2 CD_1 phenotypes in 1,173 Swedes[3].

The sera of 354 unrelated Finns were examined by unidimensional starch-gel electrophoresis, using the discontinuous buffer system described by Beckman and Holmgren[7]. The electrophoresis was applied at 6° C with 10–12 V/cm for about 3 h. One drop of bromphenol blue in the gel buffer solution was added to the filter-paper slice soaked with the test serum, so as to make the albumin band visible during the migration. This did not interfere with the staining of proteins with amido black.

The distribution of different phenotypes in the series was: *CC* 338; B_1C 9; B_2C 1; CD_1 6.

None of the CD_1 individuals was born in Lapland, nor did they have parents born in Lapland. It seems that the frequency of the B_1 and D_1 genes in the Finnish population is remarkably high. Six of the families, in which the heterozygotes were found, were investigated more thoroughly. Twelve of their children had the parent's heterozygous phenotype, while nine were of the type *CC*. The pedigrees will be published later.

We wish to thank Dr. L. Beckman for supplying us with *Tf* B_1C, B_2C and CD_1 reference sera.

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VIROLOGY

Antigenic Activity of Tryptic Peptides of Tobacco Mosaic Virus Protein

FRAGMENTS possessing immunological activity related to that of the whole protein have been obtained from several protein antigens. Enzymatic digests of silk fibroin[1], γ-globulin[2,3], and serum albumin[4,5] have exhibited such activity. A recent report from our laboratory showed that fragments of egg albumin obtained by partial acid hydrolysis of the protein inhibited both the *in vitro* interaction of the protein with homologous anti-serum and systemic anaphylaxis in guinea pigs[6]. Although active fragments have been obtained, analysis of their composition and structure is extremely difficult.

Proteins of known amino-acid sequence afford promising tools for studying antigenic determinants. Recently, Brown[7] reported on the antigenic determinants of ribonuclease. We present here our findings on the immunological activity of tryptic peptides of tobacco mosaic virus protein (TMVP), another protein of known amino-acid sequence[8,9].

The TMVP was obtained from the whole virus by treatment with 66 per cent acetic acid[10]. The protein was digested with trypsin for 2 h at 40° C maintained at *p*H 8·0 by a *p*H stat. The digest was passed through a '*G*50 Sephadex' column equilibrated and developed with M/300 phosphate buffer *p*H 8·0. Two fractions were obtained: $G_{50}S_1$ and $G_{50}S_2$, containing materials of molecular weight ranging above and below 10,000 respectively. The peptides contained in the $G_{50}S_2$ fraction were characterized by ion exchange chromatography[11,12] on a 'Dowex 1 × 2' column developed with a polygradient buffer system ranging from a collidine : pyridine buffer of *p*H 8·8 to 50 per cent acetic acid. They were also characterized by electrochromatography using the peptide mapping technique of Woody and Knight[13]. $G_{50}S_2$ contained the peptides characteristic of the tryptic digest of TMVP[12,13].

Table 1. Antigenic Activity of the Tobacco Mosaic Virus Protein and its Tryptic Peptides, as determined by Passive Cutaneous Anaphylaxis

Intradermal sensitization (0·1 ml.)	Intravenous challenge (μg)	Result
Anti-TMVP*	TMVP 100	+
Anti-TMVP	TMVP 10	+
Anti-TMVP	TMVP 1	+
Buffer	TMVP 100	−
Anti-TMVP	$G_{50}S_2$ 500	+
Anti-TMVP	$G_{50}S_2$ 50	−
Anti-TMVP	D_1 220†	+
Anti-TMVP	D_2 220†	−
Anti-TMVP	Dye only	−
Anti-TMVP + 1,000 μg $G_{50}S_2$	TMVP 10	−
Anti-TMVP + 500 μg $G_{50}S_2$	TMVP 10	∓
Anti-TMVP + 100 μg $G_{50}S_2$	TMVP 10	+
Anti-TMVP + 220 μg D_1	TMVP 10	+
Anti-TMVP + 110 μg D_1	TMVP 10	+
Anti-TMVP + 220 μg D_2	TMVP 10	−
Anti-TMVP + 110 μg D_2	TMVP 10	∓
Anti-TMVP + 22 μg D_2	TMVP 10	+
Anti-lysozyme‡	Lysozyme 100	+
Anti-lysozyme	Lysozyme 10	+
Anti-lysozyme	$G_{50}S_2$ 500	−
Anti-lysozyme	D_1 220	−
Anti-lysozyme	D_2 220	−
Anti-lysozyme + 1,000 μg $G_{50}S_2$	Lysozyme 100	+
Anti-lysozyme + 220 μg D_1	Lysozyme 100	+
Anti-lysozyme + 220 μg D_2	Lysozyme 100	+

* Containing 60 μg antibody per ml.
† 220 μg of D_1 or D_2 are equivalent to 500 μg of $G_{50}S_2$.
‡ Antiserum diluted 1 : 50; intradermal injection of 0·1 ml. of anti-lysozyme serum diluted 1/50 and intravenous challenge with 100 μg lysozyme elicited a similar degree of dye infiltration to that elicited by the intradermal injection of 0·1 ml. anti-TMVP containing 60 μg antibody per ml. and challenged with 10 μg TMVP.

Results of the experiments utilizing passive cutaneous anaphylaxis (Table 1) show that intravenous challenges with TMVP, $G_{50}S_2$ and D_1, elicited passive cutaneous anaphylaxis with TMVP anti-serum. When injected intradermally with anti-TMVP serum, D_2 and $G_{50}S_2$ inhibited passive cutaneous anaphylaxis on intravenous challenge with TMVP. The activity of the tryptic peptides was specific to the TMVP–anti-TMVP system since the $G_{50}S_2$, D_1 or D_2 did not affect passive cutaneous anaphylaxis with the lysozyme–anti-lysozyme system.

The results indicate definitely that at least one of the tryptic peptides possesses immunological properties related to those of TMVP. Assuming that under the conditions of dialysis one or more of the peptides was completely non-dialysable, the results suggest the presence of two immunologically active peptides. However, the possibility exists that conditions used during dialysis caused one or more of the peptides to be in equilibrium between its polymerized and non-polymerized form, resulting in the same peptide or peptides being present both in D_1 and D_2. From investigations with isolated muscle strips, it appears that both D_1 and D_2 fractions can combine with the antibodies of the TMVP-sensitive ileum. The passive cutaneous anaphylaxis experiments indicate that, at the concentrations used, at least one of the peptides present in the non-dialysable fraction elicits passive cutaneous anaphylaxis, while at least one peptide present in the dialysable portion inhibits the reaction.

Table 2. Effect of TMVP, $G_{50}S_2$, D_1, D_2, and Histamine on Isolated Guinea Pig Ileum Strip as determined by the Schultz–Dale Technique

Ileum source	Initial treatment			Subsequent treatments*			
	Material	Final concentration†	Result	TMVP added†	Result	Histamine added†	Result
TMVP sensitive	TMVP	0·11	+ + + +‡			0·005	+ + + +
TMVP sensitive	TMVP	0·011	+ +			0·005	+ + + +
TMVP sensitive	TMVP	0·0011	∓			0·005	+ + + +
TMVP sensitive	$G_{50}S_2$	0·11	+ + +	0·11	+ + +	0·005	+ + + +
TMVP sensitive	D_1	0·055	+ + +	0·11	+ + +	0·005	+ + + +
TMVP sensitive	D_2	0·055	+ +	0·11	+ + +	0·005	+ + + +
Non-sensitive	TMVP	0·11	−			0·005	+ + + +
Non-sensitive	$G_{50}S_2$	0·11	−			0·005	+ + + +

* Added to the system following relaxation from preceding treatment.
† All figures represent final concentration in mg/ml.
‡ No. of plus signs indicate approximate magnitude of contraction.

The $G_{50}S_2$ fraction was dialysed in the cold against distilled water which was adjusted to *p*H 7·0 with ammonia. The non-dialysable portion (D_1) and the dialysate (D_2) were lyophilized. D_1 and D_2 gave a distribution of absorbency at 280 mμ of 51 and 49 per cent respectively.

The antigenic activity of the peptides was determined by direct passive cutaneous anaphylaxis according to the method of Ovary[14]. Antisera against TMVP were obtained by immunizing rabbits with TMVP and adjuvants. The sera were pooled and contained 3 mg antibody per ml. The antibodies were injected intradermally in 0·1 ml. M/15 phosphate buffer *p*H 8·0. Three hours later TMVP or the peptides were injected intravenously in 1 ml. of 0·25 per cent Evans's blue in buffer. When passive cutaneous anaphylaxis inhibition was studied, the peptides were added to the antibodies and a total volume of 0·1 ml. was injected intradermally; TMVP was injected 3 h later. The specificity of the reactions observed were tested using a lysozyme–antilysozyme system. Results are shown in Table 1.

The activity of the peptides was also determined by the Schultz–Dale technique[15] using isolated ileum strips from guinea pigs sensitized to TMVP. The ileum strips were approximately 1 in. long, immersed in 8 ml. Tyrode's solution at *p*H 7·2, and maintained at 37° C. The test material was added in 1 ml. Tyrode's solution. Results are shown in Table 2.

The results show that tryptic peptides of TMVP possess immunological properties. Using the Schultz–Dale technique, TMVP, $G_{50}S_2$, D_1 and D_2 caused contractions (Table 2). At the concentrations used, the contraction elicited by TMVP was not inhibited when TMVP was added to the system containing $G_{50}S_2$, D_1 or D_2. TMVP and $G_{50}S_2$ were not active when tested on ileum not sensitive to TMVP.

After the manuscript of the present communication was prepared, we noted the report by Anderer[16] in which a C-terminal hexapeptide of TMVP was correlated with some of the antigenic activity of the tobacco mosaic virus.

We thank Drs. C. A. Knight and G. Funatsu of the University of California Virus Laboratory, Berkeley, for supplying tobacco mosaic virus and for help in the ion exchange chromatography, respectively.

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FORTHCOMING EVENTS

Wednesday, August 28—Wednesday, September 4

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at Aberdeen)—125th Annual Meeting.

Wednesday, August 28

At 7.30 p.m.—Sir Eric Ashby, F.R.S.: "Investment in Man" (Presidential Address).

Thursday, August 29

At 10 a.m.—Prof. J. Monteath Robertson, F.R.S.: "A Physical Approach to Chemical Structures" (Presidential Address, Section B).

At 10 a.m.—Dr. F. Fraser Darling: "The Unity of Ecology" (Presidential Address, Section D).

At 10 a.m.—Dr. S. G. Hooker, F.R.S.: "Future of the Aeroplane in Civil and Military Fields" (Presidential Address, Section G).

At 10 a.m.—Prof. J. McMichael, F.R.S.: "The Contribution of Clinical Medicine to Physiology" (Presidential Address, Section I).

At 10 a.m.—Dr. T. P. Morris: "Science and Morals in the Treatment of Deviant Behaviour" (Lister Lecture).

At 11.20 a.m.—Sir Charles Morris, K.C.M.G.: "Personal Values in Education" (Presidential Address, Section L).

At 11.30 a.m.—Prof. W. J. H. Sprott: "Problems of Penology" (Presidential Address, Section N).

At 2.30 p.m.—Sir Raymond Priestley: "Antarctic Exploration Yesterday and To-day" (York Lecture).

riday, August 30

At 10 a.m.—Dr. J. S. Forrest: "High Voltage Insulation" (Presidential Address, Section A).

At 10 a.m.—Prof. H. C. Darby: "British National Parks" (Presidential Address, Section E).

At 10 a.m.—Mr. E. J. H. Corner, F.R.S.: "The Tropical Botanist" (Presidential Address, Section K, K*).

At 10 a.m.—Prof. Martin Jones: "Providing Food for Man and Beast" (Presidential Address, Section M).

At 11.30 a.m.—Prof. O. L. Zangwill: "Cerebral Localization of Psychological Function" (Presidential Address, Section J).

At 11.30 a.m.—Prof. D. J. Robertson: "The Present Position of the City" (Presidential Address, Section X).

At 8 p.m.—Prof. C. F. Carter: "Problems of Economic Growth" (Evening Discourse).

Sunday, September 1

At 11 a.m.—Service in the West Church of St. Nicholas, Union Street. Minister: The Reverend Anderson Nicol, M.A., J.P. Preacher: The Reverend Prof. John M. Graham, D.D.

Monday, September 2

At 10 a.m.—Prof. J. H. Taylor, F.R.S.: "Some Aspects of Diagenesis" (Presidential Address, Section C).

At 10 a.m.—Prof. T. Wilson: "The Price of Growth" (Presidential Address, Section F).

At 10 a.m.—Mr. Peter Opie: The Tentacles of Tradition" (Presidential Address, Section H).

At 10.10 a.m.—Prof. H. M. Steven: "The Beneficent Forest" (Chairman's Address, Section K*).

At 2 p.m.—Dr. J. N. Murrell: "Colour and Chemical Constitution" (Kelvin Lecture).

Wednesday, September, 4

At 10 a.m.—Dr. L. Wolpert: "Growing in a Definite Shape" (Darwin Lecture).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with a degree or equivalent qualifications, and teaching and/or industrial experience) mainly for PHYSICS and ELECTRONICS, to teach at various levels to National Certificate and Higher National Diploma classes—The Principal, R.R.E. College of Electronics, Royal Radar Establishment, Malvern (August 28).

TEMPORARY LECTURER or TEMPORARY ASSISTANT LECTURER (preferably with interests in the biology of fish) IN MARINE BIOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (August 29).

LECTURER IN REFRACTORIES AND CERAMIC TECHNOLOGY—The Registrar, The University, Sheffield (August 31).

SENIOR LECTURER IN THE COMPUTING LABORATORY—The Secretary of University Court, The University, Glasgow (August 31).

RESEARCH STUDENT IN APPLIED PHYSICS—The Registrar, The University, Hull (September 1).

LECTURER IN ANIMAL PATHOLOGY in the School of Veterinary Medicine, for duties which include teaching and research—Dr. T. D. Kellaway, Secretary of the Appointments Committee, Department of Pathology, The University, Tennis Court Road, Cambridge (September 6).

SENIOR LECTURER and a LECTURER (with an honours degree in mathematics, and preferably a higher degree, and some experience of research) IN MATHEMATICS at the University of Queensland, Brisbane, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, September 6).

EXPERIMENTAL OFFICER IN THE DEPARTMENT OF CHEMISTRY in the Faculty of Sciences, to be concerned with preparative work involving the synthesis of inorganic molecules containing isotopically labelled atoms—The Registrar, The University, Manchester 13 (September 7).

LECTURER IN ANALYTICAL CHEMISTRY—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref. 8/63/6 (September 9).

LABORATORY TECHNICIANS (2) IN PHYSIOLOGY—The Registrar, University of New South Wales, Armidale, New South Wales, Australia (September 13).

FELLOW (with a Ph.D. degree in physics or metallurgy, or research experience of an equivalent standard and duration, supported by satisfactory evidence of research ability) IN METAL PHYSICS IN THE DIVISION OF TRIBOPHYSICS, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, to join a group studying the properties of defects in crystals—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 370/153 (September 14).

RESEARCH OFFICER or SENIOR RESEARCH OFFICER (with a Ph.D. degree in an appropriate field or postgraduate research experience in physics of equivalent standard and duration supported by satisfactory evidence of research ability) IN THE DIVISION OF PHYSICS, National Standards Laboratory, Commonwealth Scientific and Industrial Research Organization, Chippendale, New South Wales, Australia, to undertake research into new and improved methods of measuring temperature—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 770/257 (September 14).

SENIOR LECTURER IN CHEMICAL PATHOLOGY to undertake duties primarily teaching and research—The Secretary, St. Thomas's Hospital Medical School, London, S.E.1 (September 14).

ASSOCIATE PROFESSOR or LECTURER/SENIOR LECTURER IN THE DEPARTMENT OF FARM MANAGEMENT within the Faculty of Agricultural Economics, University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

LECTURERS or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF GEOGRAPHY, University of Malaya—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, September 15).

LECTURER or ASSISTANT LECTURER (preferably pure mathematician) IN MATHEMATICS—The Secretary, The University, Aberdeen (September 16).

MAP CURATOR (with a good honours degree in geography and preferably appropriate library experience)—The Registrar, The University, Hull (September 16).

LECTURER or SENIOR LECTURER (qualified by training and experience to help in the teaching of general invertebrate zoology and some special aspect of physiology, preferably of invertebrate animals) IN ZOOLOGY at the University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 20).

LECTURER/SENIOR LECTURER (preferably with experience in radiochemistry and/or radiation chemistry) IN PHYSICAL CHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 20).

SENIOR LECTURER IN CHEMICAL PATHOLOGY—The Registrar, The University, Sheffield (September 21).

LECTURER/SENIOR LECTURER IN BIOLOGY in any field of animal biology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 23).

PHYSIOLOGIST, for metabolic studies in the Pig Laboratory—The Secretary, Institute of Animal Physiology, Agricultural Research Council, Babraham, Cambridge (September 23).

PROFESSOR OF EXPERIMENTAL PHYSICS at the University of Adelaide—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

PROFESSOR OF INORGANIC CHEMISTRY at the University of Adelaide, Adelaide, South Australia—The Secretary, The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

READER (with appropriate academic qualifications and teaching experience) IN PHILOSOPHY at the University of Hong Kong—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, September 30).

CHAIR OF PHARMACOLOGY—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne 2 (October 12).

ELECTRON MICROSCOPIST (with at least a B.Sc. in physics or life sciences, and preferably previous experience of electron microscopy), to take charge of the New Zealand Medical Research Council's Electron Microscope Unit at the University of Otago Medical School, Dunedin—The Secretary, The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Administrative Office, New Zealand Medical Research Council, Box 913, Dunedin, New Zealand (Dunedin, October 12).

CHAIR OF BIOCHEMISTRY at Lincoln College, University of Canterbury, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

CHAIR OF HORTICULTURE at Lincoln College, University of Canterbury, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

PROFESSOR OF ENGINEERING at Newcastle University College, The University of New South Wales, Australia—The Agent-General for New South Wales, 56 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (October 31).

ASSOCIATE PROFESSOR (preferably candidate working on the integrative aspects of mammalian physiology, especially in the neuroendocrine, gastrointestinal or cardiovascular fields) IN PHYSIOLOGY—Prof. J. W. Pearce, Department of Physiology, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada.

DEMONSTRATOR, to give laboratory instruction in plant physiology and general botany—Head, Department of Biology and Botany, University of British Columbia, Vancouver 8, B.C., Canada.

ELECTRON MICROSCOPIST (scientifically or medically qualified, interested in furthering technical aspects of electron microscopy with reference to general biological problems, and preferably with previous biological experience) IN THE ELECTRON MICROSCOPY DEPARTMENT, to take charge of the work—The Secretary, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1.

FIELD ZOOLOGIST (male, single, with a university honours degree in zoology, an interest in ornithology, and preferably some research and/or teaching experience) for the post of Assistant Warden at Flatford Mill Field Centre

—The Warden, Flatford Mill Field Centre, East Bergholt, near Colchester, Essex.

Graduate in Physiology, Zoology or related sciences, for work with experimental animals in the field of toxicology—The Administrative Officer, Huntingdon Research Centre, Huntingdon.

Graduate Physicist (preferably with previous experience in hospital physics, but candidates graduating this summer may apply) in the Radio/Therapy Department, to assist with radioactive isotope studies—The Personnel Officer, St. Thomas's Hospital, London, S.E.1.

Lecturer (with a good honours degree and preferably teaching and/or industrial experience) in Physics, to teach physics and applied physics to H.N.D. and Grad. Inst. P. level—The Principal, Mid-Essex Technical College and School of Art, Market Road, Chelmsford, Essex.

Mathematician-Programmer (with an honours degree in mathematics from a recognized university, and preferably with a higher degree and possessing experience in programming and numerical analysis) at the National Research Council Computation Centre, for duties involving the analysis and programming of problems originating in the various scientific and engineering divisions of the Council, and possibly the supervision of junior programmers—The Employment Officer, National Research Council, 100 Sussex Drive, Ottawa 2, Canada, quoting File ER-282.

May and Baker Scholar (with a good honours degree in chemistry, biochemistry or botany), for research on the mode of action of herbicides at the sub-cellular level—Prof. E. O. Morris, Department of Applied Microbiology and Biology, Royal College of Science and Technology, Glasgow.

Pharmacologist or Physiologist (with an honours degree), to study active polypeptides in acute parasitic infections—The Administrative Secretary, School of Tropical Medicine (University of Liverpool), Pembroke Place, Liverpool 3.

Physical Chemist—The Dean, Faculty of Science, Haile Sellassie I University, P.O. Box 399, Addis Ababa, Ethiopia.

Research Assistant (with a good honours degree in a biological subject, and preferably research experience in human or animal cytogenetics and/or some field of tissue culture; recent graduate with no research experience may also apply) in the Cytogenetics Group of the Paediatric Research Unit—The Secretary, Paediatric Research Unit, Guy's Hospital Medical School, London Bridge, London, S.E.1.

Research Fellow (with a degree in chemical or mechanical engineering, metallurgy, chemistry or physics) in the Department of Metallurgy, for research on the engineering principles which underlie processes used for metal extraction and refining—Prof. F. D. Richardson, Metallurgy Department, Imperial College of Science and Technology, London, S.W.7.

Research Fellows (with a Ph.D. with experience in carbohydrate, phosphorus, or nucleic acid chemistry, in enzymology, or in the intermediate metabolism of nucleotides), to participate in studies in the biochemistry of neoplasia—The Director, University of Alberta Cancer Research Unit, McEachern Laboratory, Edmonton, Alberta, Canada.

Senior Physical Chemist interested in examining the properties of new protein derivatives and in applying the Flory polycondensation theory to some new reactions; and a Senior Development Chemist, to investigate uses for the new derivatives—The Director of Research, The Gelatine and Glue Research Association, 2A Dalmeny Avenue, Holloway, London, N.7.

Senior Technician (Grade I or II) in Biochemistry at the Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick—The Group Secretary, West Bromwich and District Hospital Management Committee, West Bromwich and District Hospital, West Bromwich.

Senior Technician (with H.N.C. in chemistry or equivalent, experience in general laboratory procedure and a genuine interest in analytical biochemistry), for chemical and clinical research in a newly created steroid laboratory of the Medical Research Council Unit of Neuroendocrinology—The Administrator, Department of Human Anatomy, The University, South Parks Road, Oxford.

Superintendent (with a degree in biological science or equivalent qualification, for example, F.A.T.A., and experience in some branch of animal work), to take charge of the Physiology Department's goat and small animal house—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 63/2.

Technician for the Department of Animal Husbandry—The Assistant Secretary, Royal Veterinary College, Royal College Street, London, N.W.1.

Technician or Senior Technician, to be responsible for the technical servicing of a zoological laboratory—The Dean of the School of Biological Sciences, University of East Anglia, Earlham Hall, Norwich, NOR 05G.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The J. A. Radley Research Institute. Report 1961–1962. Pp. 25. (Reading: The J. A. Radley Research Institute, 1963.) [266

The Species Unit, Unit III. By Marietta Pallis. Pp. 12. (Glasgow: Robert Maclehose and Co., Ltd., 1963.) [266

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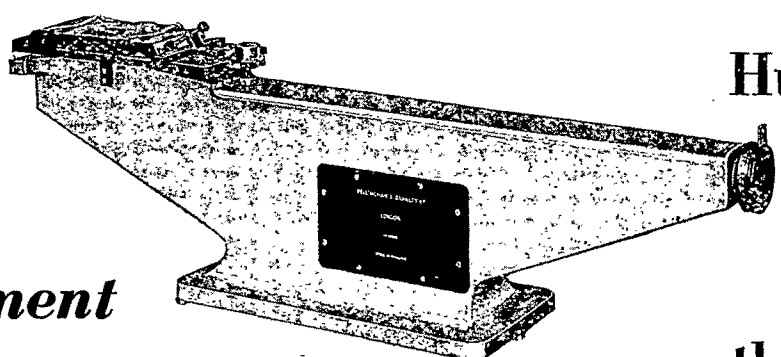
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INVESTMENT IN SCIENCE THROUGH MAN

SIR ERIC ASHBY's presidential address to the British Association for the Advancement of Science (see p. 877 of this issue) is a fitting complement to that of Sir John Cockcroft's at last year's meeting in Manchester. As Sir Eric observes at the start, the pre-requisites for investment in science are, first, scientists, and secondly, a public opinion sufficiently well informed and enlightened to give financial support to scientists. Both these are acquired through education, and, accordingly, to invest in science we have first to invest in man.

It is not only from Sir John Cockcroft, however, that Sir Eric could have obtained his lead. It is equally to be found in the emphasis placed on investment in education as a factor in economic growth by the National Economic Development Council and earlier still by the Organization for European Economic Co-operation (now the Organization for European Co-operation and Development). Indeed, the glance that Sir Eric gives at the address given in Aberdeen by his predecessor, the Right Hon. Sir Lyon Playfair, in 1885, almost implies an even earlier ancestry, and there may well be readers wishing the president had had the temerity to retail that earlier address in place of his own stimulating, but highly provocative, address. Nevertheless, any recasting of Sir Lyon Playfair's words or annotations on his theme to which Sir Eric Ashby is tempted are unlikely to be less penetrating than the address he actually delivered. However prolonged its conception, the address is entirely contemporary in its thought and exemplifies throughout that respect for the past, acceptance of a rigorous discipline and receptivity of new ideas which he himself proclaims as the unique contribution which universities make to society. He does justice to virtues in Britain's existing institutions which some critics are inclined to ignore, though without allowing any complacency. Indeed, he shatters the idea that Britain can just wait for the Robbins Committee to report as effectively as he makes nonsense of some of the wilder party political proposals. While he brings to the discussion of the plan and functions of the universities a note of urgency and realism which should force the most reluctant common room to think seriously about questions its members might rather leave alone, he never advocates change for the sake of change.

Sir Eric Ashby is concerned in this address essentially with one section only of the problem of investment in education—investment at the level of higher education, and, more especially, with the university as a social instrument for such investment. He raises many pertinent questions, to some of which the Robbins Committee is already seeking answers. Others, it is manifest, require investigations that have not even been initiated, and one purpose of the address appears to be to stimulate such enquiry so that there may be no long delay when the Robbins report appears. Here, however, Sir Eric appears rather to underestimate the attention which is now being given to educational research in Britain. If this is not as yet concerted in any such way as it is under the National Science Foundation in the United States, proposals for an Educational Research Council have already been presented to the Minister for Science; the recent annual report of the Nuffield Foundation indicates a substantial range of work in progress. Sir Edward Boyle's speech in the House of Commons on July 24 indicated that the limiting factor is not the selection of appropriate projects, but finding teams of investigators competent to execute the project, however, admittedly the bulk of such work is still concerned with education in the schools.

First, Sir Eric considers the resources of ability for this section of investment in man, and, after stressing the unreliability of the comparative figures for students and population in different countries which are used in support of arguments as to the inadequacy of provision here for higher education, he remarks that the one feature of the investment of man in higher education which has been studied scientifically merely leads to the conclusion that we could invest a much larger proportion of the age group than we do. Britain's policy for higher education is tenable only on three assumptions: first, that the techniques for selection are efficient and reliable and satisfy social justice; secondly, that the members selected each year for different kinds of full-time higher education are sufficient for the nation's needs; thirdly, that Britain offers acceptable opportunities for part-time further education to those who are not selected. Sir Eric Ashby believes that none of these assumptions is justified.

While, ultimately, the intellectual resources in a population are limited by its genetic structure, there is abundant evidence that inequalities in Britain's society and inadequacies in her educational system, and not genetics, at present limit her investment in man. He describes Britain's system of selection as a system for distributing challenges: attempts are made to select not those likely to profit from higher education, but to select from all these, the candidates to whom it should be offered. Sir Eric rightly suggests that much more information is needed about the success or failure of those rejected. It is in such a context that the suggestion which has already been made for some separate institution of university quality to take the candidates who have failed to obtain entry might be worth examining. Despite his assertion as to the existence of this larger supply of potential university entrants, in support of which he quotes some evidence, Sir Eric does not, however, consider the possibility of Britain's present methods directing to the universities those students whose potentialities would be more fully and effectively developed in other institutions, such as colleges of technology.

The existence of some opinion to that effect is not, however, inconsistent with Sir Eric's rejection of this first assumption. He is in fact critical of the efficiency and adequacy of Britain's system of higher education apart from the universities and, particularly, of the systems of part-time further education integrated with employment. These are the main reasons for regarding as unsound the second and third assumptions on which Britain's policy for higher education is based. The high rates of wastage in part-time study are the fault of a social system which denies to the part-time student an opportunity to learn well.

Nevertheless, Sir Eric does not advocate indiscriminate inflation of the volume of investment in higher education. Such expansion should be based on far firmer knowledge about the pattern of higher education which is likely to be

relevant to the lives the students are to live. It is this knowledge that we should be acquiring, and it is here that Sir Eric Ashby enters his central theme and begins to ask searching questions about the appropriateness of institutions, their methods and functions, their use and obsolescence to the needs of the community they exist to serve.

At their best, Sir Eric Ashby reminds us, universities endow the men and women who pass through them with the capacity to reconcile orthodoxy and dissent. They not only pass on a corpus of knowledge and ideas, but also train students to disclose errors in knowledge and to question ideas. In so far as they also do this, the colleges of technology also are rightly regarded as university institutions; the dichotomy between technological institutions and universities which Sir Malcolm Knox suggested to the Vice-Chancellors of the Commonwealth Universities in welcoming them to St. Andrews is misleading and dangerous. Both alike have to equip their students to cope with the known. Both have also to encourage the critical temperament which alone will enable its possessor to cope with the unknown.

Sir Eric Ashby is on sound ground in suggesting that the unique contribution which universities make to society is to train young people in this dialectic between orthodoxy and dissent, and that any change in the pattern of universities which endangers this function endangers the society which the university serves. This, he remarks, is the real reason why the universities have to combine teaching and research, and why also they must seek to include among their students those who have the combination of temperament and intellect to profit from this particular approach to learning. But, while he adds, quite rightly, that the universities have also the task of training men and women for the professions and that they will largely be judged by the efficiency with which that task is discharged, his words equally apply to the technological institutions. Their work, too, is endangered when attendance at a university becomes a status symbol, and investment in institutions of technology and universities is ultimately determined by the extent to which the public judges they fulfil their function efficiently.

For such reasons it is important that the distinction between technical and technological institutions should be made clearer, and here again the association with such distinction of any suggestion of status should be avoided. The distinction is one of function and, on the part of the student, of capacity also. It might well be better understood if we knew more about the effectiveness of our educational institutions generally, let alone the efficiency of processes of teaching and learning in universities to which Sir Eric Ashby directs attention. Without a sustained programme of research into higher education it is not possible to provide the answers to the essential questions as to the means and instruments by which we should increase our present investment. Even the present annual expenditure on universities of more than £70 million is too high to expect that investment to be forthcoming—with all that is implied in reduced expenditure or investment elsewhere—unless it can be made on assumptions that are known to be valid.

Whatever difference of opinion there may be as to the scale of expansion or the distribution of that expansion as between the universities and the colleges of technology, for example, Britain is already committed to a large expansion of higher education. The uncertain factor is, Where can be found the 8,000 or more teachers that will be required for the universities alone? Sir Eric is of the opinion that in mathematics, science and technology there is good reason to fear either a shortage of teachers or a decline in quality. Whichever option is elected—and both could be forced on us—Sir Eric is undoubtedly right in suggesting that under such conditions the only way to maintain standards in the university is through the use of operational research on the use made of teachers and on methods of teaching. At present we know little, for example, as to the possibilities which the advent of the film, closed-circuit television and the tape-recorder offer in the reduction of the ratio of staff to students. We do not even know what size of small groups is most effective for teaching by discussions. We have not even explored, in the light of the new possibilities that audio-visual aids, for example, offer, how far we can proceed in the direction to which Comenius pointed three centuries ago, of finding a method by which the teachers teach less and learners learn more.

The brief reference, given by Sir Eric Ashby, to the technical devices now at the disposal of the teacher, suggests something of the exciting possibilities which could follow if the university teacher used them to give himself more time for the intimate encounter of discussion which no modern technique can replace—techniques or possibilities which go far to safeguard and encourage the characteristics which the university must always seek to cherish. It is not the unexamined adoption of new methods that Sir Eric Ashby urges: rather he challenges the unproved assumption that we have nothing to learn about the techniques of teaching or the processes of learning. So too, like Prof. B. R. Williams in a recent even more forthright article, he would examine the efficiency with which we use the capital we invest in universities, not merely the design of the laboratories on which the bulk of the expenditure is made, but the assumptions on which this design is based.

In spite of the evidence elsewhere of the value of research into the utilization of laboratories, we do not know the answers to such questions as whether we should continue to build scientific laboratories on the pattern traditional in British universities or whether the capital so invested could now be put to better use; there is little indication that we are attempting to find the answers. Even more important, as Sir Eric justly observes, are the unscrutinized assumptions we make about the function of a university, and in spite of a growing volume of attention to the content of school curriculum, the university curriculum as yet has scarcely been examined from this point of view. There are notable exceptions, it is true, but by and large it is fair to say, as Sir Eric does, that the pattern of courses still presupposes that students are to become professional scientists and scholars, or that students who are not to become professional scientists and scholars should receive the same academic training.

Both these assumptions have in fact been challenged in discussions on the B.B.C. Third Programme, and it may be confidently expected that the Robbins Committee will make some trenchant comments on their validity. There are also untested assumptions about the content of courses, and here Sir Eric makes an important point which he has already expounded at greater length in his book, *Technology and the Academics*—the importance of diffusing technology into the common culture. Education has still to accept technology as a form of humanism.

Finally, Sir Eric attacks the assumption built into Britain's present policy for investment in man that a

three-year undergraduate course is sufficient to set up a man for a life-time. Doubtless that assumption was never or entirely true as Sir Eric implies, for it is fundamentally inconsistent with success in the primary task of a university of imparting a critical and enquiring mind. The student who has learnt to enquire will never entirely lose his or her zest for learning. But the need for measures to combat obsolescence is imperative and urgent.

More is in fact being done than Sir Eric Ashby seems to allow. The professional institutions, like the Roya Institute of Chemistry, as well as the Nuffield Foundation, are already playing a part and the section on education in the latest annual report of the latter body is a fitting counterpart to Sir Eric's remarks on educational research and obsolescence. Nevertheless, as he points out, re-education requires challenge and response, an encounter between teacher and student; what is already being done is on nothing like the massive scale required to meet a need that steadily grows in magnitude as well as complexity. There is perhaps no more important task of higher education than, to use words which Sir Eric Ashby quotes from J. W. Gardner, president of the Carnegie Corporation of New York, to provide a framework within which continuous innovation, renewal and re-birth can occur.

Sir Eric Ashby opened his address with a reference to Sir Lyon Playfair's address in 1885 on the relations of science to the public need. The sub-headings of that address—science and the state, science and secondary education, science and the universities, science and industry, abstract science the condition for progress—cover subjects which are the subject of present-day debate, and despite the changed conditions there is still much truth in many of Sir Lyon Playfair's observations. A Ministry of Education has long since arrived, but the very argument over educational research and the need for a 'Council for Educational Research' illustrates our uncertainty as to how far the responsibilities of such a Minister should extend, though it is clear enough from what Sir Edward Boyle said at Belfast on July 5 that the present Minister would go all the way with Sir Eric Ashby in his call for educational research. But can we be sure that it can no longer be said of the British Treasury that it clings to the view indicted by Sir Lyon Playfair in 1885 that there is a finality in science and in expanding knowledge ?

It would be rash indeed to assert that the nation has really grasped the truth and implications of Sir Lyon Playfair's closing words that abstract discovery in science is the true foundation on which the superstructure of modern science is built, for the sorry tale of frustration and shortsightedness and shortage of finance could scarcely then persist. Moreover, Sir Lyon Playfair himself took as his text some words from the presidential address of his predecessor, the Prince Consort, in 1859: "We may be justified in hoping . . . that the Legislature and the State will more and more recognize the claims of science to their attention, so that it may no longer require the begging-box, but speak to the State like a favoured child to its parent, sure of his paternal solicitude for its welfare; that the State will recognize in science one of its elements of strength and prosperity, to foster which the clearest dictates of self-interest demand". This makes a fitting tail-piece to Sir Eric's address, for just a century later a Minister for Science was appointed, but even so we still await the fulfilment of the hopes of Prince Albert. Moreover, the present debates in both Houses of Parliament, for all the evidence they afford that Sir Eric Ashby's words may fall on fertile ground, do not indicate that there is any real and widespread appreciation of the price that must be paid if Britain is to make the investment in man to which Sir Eric calls, and make that investment wisely and efficiently.

SUPPORT OF RESEARCH IN U.S. UNIVERSITIES AND COLLEGES

The Effects of Federal Programs on Higher Education

A Study of 36 Universities and Colleges. By Harold Orlans. Pp. xvi+361. (Washington, D.C.: The Brookings Institution, 1962.). Paper 2.95 dollars; Cloth 5 dollars.

THIS volume is a carefully documented survey of the effect of U.S. Federal expenditure in the Universities and colleges. Although the university system is so different from that in Britain, surveys of this kind are of immense value to those interested in similar problems in the U.K. There is much to be learned from this survey since some well-defined tendencies in Britain are along lines pursued in the United States for many years. The total expenditure by Federal Government, both from civilian and defence agencies, is no less than 1,000 million dollars a year. The report does not state what the total cost of running all the universities and colleges in the U.S. amounts to, and thus it is impossible to estimate what fraction comes from Federal funds. However, in some of the leading institutions it is known that 50 per cent of their income is derived in this way.

It would clearly be impossible to do this kind of job other than by taking representative samples from State and private universities and liberal arts colleges. This is done by examining twelve institutions of each type, and the general results are given in tabular form in the report so that it is easy for the reader to examine for himself whether the right conclusions have been drawn.

Commenting on the quality of staff, and on the teaching done, the clear conclusion is that these have not suffered. In fact, rather the opposite is true, for these funds have enabled the institutions to retain staff of high quality in spite of the strong recruiting drives from U.S. industry. Generally the smaller institutions with smaller contributions from Central Government funds fare worse. Their staff salaries are lower and their teaching load is higher. The result naturally is that even more funds go to the bigger institutions. In this struggle the private universities fare best. The result is that research as such is regarded as a prestige occupation and teaching something to be done by others, particularly at a lower level. One might therefore wonder how students of ability receive a good education if the leaders in science do not take some part in it. Maybe, the answer is that with high student mobility, the better class institutions can pick and choose with care and so succeed eventually in getting the best brains into their graduate schools.

One of the most surprising facts elucidated in the survey is that this vast sum of money does not necessarily induce the best brains to take a science training. The arts and social sciences get their full share of what is going. It has even been possible to gain some idea of where the best students go. At undergraduate level the liberal arts colleges top the list. In the graduate schools the order is private universities with large federal funds, private universities with small federal funds, public universities with large funds, and public universities with small funds. Thus money itself does not necessarily attract the best. One noticeable result clearly shown is that there is a tendency for professors, in the American sense, to do less teaching of undergraduates. In fact 12–18 per cent of the staff do no undergraduate teaching at all, though it must

be remembered that graduate teaching is far more developed than it is in the U.K., and that the first degree in science is taken at a lower level than it is in Britain.

The report itself is critical of the way in which the funds are dispersed leading to a wider and widening difference in quality between the top and the bottom. On the whole the agencies responsible for providing the funds take the view that their money must be most effectively spent and therefore the better institutions are more favoured. All this can lead to the business of grantsmanship on a really big scale, particularly so when the salary of a member of staff may partially depend on his personally getting support from the agencies.

There is so much information in the report that it is impossible to comment completely, but since the United States is more inclined to engage in experiments of this kind than we do in Britain, it is extremely valuable to have the results of such a survey so that we in Britain may have the benefit of the effect of these methods of working in determining our own domestic programmes and in avoiding errors of judgment which can become of great academic importance. H. W. Melville

A JUBILEE TRIBUTE

Community of Universities

An Informal Portrait of the Association of Universities of the British Commonwealth 1913–1963. By Sir Eric Ashby. Pp. viii+118. (London: Cambridge University Press, 1963.) 12*s*. 6*d*.

THIS slim but important volume was written to herald the ninth quinquennial Congress of the Association of Universities of the British Commonwealth (held in London in July) and in anticipation of the granting of a Royal Charter to the (renamed) Association of Commonwealth Universities. Though based on such records as were not destroyed in 1940, it is not strictly an 'official' history and is refreshingly free from the pious and arid pedantry which often mar such works.

The main purpose of the book is to trace the development of the organization under its different names from the conference convened by Gilbert Parker, member of parliament for Gravesend, in the rooms of the Royal Society in 1903 when it was resolved that: "it is desirable that such relations should be established between the principal teaching universities of the Empire as will secure that special or local advantages for study, and in particular for post-graduate study and research, be made as accessible as possible to students from all parts of the King's Dominions". The first Congress of the Universities of the Empire was held in 1912, a Universities Bureau established, and the publication of a *Yearbook* undertaken. The early years were not without growing pains; yet it was under the auspices of the Bureau that in 1917 a conference commended to the universities 'as a basis for common action' the desirability of establishing the Ph.D. degree.

Even as late as 1931 fears were expressed that the organization might attempt too much in the way of a regimentation of the universities. To-day, the Association is a thriving institution which enjoys the confidence of the universities and is recognized by them as indispensable. The latest edition of its *Yearbook* runs to 1,825 pages and is a major work of academic reference (see *Nature*, 199, 316; 1963); the Association assists in the making of more than 900 overseas appointments annually; it plays a big part in the administration of the scholarship schemes of the Marshall Aid Commemoration Commission and the Commonwealth Scholarship and Fellowship Plan; it organizes the comprehensive quinquennial Congresses, smaller inter-congressional conferences in various parts of the world, and the annual Home Universities Conference; it provides a secretariat for the Committee of Vice-Chancellors and Principals; and, not least, its office in Gordon Square is the Mecca to which the steps of countless academic visitors are directed whenever help and advice are needed.

For many readers a particularly interesting chapter will be the one on the Vice-Chancellors' Committee, a body about whose casual origins and present functions Sir Eric provides much invaluable information. Occasional meetings of groups of Vice-Chancellors or Principals can be traced back as far as 1887. It was a simple matter of convenience that the Vice-Chancellors of the home universities were appointed in 1910 as a committee of the Bureau to take charge of the academic side of the 1912 Congress; and it was this perhaps that triggered off the proposal of W. M. Childs of Reading in 1912 for 'devising more effective machinery for joint action'. It was, however, a conference at the Foreign Office, convened in May 1918 by A. J. Balfour with the concurrence of H. A. L. Fisher to discuss the Ph.D. question, which really brought the Vice-Chancellors' Committee into being under the auspices of the Bureau. Since that date, the constitutional position of the Committee has several times been clarified and the range of the topics with which it has perforce to concern itself has grown immeasurably. Sir Eric's judgment on its present status and activities is worth quoting: "The Committee to-day is as informal as ever it was. But its influence is very great indeed. It rarely makes pronouncements, but the pronouncements it does make carry great weight. Far from infringing university autonomy the Committee is a powerful instrument for preserving it".

This book is a delight to read, with never a dull page. It has all the pithiness of style, sense of perspective and penetrating comment which we have long come to associate with Sir Eric's writing. The facts are marshalled with an easy mastery and the main themes never obscured. Especially valuable features are the extensive quotations from a number of vital documents (such as Balfour's letter of 1918) not easily accessible otherwise and the succinct summaries and comparisons of the topics discussed at the various Congresses. There are eight short appendixes (including one of brief biographies of some outstanding personalities in the history of the Bureau and the Association) and a short index.

For everyone involved in the work of universities at home or overseas or generally interested in their welfare this book should be regarded as essential reading. The fascinating story it unfolds should inspire in the universities a quiet confidence during the period when they live under the shadow of Robbins and afford them encouragement as they face the glare of whatever 'new deal' may await them. James Mountford

UNITS OF EVOLUTION

Animal Species and Evolution

By Prof. Ernst Mayr. Pp. xiv+797. (Cambridge, Mass.: The Belknap Press of Harvard University Press; London: Oxford University Press, 1963.) 70*s*. net.

THIS is a magistral book. It is certainly the most important study of evolution that has appeared for many years—perhaps even since the publication of *The Origin of Species* in 1859.

In any event, it resembles the *Origin* in focusing on the species as the key to evolutionary understanding. The species concept is certainly central, as Mayr shows in his chapter on the history of the subject. He reminds us that Darwin considered it a purely arbitrary one: "I look at the term as one given for the sake of convenience to a set of individuals closely resembling each other". For him the species had no objective existence. His prime concern was to show that they were not fixed, but could be "transformed"—in other words, evolve. Lin-

næus, through his emphasis on the essential distinctness or "fixity" of species, with its implications of their non-arbitrary nature, was able to defeat what Mayr calls "the folklore belief of the easy mutability of species", and for the first time posed the scientific problem of their origin.

The central thesis of Mayr's book, supported by a vast array of facts from every field of biology, is that species are *not* arbitrary creations of the taxonomist, but objective entities—the units of evolution, to be recognized by their distinctness rather than the degree of their difference. Furthermore, they are highly organized entities. Far from being just an agglomeration of separate mutations, or a mere collection of variant individuals, they are populations with a closely knit genetic structure, an integrated and cohesive gene-pool, homeostatic and change-resistant. This is a repudiation of the "beanbag" view, as Mayr calls it, which was in vogue in the early years of genetics. In the modern view, genes are not isolated entities with fixed properties; the selective value of a gene differs both in relation to genetic background and to environmental conditions. Although the ultimate source of evolutionary novelty is mutation, by far the largest immediate source is genetic recombination.

From these principles and from the vast body of taxonomic fact, certain conclusions follow. The most important is that we have to do something which Darwin never attempted—namely, to distinguish two very different methods of "transformation of species" or evolution—on one hand, phyletic evolution, resulting from the internal transformation of a single species, and on the other hand what Mayr calls "true speciation", the splitting of one original species into two. The long-term continuation of such splitting underlies the major evolutionary process of branching taxonomic differentiation and consequent adaptive radiation to which Rensch has given the useful name of 'cladogenesis'.

Internal transformation of species is of course widespread. It is correlated with spatial and temporal differences in the environment, and its results are manifested in the population structure of the species, notably by its network of adaptive clines, its genetic polymorphisms, and its geographical differentiation into subspecies. In the long term, internal phyletic change may so transform a population that it merits being categorized as a new species: this has been demonstrated in fossil echinoderms and a few other lineages.

The most interesting result of Mayr's analysis is the demonstration of the difficulty and rarity of true or multiplicative speciation. The cohesion and integration of the gene-pool of a species is so high, its genetic and epigenetic homeostasis so considerable, the pressure of gene-flow within the interbreeding unit so great, as to make it virtually impossible for a new integrated genetic pattern to arise within it. This applies even to peripheral parts of the species range, where environmental conditions are different, so that density-independent factors often become more important than density-dependent ones: the old cohesion is too powerful to be overridden by the new selection-pressures. However, there is one exception—the situation where peripheral populations become effectively isolated from the main body of the species. There is then no pressure of gene-flow from the co-adapted centre, so that the new selective pressures from the distinctive environment can take over and remodel the genotype to become the basis of a new species-unit.

For various reasons, this radical genetic reorganization may involve very little remodelling of phenotypic appearance. We then have a pair of sibling species. Mayr gives numerous examples and concludes that so-called "biological races" are in reality true but sibling species.

After a masterly review of the geographical differentiation of populations, in which he dismisses drift as unimportant, but puts in a plea for the founder principle, Mayr makes out a strong case for believing that, in animals at least, geographical (spatial) isolation is a prerequisite for cladogenetic speciation. The only exception is that forms with exceedingly specialized ecological requirements, such as monophagy, may possibly speciate without benefit of geographical isolation.

He is careful to point out that subspecies do not have the same objective reality as species: they are not evolutionary units of the same kind as (but on a lower taxonomic level than) species. On the other hand, the concept of the subspecies is an important taxonomic tool which in the hands of competent systematists can throw much light on the problems of intra- and trans-specific differentiation.

In this field, comparative taxonomy is beginning to give interesting quantitative results. Thus there are about 8,600 species and 28,500 sub-species of birds, giving an average of 3·3 subspecies per species. The figure varies from family to family, being low (2·6) in swallows and high (5·1) in larks. In Tiger-beetles the subspecies/species ratio is about the same as in birds, but in small rodents and especially in tropical land-snails it is considerably higher.

For many general biologists, the most novel part of Mayr's book will be his chapters on the genetic organization of populations: "The Population, its Variation and Genetics"; "Factors Reducing the Genetic Variation of Population"; "Storage and Protection of Genetic Variation"; "The Unity of the Genotype" (with a full discussion of pleiotropy, genic interaction, and correlated characters); and the "Population Structure of Species".

One of the most surprising evolutionary discoveries of recent years is that wild species, far from being genetically uniform, contain a vast reservoir of variability, some overt, some concealed or stored but capable of being released in response to selective pressures.

This store of variability includes recessive mutants, isoalleles, and the huge array of morphs involved in balanced genetic polymorphism, both phenotypically conspicuous and cryptic. One important conclusion is that correlated responses in other characters to selection for one character are not only widespread but often deleterious (for example, decreased fertility and viability in response to selection for changed bristle-number in *Drosophila*, or for commercial qualities in domesticated turkeys). A further consequence is the impossibility of accumulating more than a certain number of variability-storing mechanisms (such as heterotic combinations, balanced morphisms, supergenes, and co-adapted gene-systems) in a single gene-pool: this is illustrated by such facts as the narrowness of secondary hybridization belts, and the conversion of heterozygous advantage into disadvantage in double heterozygotes.

In regard to the complex subject of polymorphism, I feel that Mayr has not sufficiently stressed the biological value of sharp morphisms in enlarging the organism's effective niche, and the consequent necessity for establishing a strong bond between the alternative genes concerned in sharp balanced morphisms, so as to avoid one or other being lost through sampling accidents. Nor has he mentioned those cases where diversity is advantageous *per se*, in impeding recognition by predators, as in some grasshoppers and various shallow-water bottom-living marine organisms like bivalve molluscs. In the next edition of his book, he will doubtless take more account of allotypy—the fact that most or all polynucleotides, polypeptides and polysaccharides, at least in higher vertebrates, exist in a large number of chemical forms, the differences of which range from large to extremely slight. Recently a cytogenetic mechanism has been suggested which would account for the frequency of such allotypic mutations. It may be suggested that allotypic mutants of considerable extent will either be eliminated, or utilized in establishing balanced morphisms of the classical type (as in thalassemia or the major blood-groups), whereas

those the effect of which is very small will not be subject to selective pressures and will persist and accumulate, thus increasing the flexibility of the gene-complex.

Here I must bring up the topic of 'fitness'. It is now fashionable among population geneticists to define fitness solely in terms of differential reproductive advantage, without reference to phenotypic fitness involving differential individual survival. Although Mayr repeatedly stresses that selection acts on the phenotype, and that all characters are the result of compromise between different kinds of selection-pressure, his general adoption of this terminology tends to confusion. Of course, the efficacy of natural selection can only be measured in terms of differential reproduction. But the kind of biological fitness (or improvement, to use Darwin's term) which it promotes depends on the kind of selection which is operating. The confusion can only be clarified by redefining the problem in terms of selection, as J. B. S. Haldane and R. A. Fisher have already suggested, and as Darwin himself adumbrated by proposing sexual selection as a separate selective agency. It then becomes clear that two distinct modes of selection are operating in Nature—survival selection involving differential survival of individuals and reproductive selection involving differential production of offspring.

In the process of biological evolution, survival selection is the more important. It produces evolutionary effects because, as Darwin pointed out: (1) the majority of individuals which survive to maturity will leave offspring; (2) the phenotypic variance promoting individual survival has a considerable genetic basis.

Reproductive selection seems to have few biological results beyond promoting optimum progeny-number and efficient mating mechanisms, except where, as in polygamous birds and mammals, there is intense intrasexual competition and therefore a high premium on mating success. In man, on the other hand, differential reproduction is becoming increasingly important, but survival selection is becoming increasingly relaxed, with serious dysgenic consequences.

I have no space to discuss Mayr's interesting views on developmental homeostasy and canalization, or on the limitations imposed on evolutionary possibilities by the organized structure and mode of action of the genotype, except to say that I think he underrates the importance of Waddington's discovery that Lamarckian inheritance can be simulated by "genetic assimilation". I also feel that he underrates the consequential effects of differential growth (allometry). Although genetic patterns of allometric growth can be modified by selection, they still have marked evolutionary effects, as in the well-known cases of horn-development in deer, titanotheres and other mammals. The most important of such consequential effects is the increase of 'intelligence' and other behavioural capacities with absolute body-size, which, as Rensch has demonstrated, depends partly on the differential (allometric) growth-rate of the 'higher' and later-evolved regions of the brain.

I must pass to Mayr's two final chapters. That on "Man as a Biological Species", besides giving a valuable summary of the latest work on the hominid origins of man, stresses the extreme anatomical and biochemical variability of modern man, as demonstrated by Roger Williams, and emphasizes the adaptiveness of human "racial" differentiation. However, he does not point out the possibility that quite slight genetic differences between races or ethnic groups in mean mental capacity may have considerable effects on psychosocial evolution, though producing a marked increase in the supply of exceptionally capable individuals. He closes with a valuable discussion of eugenics, overpopulation, and the possible dangers of excessive outbreeding.

In the other chapter, he undertakes the important task of extending his biological arguments to trans-specific evolution—the origin of higher categories, convergence, grades, evolutionary potential and limitation, mosaic evolution, preadaptation, and the emergence of new types and evolutionary novelties.

Throughout, he emphasizes the indispensable role of the species. All the new radiations that constitute higher taxa arise from some single new species. However, though every new species is a pioneer, making an evolutionary experiment, the great majority soon reach a dead end. It is the sheer multitude of new species which makes it possible for an occasional one, perhaps only one in a hundred thousand, through chance or circumstance, to give rise to a new Order or Class. However, the key problem of trans-specific evolution is the steady rise in level of organization seen during the entire process, and especially the increased organization and quantitative differentiation of "mind" and awareness—the mental or psychological properties of organisms.

We may hope that within a few years it will be possible to deal with the problem of trans-specific process and progress in the same comprehensive way as Mayr has dealt with the subject of species in evolution. Meanwhile, I unreservedly commend this book to all biologists. It contributes notably to a fuller understanding of the objects of their study—the processes and products of life.

Julian Huxley

THE SUN

The Sun and the Amateur Astronomer

By W. M. Baxter. (Amateur Astronomer's Library, Vol. 3). Pp. 165+24 plates. (London: Lutterworth Press, 1963.) 25*s*.

The Sun

By G. Abetti. Second edition. Translated by J. B. Sidgwick. Pp. 358+149 plates. (London: Faber and Faber, 1963.) 63*s*.

Mr. Baxter's book is the most welcome contribution from a member of the British Astronomical Association to be published for some time. He observes the Sun because he likes to observe the Sun: he is not worried by the fact that sunspots are photographed by professionals as part of their regular duties. For him astronomy is, "a pleasing and satisfying hobby". His sunspot photographs, 25 of which are included, certainly gave me pleasure and proved again the folly of wondering whether or not the independent observer catered for by the British Astronomical Association should worry about doing 'useful' work. Mr. Baxter clearly shows how the Association should provide for the amateur while at the same time retaining the respect it has long held among professional societies but which it is in danger of losing. If I am right, the Association's function is educative in the first instance and, Mr. Baxter, it's secretary, elucidates the use of small telescopes and cameras as they observe the Sun. This is an admirable book for the beginner.

Not content with mere photography, Mr. Baxter also uses a Beck-Thorp direct vision diffraction prominence spectroscope, which he describes in some detail together with illustrations of prominence changes observed by himself. For me personally, the chapter on the spectroscope and the spectrohelioscope is a little disappointing for although much detail is given, enthusiasts would want much more, for example, measurements—size, etc. All would have been forgiven if reference had been made to the detailed constructional data of a spectroscope provided in the booklet by F. J. Sellers, published by the British Astronomical Association. I was also disappointed that, in noting Prof. Ellison's encouragement of the amateur, Mr. Baxter did not illustrate his work with references, photographs or drawings from his published work in the Associations journals.

Apart from some very minor quibbles, such as the inclusion of photographs of galaxies which appear in so many books, some poor diagrams due, rather to size of reproduction than draughtsmanship, a limited bibliography and a 'typical' instead of 'actual' graph of sunspot activity, I feel that Mr. Baxter has achieved his purpose admirably. Perhaps next time he will include a chapter for the amateur radio astronomer, for as yet we have little detailed discussion of this most promising field of enjoyable amateur activity. It is a book which should not only inspire the individual enthusiast but also should be used by schoolmasters for advanced projects taking pupils beyond the laboratory. It is entirely complementary to Prof. Ellison's book, *The Sun and its Influence*.

Also complementary to *The Sun and its Influence*, is the well-known text on *The Sun* by G. Abetti. This is a revision of the second edition already widely reviewed. The revision takes the form of a number of additions to the individual chapters, but contained in one appendix. There is also a short new section on the Sun and cosmic rays. Doubtless this technique has enabled the publishers to produce the book cheaply. It is perhaps worth reminding the reader that *The Sun* contains the most detailed account of solar physics at the descriptive level published in Britain. Further, it contains the unusually large number of 149 plates, most of which are worth including. With the large number of astronomical books on the market I wonder if writers could stop inserting photographs of objects or apparatus which are largely irrelevant. Baxter includes four photographs of distant galaxies—Why not four more of his own photographs or a very detailed layout of a spectroscope? Abetti includes the Jodrell Bank radio telescope and a large dish used in Australia: it would have been better to have inserted a photograph of a rhombic or the Australian solar radio spectrohelioscope. Radio observations of the Sun are not dealt with satisfactorily in this revision. The tables classifying solar radio waves on pp. 314 and 339 are not well explained, a fact which can only be put down to lack of space.

A new edition of *The Sun* ought to have included a detailed chapter on solar radio astronomy. It should not have increased the cost of the book greatly. Indeed, fifteen extra pages come available if the list of illustrations is omitted from the beginning: each one is labelled in the text; this is quite sufficient.

The book is written in a form which makes revision easy and is likely to be a best seller for many years. However, I make one plea for future editions—a better bibliography!

J. HEYWOOD

THINKING ANEW ON MODERN PHYSICS

Understanding Physics Today

By W. H. Watson. Pp. xiii+219. (London: Cambridge University Press, 1963.) 30*s*. 5.50 dollars.

THIS new book by Prof. Watson was stimulated, as he says, by the reprinting of *On Understanding Physics*, written twenty-five years ago, and by a period of philosophical reflexion during the past three years. It is not a text-book on physics in the ordinary sense but a serious discussion on where the physicist stands today in his approach and attitude towards the subject in the light of the advances made in micro-physical phenomena.

The author's basic quest is for an intuitive grasp of atomic existence, and he discusses very fully the relation between atomic and modern physics. His attitude is that physics is not based on any formal philosophy, but depends on training and practice, and on human behaviour that has evolved with the growth of experience in doing physics, a point of view from which very few would dissent.

Throughout the discussion Prof. Watson very cleverly introduces simple analogies to make his points, and to show the confusion into which a student might be drawn, he poses many pertinent questions. For example, in the chapter on atomic existence he asks: "When an electron is detected in a counter, on what grounds are we willing to accept the triggering pulses that actuate the electronic recorder as signifying events in the crystal or gas counter due to the electrons we wish to count?" This and similar questions are certainly thought-provoking, and there is no doubt that Prof. Watson fervently desires that physicists should think deeply on these things, as he undoubtedly has done himself.

The first three chapters of the book are intended to prepare the reader for the basic questions concerning existence, substance, motion and atomic connexion which are discussed in the remainder of the book. As is to be expected, quantum mechanics is well to the fore in the discussion, but the book is not intended to explain this topic or how it is applied; the aim is to look on the mathematical system of quantum mechanics not merely as a mathematical invention but as the reasonable way to represent atoms in the continua of classical physics.

Although it has been said earlier, this is no ordinary text-book on physics, yet, apart from the thought-provoking discussion, there is a wealth of knowledge in every chapter from which the young student can derive great benefit.

Undoubtedly the book is a brilliant piece of work and the discussion developed in a highly successful way; it is well written, and I am convinced that it is the result of very deep and sincere thinking. It should be in the hands of all physicists young and old, for by studying its contents they will certainly be stimulated to think anew.

[The late] T. M. YARWOOD

AFRICAN STRATIGRAPHY

The Stratigraphic History of Africa South of the Sahara

By Dr. S. H. Haughton. Pp. xii+365. (Edinburgh and London: Oliver and Boyd, Ltd., 1963.) 63*s*.

THE geographical, economic and political factors which, to a greater or lesser extent, govern the pace and direction of the development of any branch of science have had a striking influence on the course of geological research in Africa. For obvious reasons less is known about the geology of most of Africa than of the more highly developed countries, but the inaccessibility of some of the literature and the limited number of geologists who are able to work astride political boundaries means that much valuable information, which has already been gathered, is not being used to the full.

One of the organizations which has devoted itself to the dissemination of geological knowledge is the Commission for Technical Co-operation in Africa South of the Sahara. It is fitting that Dr. Haughton who, until recently, was the Commission's international scientific correspondent for geology, should bring together the main features of the stratigraphy of the area, which fell under the ægis of the Commission, into one volume.

The Precambrian accounts for the first nine chapters of the book. Seven of these describe the stratigraphy on a regional basis while the other two discuss general topics. Further chapters are devoted to the Palæozoic; the Karroo System; the Jurassic and Cretaceous Systems; Mesozoic igneous activity; the Tertiary and Quaternary sedimentary rocks; and the Tertiary and Quaternary volcanicity. These groupings may appear surprising to the geologist more accustomed to working in the northern hemisphere, as may the amount of space allotted

to various systems and events. Thus the first half of the book is devoted to the Precambrian, while the following chapter on the Palæozoic occupies only eighteen pages. These features, in a well-balanced book, reflect the contrast between the tectonic behaviour of Africa and Europe in post-Cambrian times. The work is essentially a compilation of facts and in each of the sections the lithologies, thicknesses and distribution of the appropriate rocks are considered region by region. Where an understanding of the distribution and stratigraphy of the rocks is dependent on knowledge of the structure this is briefly outlined. In spite of the emphasis on the summarizing of factual information rather than on synthesis, the book, through its clear presentation and careful arrangement, is very readable and is not likely to be treated solely as a convenient work of reference.

The illustrations are good and on the whole well selected, though the general reader will probably find the locality maps, which show place names rather than geological information, of doubtful value. Perhaps the space could better have been utilized to display some geological maps of wider areas—there is no map covering the whole of the region embraced by the title. Maps showing, for example, the distribution of the Karroo System, or the marine facies of the Jurassic and Cretaceous Systems would be most instructive.

The book is balanced, up to date, clearly written and well produced; it fills a real need at the present time.

R. L. Johnson

MOSQUITOES OF THE SOUTH PACIFIC

The Mosquitoes of the South Pacific (Diptera, Culicidae). By John N. Belkin. Vol. 1: Pp. xii+608. Vol. 2: Pp. ii+412. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1962.) 160*s*. net the set of two volumes.

THE first of these two very handsome volumes contains the text. The second is devoted to distribution maps and tables and taxonomic figures, mainly of male terminalia, pupæ and larvæ. The descriptive work has been undertaken with great thoroughness and in this respect the book can be taken as fully authoritative. No serious student of Pacific mosquitoes will be able to dispense with it. In recommending it to students of Pacific science in general, however, it must be said that the author's views on a number of subjects are highly personal and would not always command the assent of the majority of mosquito workers.

Taxonomically, Dr. Belkin is a 'splitter'. He distinguishes 12 tribes of true mosquitoes as opposed to the 3 recognized at present by most workers, including Stone, Knight and Starcke, whose world catalogue will probably continue to be followed by the majority. The advantages of consistency are such that the prudent will, probably, also await a decision by these authors regarding the various monotypic and oligotypic subgenera introduced by Dr. Belkin. In brief, it is likely that the system used in this book will be generally accepted as an expression of Dr. Belkin's personal opinion regarding the probable phylogeny of the Culicidae rather than as a universal recipe.

The general sections are too discursive for easy reading. Good points are made here and there but there is much that is uncritical, in particular a disproportionate amount of highly speculative phylogeny, surely unsuited to an authoritative monograph. Too little space is devoted to bionomics. It is true that relatively little work has been done on the biology of Pacific mosquitoes but this is all the more reason for directing the reader to sources of information on the biology of Pacific groups as studied in other areas. The distribution maps seem for the most part to be comprehensive and reliable, but it is noted that the very important *Aedes scutellaris* group is not shown as occurring farther west than the Andamans and Nicobars whereas, in fact, it has certainly been found in the Maldives and it has been recorded, apparently on good authority, from Ceylon. The treatment of disease relationships is cursory to a point at which it could sometimes be misleading. Thus no mention is made of the considerable body of evidence incriminating *Culex annulirostris* as a major vector of Murray Valley Encephalitis.

Nevertheless, whatever its other shortcomings, this book is an outstanding contribution to the descriptive taxonomy of the mosquitoes and the author deserves the gratitude of all of us for the untiring exertions which have made it possible.

P. F. Mattingly

AGRICULTURAL ENTOMOLOGY

Entomologie Appliquée à l'Agriculture

Traité publié sous la direction de Prof. A. S. Balachowsky. Tome 1, Vol. 1: Coléoptères. Pp. xxvii+564. 1962. 132F.; Vol. 2: Coléoptères. Pp. 565–1391. 1963. 162F. (Paris: Masson et Cie.)

PROF. A. S. Balachowsky has planned a monumental treatise on agricultural entomology, dealing with insects and other pests of Europe (excluding Russia), the Middle East, and Africa north of the Sahara. The first 'tome', divided into two volumes, includes 1,392 pages and describes the beetles. The remaining seven parts of the treatise will deal with the other orders of insects, with mites, myriapods, molluscs and (in the final part) with nematodes. Some sixty French-speaking scientists are collaborating in this work. They include not only well-known entomologists, but also many younger workers actively engaged in research.

The beetles of agricultural importance are described family by family. First there is a general description, then an account of the anatomy of all stages from egg to adult. Up-to-date accounts of the results of biological and physiological research are included, with some notes on control. The space given to a family usually reflects its economic importance in Europe; thus about 20 pages are devoted to wireworms and 100 to the Colorado potato beetle. The accounts of stored product pests are rather brief. Descriptions are clearly expressed and well illustrated. These two volumes contain 784 figures, many originals, others copies of well-known, but nevertheless useful, drawings or photographs.

Each volume contains an extensive bibliography, again divided into families. This contains most if not all important references to papers published up to 1961; there are, for example, nearly 200 papers on the Colorado potato beetle quoted. Non-French sources are included equally with those in the French language, and there is no suggestion that many trivial French papers have been included unnecessarily. The bibliography should be very useful to research workers unfamiliar with any family of beetles.

We are becoming used to expensive books, but few will be prepared to pay some £22 for this, the first part of an eight volume treatise. It is difficult to think for whom it is designed. Although each section contains more information than can be found in any existing text-book of agricultural entomology, yet research workers will have to supplement this with much further reading. For example, some of the results of sampling soils for pests are given, but there is no room for any description of the techniques used. The bibliography, as I have said, is excellent, but this will be out of date in a few years. Nevertheless, reference libraries should contain this treatise, if they can afford the outlay.

K. Mellanby

NEW DEVELOPMENTS AT GLASGOW'S ROYAL COLLEGE

By Dr. S. C. CURRAN, F.R.S.
(Principal)

A RECENT Government announcement to the effect that the Royal College of Science and Technology, Glasgow, was to be the fifth University in Scotland was historical in several respects. Very obviously it marked the end of a long and frequently difficult passage towards full university status. Few people realize that the College was founded in 1796, and that it was the second of the major technical colleges in Europe, following the École Polytechnique (1794). From the start the Trustees attempted to create a college with the status of a university as this was indeed the expressed desire of the remarkable Prof. John Anderson, whose Will gave vent to his strongly critical views of some of the practices of the universities of his time, and his conviction that applied science was of very great importance indeed and worthy of university recognition. In fact the College was literally called Anderson's University from 1828 until 1877.

The history of development of the College proved eventful and was marked by many changes of fortune, yet over the years it progressively grew in importance and clearly filled a very real need in higher education. At one time it contained within itself a medical school (later Anderson College of Medicine) which nurtured the great Scottish medical missionary and explorer David Livingstone, and it contained likewise a College of Agriculture. Outstanding figures feature in its records, among them Birkbeck, Thomas Graham, Young, Reith, Baird and many others. A veritable host of distinguished Scots benefited from part-time studies at the College.

Perhaps less obviously the announcement represented one aspect of the coming of age of technology. In the case of engineering studies the extent of academic recognition has rather slowly yet steadily grown, but the applied sciences have proved more difficult to assimilate in the universities of this country. On the other hand, the Glasgow College had tended to narrow its scope until at the beginning of the century these subjects of engineering and technology were the only ones of real importance. The medical part of the College work was separated in 1886. The early years of this century were not the best in which to seek degree-awarding powers in technological centres, and in the light of this fact the affiliation of the College with the University of Glasgow in 1912 can be seen to be the beginning of a new period. From then onwards the College tended to devote its whole energies to work of degree-level and gradually passed more and more of its heavy load of responsibility for the more elementary work to other Institutions. Indeed, by 1919 it was in a position to receive direct financial support from the new University Grants Committee, a distinction which it shared only with the Manchester College of Science and Technology.

Thus the debt owed by the College to the University of Glasgow is a very real one. The interest of the University in the activities of the College was a constant spur to the College staff to emulate and if possible surpass the standards of those in the ancient and world-renowned University. However, the affiliation machinery by which degrees in a steadily increasing variety of subjects could be taken by students at the College proved increasingly unsuitable as a link between two major centres of learning, particularly when the academic staff of the College reached the hundreds. The new ideas born in the College were inevitably delayed in coming into operation by the more tortuous machinery involved so that some sense of frustration was engendered. More importantly the devotion of the College to technology was increasingly in harmony with the views of powerful minds such as those of Lord Cherwell, who firmly believed in technological universities. Indeed, by 1954 it was one of the three British technological Colleges singled out for special development and this increased the strength of the ambition to be a separate university.

Even so two main obstacles seemed to stand in the way of recognition of the College as a university with full university status and powers. First, there seemed to be a clash with the concept of a university as a multi-faculty institution; secondly, the College was geographically situated close to the ancient University of Glasgow. The first difficulty was more apparent than real. Many major technological colleges were anxious to introduce the study of the social sciences and subjects such as foreign languages normally within an arts faculty. Indeed some, such as the Massachusetts Institute of Technology, had made very substantial progress along these lines but most had not been permitted to follow their desire in this matter. It seemed, therefore, that if one was prepared to visualize a science-based university with faculties of science, technology and engineering and arts and social studies then this problem largely disappeared. In the second place, the rising level of demand for university places made it obvious that a populous region such as the Clyde Valley could well have two universities with great advantage. Indeed, the accepted principle of students going anywhere within Great Britain to the university of their choice made location a matter of the philosophy of the institution itself. The views held in the College were clearly in favour of a university that placed emphasis on science and technology and its existence within an industrial region and preferably right in the heart of a major city was particularly appropriate.

At the present time a modern university is well on the road to full development of its potentialities. The necessary physical space is available, and some 25 additional acres have been earmarked for the academic precinct of the new University, the main centre of which will be self-contained and extending over approximately 30 acres. The College playing fields are about to be extended, and in due course they will cover an area of 80 acres. They are sited about three miles from the College itself. Some outlying properties will be centralized, but the residential Chesters School of Industrial Administration (Management Studies) at Bearsden on the outskirts of the city will obviously continue to provide for special postgraduate instruction. At the moment the number of general student residential places is severely limited, but as soon as possible provision will be made for something like 1,000 places either within or very close to the precinct. In many other matters of student amenity a satisfactory position has been achieved. Thus the students entered a modern Students' Union Building in 1958 and the gymnasium is being extended to include a swimming pool.

Turning to the more basic matters, the College has always stressed the importance of staff teaching effort and with a very favourable staff/student ratio the interest of the students are safeguarded. The list of departments exceeds twenty, covering science, pure and applied, engineering and arts and social studies. The last of these contains six departments at present, these being: Econo-

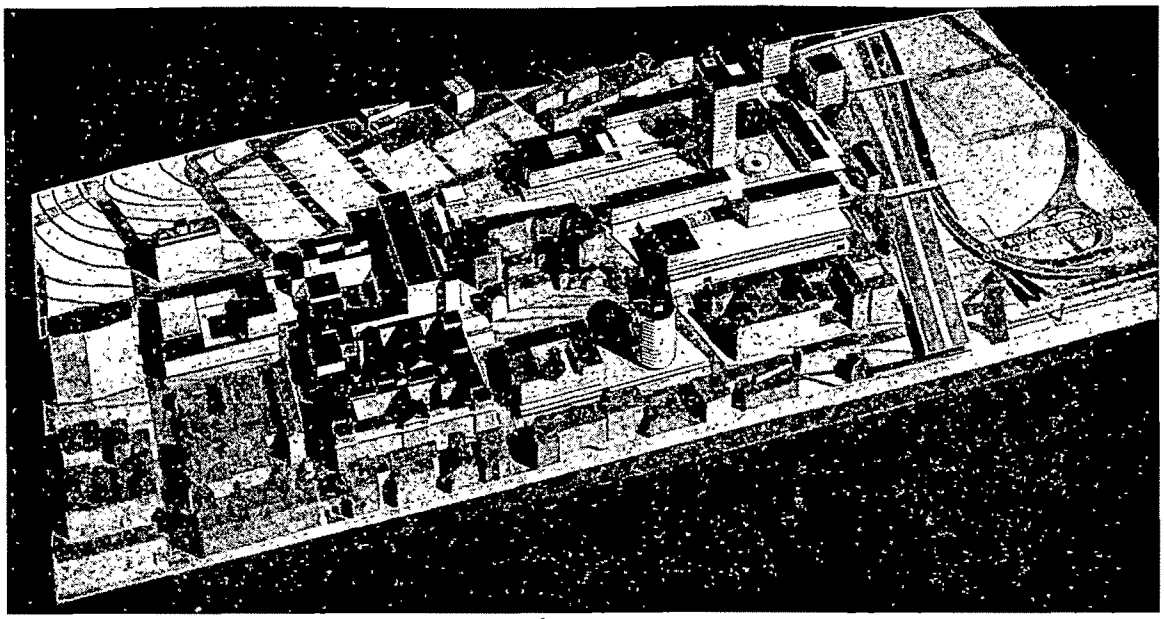


Fig. 1. Model of precinct of the new University

mics, Economic History, Political Theory and Institutions, Psychology, General Studies and Administration. The first students for degrees in these subjects or combinations of these subjects are being enrolled at present, and the first classes will begin in October of this year. The basis of admission will be the attestation of fitness of the Scottish Universities or the equivalent of the Northern Universities Entrance Board Matriculation requirements. With suitable advanced level qualifications a student can anticipate taking an honours degree in three years.

The ideas that led to the establishment of a Faculty of Arts and Social Studies may be explored as they illustrate clearly the probable method of academic development of the new University. By virtue of the nature of a Scottish Central Institution it was necessary for the College to confine itself largely within the bounds of science, particularly applied science and engineering. Thus in the later 'fifties the total number of departments was fifteen and some of these were very large. Among these large ones might be mentioned Mechanical Engineering, Civil Engineering, Chemistry and Pharmacy. Some in new applied sciences, such as Food Science and Microbiology, were in their earlier formative stages and growing, while among those of intermediate size were such departments as Textiles, Applied Physics, Applied Mathematics, Electrical Engineering and Chemical Engineering. Alongside these departments of science and engineering stood a strong department of Architecture, and this in some ways represented the nearest point of approach to the Arts. It was in the light of an obvious need to widen the horizons of the almost exclusively vocational education of the students that a strong postgraduate and residential centre was first created in 1954 and this was later centred at Chesters. This centre has been primarily concerned with the subject of industrial administration, but in catering for a residential study body of nearly 60 it engaged in a rather extensive range of tasks, collaborating with members of staff of the Engineering departments particularly well so that the students in those departments learned a good deal about Industrial Economics and a variety of other subjects, some closely relevant and others loosely linked to their main studies. The same department organized within itself a sub-department, known as General Studies, and this sub-department now stands as a department in its own right. The staff have many disciplines at their command including, for example, English, history and geography. They included within their responsibilities a number of special tasks such as the teaching of history of science, contributing to special postgraduate courses such as environmental engineering, while fulfilling their main task of stimulating the interest of almost all College students in the achievements of Western civilization. These courses seemed to be welcomed for their own sake by the great majority of students. The lesson drawn from these ventures and indeed from the well-established principles of sound education was that a much greater effort should be made to introduce social studies and arts for their own sake. Only thus could it be expected that really first-class teachers and research workers in those fields could continue to be recruited to the staff in adequate numbers.

A substantial start is now about to be made and in the first instance the new departments will be: Economics, Economic History, Political Theory and Institutions, Psychology and, as already noted, General Studies and Administration (complementing the postgraduate Industrial Administration). It is expected that the number of departments will grow until very strong Schools of Social Studies and of Arts will be functioning alongside the strong and vigorous Schools of Science and of Engineering.

The method of growth is a completely logical one. It can, in fact, be regarded as an extension of the province

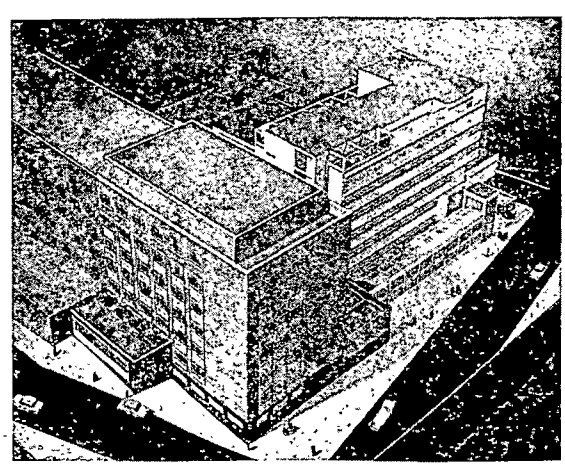


Fig. 2. Extensions to Mechanical and Engineering Departments in Cathedral Street

of science, using that word in its broadest sense. Obviously it will be desirable that the approach to the social sciences, and if possible the arts, will be such as to inject into their method as much as possible of the scientific techniques and method. With some of the subjects such as economics and psychology it is obvious that this can be done, and econometrics, for example, will feature strongly. Clearly many of the subjects can benefit from a maximization of the use of mathematics, particularly such branches as statistics. The massive application of computer techniques obviously can open up fresh horizons in some of the social sciences. The same desire to innovate can be seen in the construction of a television studio in the new social science building due to open this month. The whole subject of communication is changing at present at a very great rate indeed, and it would seem most desirable that a new university claiming a real concern for the sciences should be prepared to experiment in its approach to some of the more traditional branches of learning. The complementarity of the arts and sciences must be stressed and the integrating forces revealing the unity of knowledge and understanding given full play. This can prove to be one answer to the problem of the so-called two cultures.

Students are at present being enrolled for a range of degree courses in social studies and a handsome new building which can accommodate at least 400 students and 50 members of staff will shortly be completed. It is probable that the new university will have 2,900 students by the end of the present quinquennium in 1967, and it aims to have approximately 4,000 by the early 1970's. The number of postgraduate students, at present about 10 per cent of the total, will rise steeply with the increasing need for such degrees as M.Sc., and later some 25 per cent of the students may well be doing postgraduate work.

It is a time of great expansion, indeed, almost a time of revolution in the universities. The College joins the new universities, and although it has a long history behind it there is no question of the freshness of its spirit and enthusiasm. It is determined to play a full part in the adventure of making fresh approaches to higher education. Starting with technology firmly established, it will in some ways be in a unique position, able to develop in a fashion not unlike that discussed by Sir Eric Ashby[1].

There is obviously a very great need for more university places, though at this moment it can be questioned if the need is proportionally so great in Scotland. Most authorities agree that the demand for places in technology is not increasing at as high a rate as in other fields, particularly in arts. It is to be hoped that a new view of the technologies, different from the somewhat unfortunate one deduced from the recent study, *Technology and the Sixth Form Boy*[2], will be observed soon. As to the overall size of the Scottish demand for places many believe that the ordinary and advanced grade certificates will prove in due course very stimulating and lead to a rapid increase in the number of those ambitious to go on to University. In the meantime there may be increased opportunity for those English students who believe in the value of the education that the new University is able to offer. Many students come from overseas to the College, indeed, rather more than 15 per cent of the students come from more than 30 countries, but a relatively small fraction comes from England. This may be due to the erroneous picture of Glasgow and its environs held by many.

All concerned are determined to succeed in their new tasks. The College has prospered for sixty years as a senior central institution under the wise dispensation of the Scottish Education Department and as a College affiliated to the University of Glasgow for the past fifty years and benefiting from the care of the University Grants Committee. As it transforms to the first university founded in Scotland in nearly five hundred years, it is conscious of its responsibility and exciting opportunity.

[1] Ashby, Sir Eric, *Technology and the Academics* (1958).
[2] Oxford University Department of Education (1963).

REGULATION OF GROWTH IN SIZE IN MAMMALS

By Dr. J. M. TANNER*

Department of Growth and Development, Institute of Child Health, Hospital for Sick Children, University of London

ALMOST striking characteristic of the growth of mammals is that it is self-stabilizing, or, to take another analogy, 'target seeking'. Children, no less than rockets, have their trajectories, governed by the control systems of their genetical constitution and powered by energy absorbed from the natural environment. Deflect the child from its natural growth trajectory by acute malnutrition or a sudden lack of a hormone, and a restoring force develops so that as soon as the missing food or hormone is supplied again the child catches up towards its original curve. When it get there it slows down to adjust its path on to the old trajectory once more.

This article is concerned with the problem of how the child 'knows' that it has reached its old trajectory and should slow down. More generally, it elaborates a hypothesis as to how growth in size is regulated in mammals, and seeks to explain the mechanism by which velocity of growth is rapid in early childhood and becomes progressively less. I am frankly speculative but the hypothesis is presented for test, since one of the reasons for the relative neglect of experimental work on growth seems to be the lack of appropriate models.

* Substance of a portion of the first Harold E. Jones Memorial Lecture of the Society for the Study of Child Development, delivered at the University of California, Berkeley, April 1963.

In normal circumstances, the growth of a healthy well-nourished child is very regular. The upper section of Fig. 1 shows the measurements of height of a boy in the Harpenden Growth Study taken every six months from age 4–10 years by a single observer, Mr. R. H. Whitehouse. The circles represent the measurements, and the solid line is a simple mathematical curve of the form $h = a + bt + c \log t$ (where h is height and t age) fitted to them. None of the measurements deviates more than 4 mm from this line, although the experimental error of measuring height may be 3 mm even in experienced hands. The lower section of Fig. 1 shows the same data plotted as six-monthly velocities; the continuous curve is the first derivative of the fitted curve in the upper section. At adolescence a different curve is needed, but the Gompertz function, which is a sigmoid with four parameters, fits just as well at that time as the simple curve does earlier.

When a child or young animal is starved, however, growth slows down, and when it is fed adequately once again a 'catch-up' period occurs during which it grows at (in the human case) up to four times its natural velocity for its age. Fig. 2 illustrates the effect on height (or length) growth of two periods in which a young child's food intake was much reduced for psychological reasons. Above is the curve of body-length at successive ages ('distance' curve)

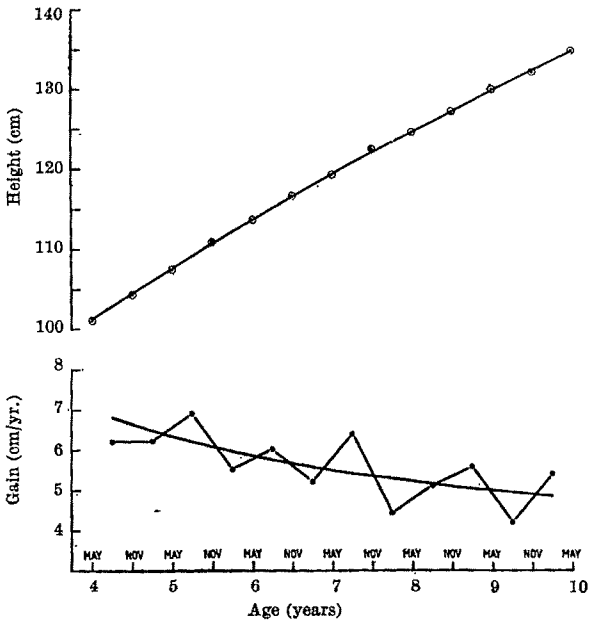


Fig. 1. Growth of a boy in the Harpenden Growth Study measured every six months by R. H. Whitehouse
Above, 'distance' plot of height achieved at each age; below, velocity plot of average velocities each six months. Solid lines: above, curve of form height = $a + bt + c \log t$ (t = age) fitted to individual's height-achieved points; below, the derivative of the fitted curve (from Tanner, ref. 10)

and below the average rates of growth at successive periods plotted against the 50th percentile lines for length-growth velocity (for details see Prader *et al.*[1]). The catch-up is apparently complete in that the child is quite normal in both length and velocity of length growth by age 5.

Two further examples are given in Figs. 3 and 4. Fig. 3 represents the growth of a girl with an adrenal tumour which secreted excessive quantities of cortisol, a growth-retarding substance (Cushing's syndrome). At the age of four the tumour was removed. The catch-up was initially very rapid, and steadily decreased in velocity. By the time she was eleven years old this girl had only reached the 20th percentile for height, so perhaps the regulatory forces had been insufficient to make good the effects of the long period of deflexion from her natural trajectory.

Fig. 4 concerns a boy who was hypothyroid from his seventh year or before up to the age of twelve years, when thyroid administration was begun. In early childhood height velocity had been average, but from seven to ten years it dropped and from ten to twelve actually became zero. During the first eight months after thyroid hormone was given the height-velocity reached more than twice average for chronological age. The boy's growth soon moved back to the normal pattern and he underwent an adolescent growth spurt which was average in intensity and occurred at the nearly average skeletal age of fourteen years. His chronological age was nearer sixteen, however. It seems that some of the years of hypothyroid arrest had been eliminated from his biological calendar, so that he was still two years behind; but by the age of twenty when his skeletal maturation was practically complete he had caught up presumably completely in height.

In all these examples, the catch-up is at first rapid and then decreases, the catch-up velocity curve resembling a telescoped version of the general mammalian growth-curve. Before setting up a hypothesis to explain this we should note that during a typical catch-up the whole organism grows rapidly and in at least approximately a proportionate manner. It is difficult (though not impossible) to see how this could happen unless the stimulus to catch up is a systemic one circulating with the blood to all parts of the body. In everything that follows it must be remembered that we are dealing with growth in size and not in shape. So far as the present results go, shape in the human being is little affected by a slow-down and subsequent catch-up of growth. Differential effects of malnutrition on limbs and trunk, for example, have never been proved to occur, though they have been suggested by analogy with some results on cattle. Growth in shape must clearly be regulated by peripheral mechanisms rather than the central mechanism for size postulated here.

We do not know what the catch-up stimulus is; suffice it to say here that it seems likely to be a balance of several hormones, released or co-ordinated by the pituitary. The factor or factors must be released in response to a signal, and it seems to me that the characteristics of catch-up require this signal to represent the degree of mis-match between the actual size of the organism and the size required at that age by the hypothetical built-in or 'target' growth curve. As the target curve is approached the mis-match diminishes and the catch-up slows down. For the mis-match to be read, both actual size and 'target' size must somehow be represented in the organism. At

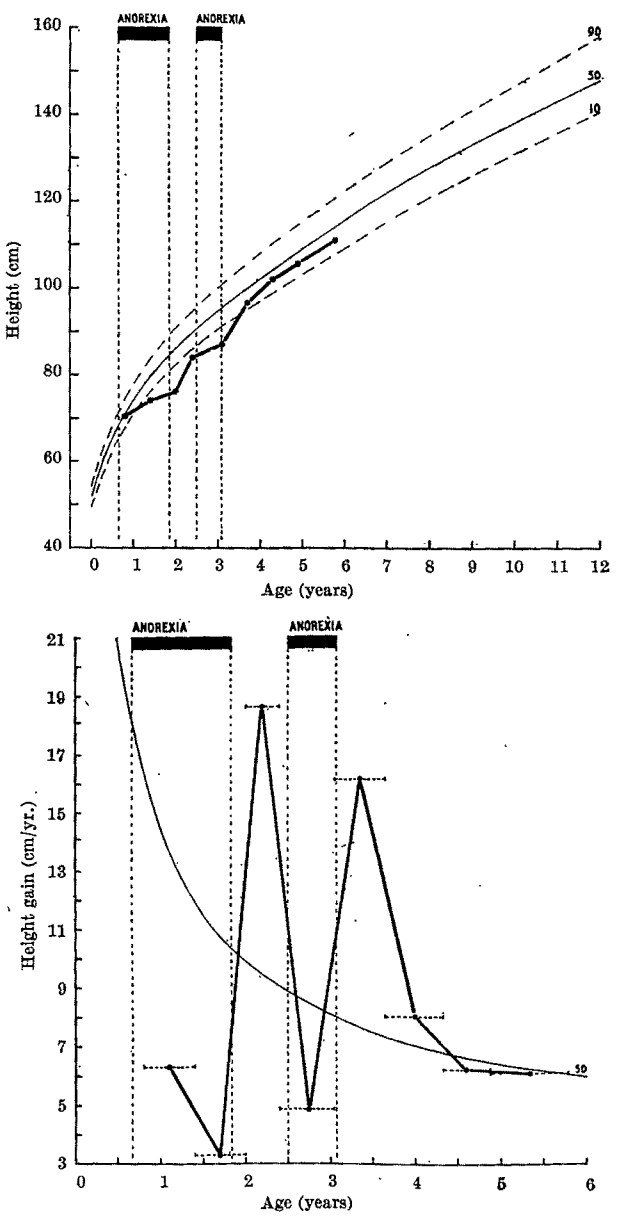


Fig. 2. Two periods of catch-up growth following episodes of anorexia nervosa in a young child (from Prader, Tanner and von Harnack, ref. 1)

present we do not know how either representation is made. It is possible that the mis-match is a peripheral phenomenon, and occurs in all cells in all tissues, the cells themselves each carrying the code for their own maturity. The catch-up in treated hypothyroidism might indeed be explicable on this basis. But it seems an unlikely hypothesis for explaining most catch-ups, and for explaining the normal control of growth in size, which is our ultimate objective.

Consider 'target' size. Suppose that somewhere in the brain a tally is kept of the time passed since conception, or rather since the age, perhaps about three months after conception, when the mechanism of the tally begins to function. This tally can represent the target curve, for both are fundamentally series of signals made against a continuing time-base. Suppose, purely for the sake of simplicity, that the tally consists of a steady increase in the amount of a substance in certain nerve cells, then the form of the growth-curve may be represented at any time by (some function of) the concentration of this substance (Fig. 5).

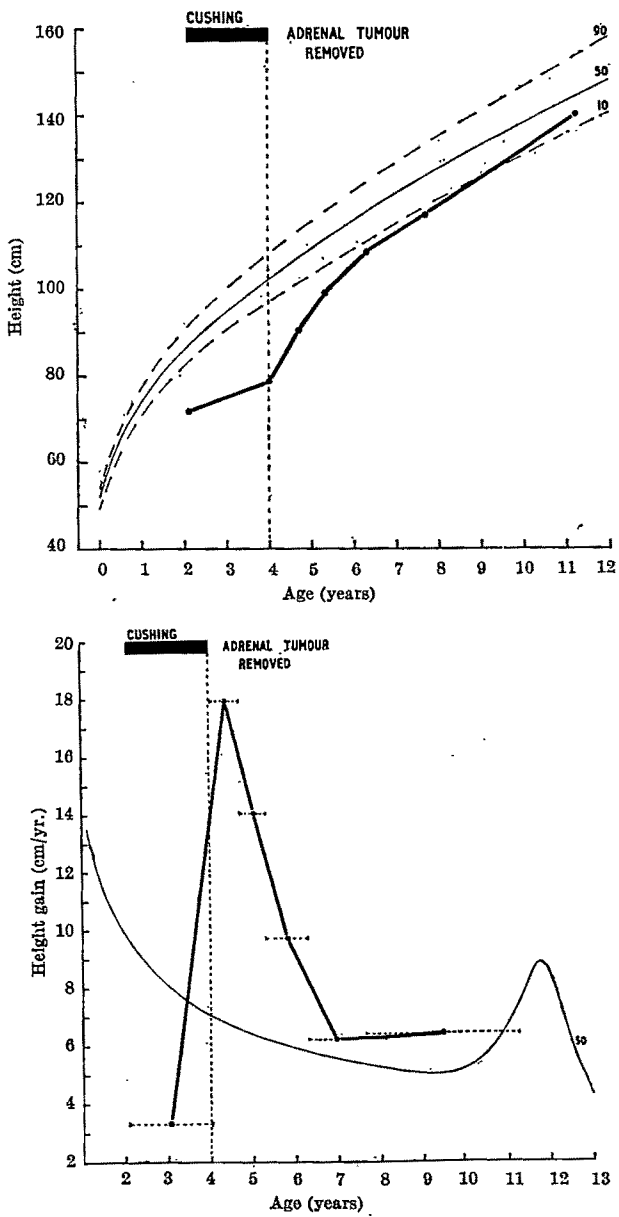


Fig. 3. Girl with Cushing's syndrome showing catch-up after removal of adrenal tumour at age 4 (from Prader, Tanner and von Harnack, ref. 1)

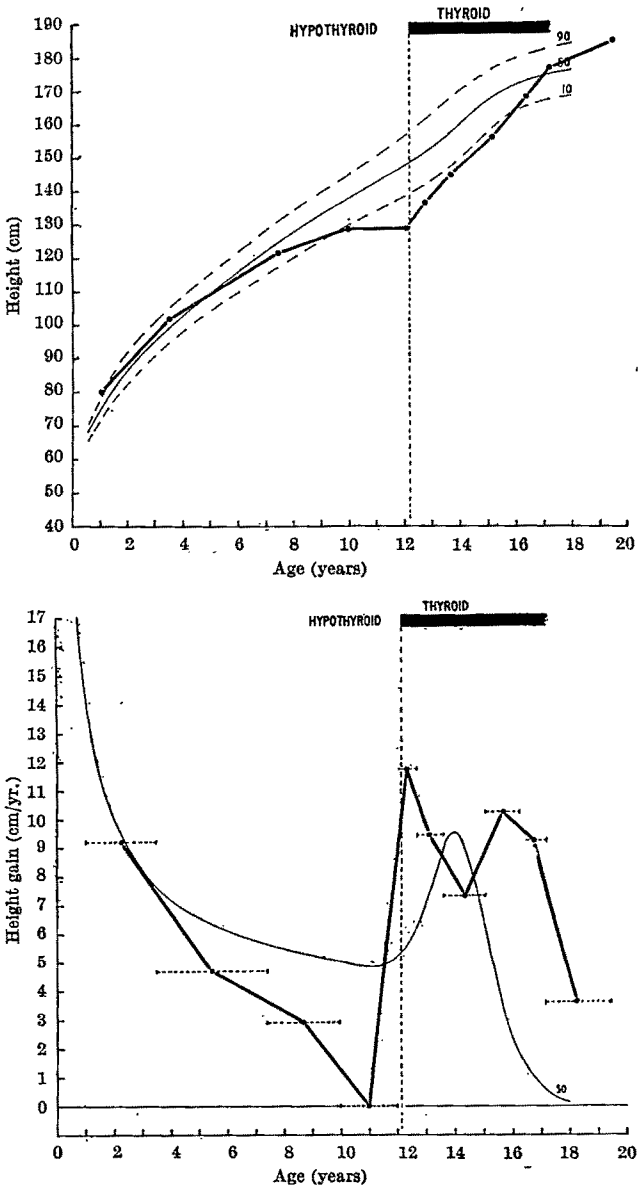


Fig. 4. Catch-up in a hypothyroid boy following treatment at 12 years old (from Prader, Tanner and von Harnack, ref. 1)

Now as to actual size: it is scarcely conceivable that the body can represent its actual extension in space. Suppose instead that the organism measures its actual height or size by the concentration of some form of circulating substance produced by cells as an inevitable accompaniment to the process of growth or protein synthesis. In this supposition I follow the most provocative and stimulating ideas of Paul Weiss[2], except that he envisages these substances—which he calls anti-templates—as numerous and tissue-specific and acting directly on peripheral cells, whereas I prefer to think primarily of a single substance acting at the brain-level. In the simplest model then, the concentration of this substance (which we will call 'inhibitor') would be proportional to the size of the organism. Its actual concentration can be measured against the concentration expected on the basis of the time tally and the discrepancy used as the mis-match signal for release of growth-stimulating factor. If also for the sake of simplicity we suppose that the time tally consists of a steady increase in the number of receptive sites for growth-inhibitor substance, then the mis-match signal would consist of the number of unoccupied sites.

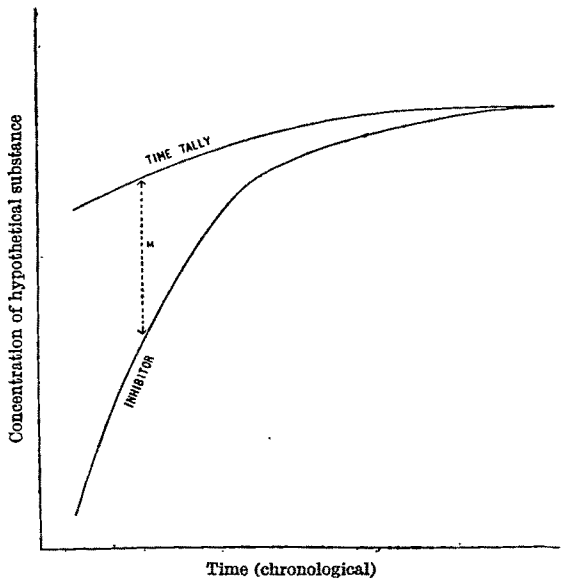


Fig. 5. Hypothetical relations underlying supposed growth-controlling agents. The quantity m represents the mis-match signal

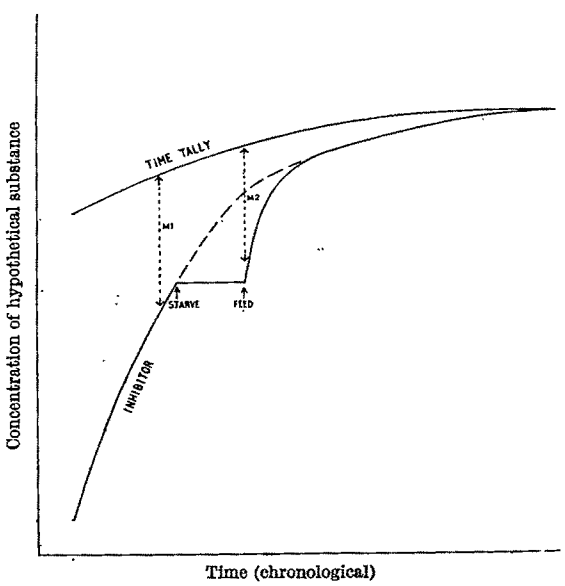


Fig. 6. Velocity expected in catch-up growth, assuming control system of Fig. 5. Catch-up velocity is proportional to the mis-match m_2, which is greater than the mis-match when starvation began, and equal to mis-match at an earlier age, as represented by $m_1 = m_2$

The tally need not, of course, represent clock time; on the contrary, it will represent the maturation rate for each individual organism. The rate of tally, and rate of change of that rate, will be an individual, presumably inherited, characteristic.

The growth inhibitors are purely hypothetical substances. Neither their origin nor their site of action is known. Weiss supposes that each type of tissue produces inhibitors as it grows, and these diffuse out into the blood and inhibit the growth of the same tissue elsewhere in the body by local action at the cellular level. This is doubtless a valid model for lower animals. It may also be valid for certain aspects of shape in mammals. One might even suppose at least *a priori*, that the general correspondence of development throughout the whole body of a given individual of a tissue such as muscle or bone[3] might be brought about in this way. But in the control of mammalian growth, certainly in size and probably even in bone and muscle, it seems more likely that the inhibitors act centrally. Furthermore, it seems unlikely that many tissues produce tissue-specific inhibitors (though kidney and liver may). Clearly the limbs contribute nothing important, for children with poliomyelitis, or even entirely missing limbs, grow perfectly normally in the rest of their body: they do not hypertrophy due to lack of inhibitors. Thus bone muscle and fat seem not to be the source of inhibitor production. The liver is the obvious choice; but it is very difficult to provide evidence for this, since damage to the liver diminishes the ability to metabolize substances essential for growth and hence may result in a slowing-down of growth even if inhibitor production is also diminished.

We may reasonably think that the same mechanism underlies the form of the normal velocity curve of growth, wherein a baby grows faster than a nine-year-old child. Suppose, again for simplicity, that the tally accumulates at a constantly decreasing rate as illustrated in Fig. 5. Suppose also that the inhibitors occur in proportion to the synthesis of protein in the cells, that for each amino-acid assembled into protein a molecule of inhibitor is produced. Then the inhibitor concentration would rise fast at first and slowly later. The mis-match (m) between these two concentrations would be large at first and decrease after the manner of the growth velocity curve. In this simple model the concentration of growth-stimulating factor is directly proportional to the mis-match and the velocity of catch-up is supposed to be directly proportional to the concentration of growth-stimulating factor. (Really one or other of these relationships might well be logarithmic.)

As Fig. 6 shows, the model represents the chief feature of catch-up correctly. The catch-up velocity seems usually to be not only greater than the velocity expected at the age of catch-up, but also greater than the velocity expected at the age at which growth stopped[1]. The model predicts that the mis-match and hence the catch-up velocity will be the velocity appropriate to a younger age than this, how much younger depending on the relative curvatures of the time-tally and inhibitor lines. The model also predicts that catch-up growth, following a given time of growth arrest, will be more intense at young ages than at older ones. This is generally thought to be the case, although my own results give no direct evidence for it.

I should not press such a crude model too far; but one cannot help noticing that the relation between the time-tally curve and the inhibitor curve resembles that between the growth of the central nervous system and the growth of the rest of the body[3]. Perhaps one of the reasons that

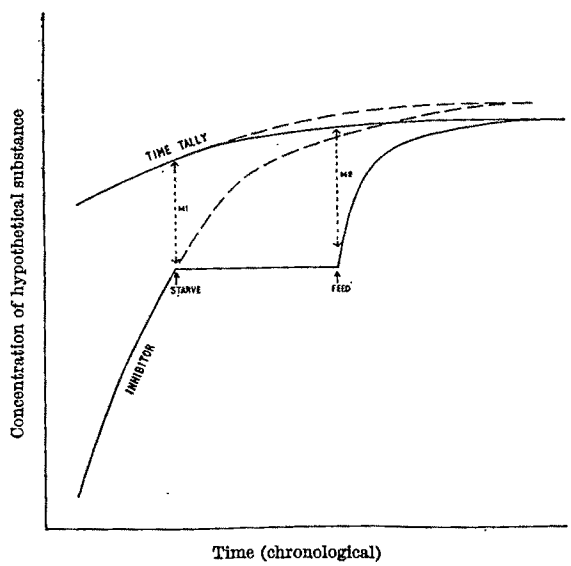


Fig. 7. Hypothetical explanation of incomplete catch-up following prolonged starvation. Time-tally velocity is supposed to be reduced irreversibly under these conditions

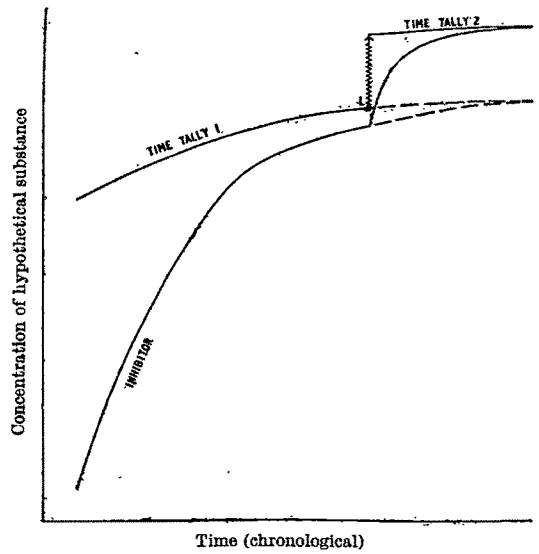


Fig. 8. Hypothetical linkage of two systems. Switch to new system occurs at L, a point supposed governed by velocity or integral of time tally

the central nervous system develops ahead of the remainder of most of the body is that the time-tally is located there. It would be logical if the earliest developing part of the organism carried the programme for the growth of the remainder.

Of course, this model may be quite wrong, and if not quite wrong, is certainly very much over-simplified. The true situation is without doubt more complex. For example, I have assumed that the tally continues unaltered even in circumstances which stop the rest of the organism growing altogether. This is somewhat unrealistic. Catch-up is probably incomplete if the period of growth arrest by starvation, say, is prolonged. To explain this we may assume that the time-tally is somewhat affected by the starvation though to a much lesser extent than the rest of the body. It may fail to resume its old rate at re-feeding (Fig. 7) or it may return to its old rate but without a catch-up. Alternatively we can assume that catch-up depends not only on mis-match but also on the velocity of time-tally (or its integral or total amount).

I have so far ignored the existence of the adolescent spurt and dealt with growth as though it gradually ceased at the end of its exponential pre-adolescent curve, as it does in fishes and reptiles. In primates particularly there is a secondary growth system superimposed on this basic one, causing a new increase of growth rate to occur just when the impetus of the basic system is nearing its end. This secondary system, be it noted, is not a necessary condition for growth to cease, even in man. Children who suffer gonadal or gonadotrophic failure at adolescence may not close their epiphyses, but their growth gradually ceases all the same (see, for example, cases *C* X and *C* XII of Bayer and Bayley[4]). Rats never close their epiphyses and reptiles do not have epiphyses to close. Evidently, it is the dwindling of the mis-match signal which causes growth to slow down; finally, the signal becomes vanishingly small, and the organism remains in a steady state of equilibrium. There is no reason to suppose that the adolescent growth cycle is organized in a different manner from the basic cycle, but it uses different substances. Its time-tally and its inhibitors must be distinct from those of the basic cycle, just as its growth-stimulating factor is also distinct. But it is also linked to the basic cycle in that its beginning occurs when the basic cycle has reached a certain point in its evolution.

Linkages such as these occur between many systems during growth. The simplest way in which the linkage could occur is illustrated in Fig. 8.

Here it is supposed that when the threshold point L on the time-tally is reached a new tally is activated and the switch made. L might be specified either by the velocity or the integral or accumulated amount of the time tally. We could explain early and late maturing by variation in the place of L on the curve. We could account also for the fact those who mature early have a greater adolescent spurt velocity than those who mature later; since when L is placed nearer the origin of the curve the total mis-match will be greater than when it is placed far from the origin.

But this simple linkage alone does not seem able to account for the postponement of puberty after starvation. Though the facts here may be a little questionable, it seems that if a period of starvation occurs at the normal time of puberty, puberty is delayed, and that on re-feeding puberty does not occur at once (as demanded by the simple linkage model), but only after a certain amount of catch-up growth has occurred. We could account for this by assuming that the switch mechanism is dependent not only on the velocity of the tally reaching a critical value

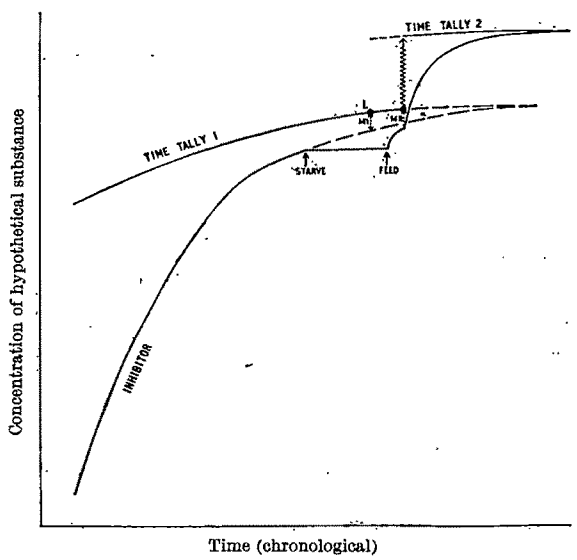


Fig. 9. Hypothetical linkage of two systems under conditions of temporary starvation. The distances marked m_1 and m_2 are supposed equal

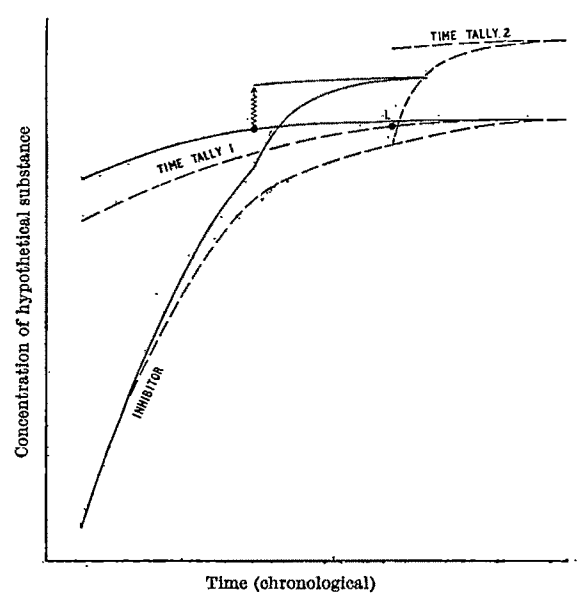


Fig. 10. Hypothetical curves for precocious puberty, assuming shape of time-tally changes, and point of switch L still occurs at usual velocity on tally. Dashed lines, normal child; solid lines, precocious puberty

L, but also on the mis-match reaching a certain value m_1 (Fig. 9). In this case ordinary catch-up would occur until the mis-match reached m_2, equal to m_1. As the scanty experimental and clinical data make it seem probable that in fact puberty occurs before the catch-up velocity has actually fallen the whole way to the usual pre-adolescent velocity, we may think that the relationships are quantitative, and that a further lowering of the tally velocity after L is passed makes possible a higher mis-match, the combination of the two being the quantity required to reach a threshold value. A two-factor linkage such as this seems more typical of events in growth than a single-factor one, in that it permits greater variability among a number of individuals. In this sense it is a 'looser' coupling. Actually the recent results of McCance[5] on starvation and re-feeding in pigs agrees with a fairly simple direct linkage; for puberty occurred in the starved and then fed pigs much before the body has caught up to its usual puberty size.

The model must be capable of explaining the disorder of precocious puberty. In some cases pubertal changes occur early, at a nearly normal size for chronological age; the children then finish their growth as very small adults. Essentially the same can be brought about in rats by destruction of an area in the hypothalamus; in an experiment of Donovan and Werff ten Bosch[6] vaginal opening occurred at 33 days instead of 40, but the growth in size of body was not accelerated. In this case, we may suppose that L has been drastically moved back along the tally curve, that is, that the linkage occurs at a higher tally velocity than normal. Physiologically, it may be that the inhibitory impulses to the pituitary are steadily diminishing as the velocity of the time tally diminishes (that is, as the maturity of the cells increases). If some of the inhibitory cells are destroyed, then the total effective inhibition diminishes, and the threshold is reached at an earlier point on the tally curve.

Other cases occur in which the child grows excessively fast from an early age, at first without any signs of puberty. He reaches puberty early, however—very early as regards chronological age and somewhat early as regards size. The growth regulation of such a child is represented in Fig. 10. The simplest supposition is that the form of the tally curve is distorted so that its velocity is at first abnormally large. This would give rise to an abnormally large mis-match at the beginning of growth and hence to the accelerated growth. The point L would occur early in chronological time, if it reflects the same critical velocity as usual in the time tally. In this form of precocious puberty therefore, puberty would occur early without any shift in time-tally threshold.

A striking example of experimentally produced disturbance of the regulatory mechanism has been given by the work of Kennedy, McCance and Widdowson in rats[7,8]. Rats which are malnourished during the first 21 days following birth—which corresponds very approximately to the period from the fourth month of fœtal life onwards in man—never catch up afterwards on their well-nourished litter-mates' curve, although they do show some catch-up growth on unrestricted feeding. When rats are starved at older ages catch-up does occur. In terms of the hypothesis presented here, the malnutrition occurring while the brain was still relatively rapidly growing apparently resulted in permanent damage to the time-tally mechanism. The hypothalamus seems the most likely location of the mechanism, particularly in view of the work of Harris and Jacobsohn[9], who showed that a new-born pituitary grafted into an adult hypophysectomized rat functioned as an adult pituitary as soon as vascular connexions were established. Thus information as to maturity is carried in the hypothalamus, or at some higher point in the brain.

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IN THE SERVICE OF SCIENCE, INFORMATION AND THE ENJOYMENT OF LEISURE

THE Lord Mayor of Oxford, in opening the Robert Maxwell Bookshop at Oxford housed in the Magdalen College Waynflete Building, described it as a bold enterprise and a most imaginative venture.

This new and most remarkably designed bookshop is also a cultural and information centre. Its 10,000 sq. ft. of space holds seven separate shops: including a general bookshop; a scientific, technical and medical bookshop; a paperback bookshop; a record and music shop; and finally, a stationery, office equipment and systems shop. In addition, there is an area designed for lectures, exhibitions and live recitals.

Service—not only to the people of Oxford but also to visitors in general—is the keynote of Maxwell's seven bookshops in one, planned carefully for more than three years. Its design and facilities incorporate the most modern American, British and Scandinavian techniques of displaying, recording and disseminating information of the printed and recorded word.

Students in Britain, elsewhere in the Commonwealth, and most other countries of the world will have the benefit of a new service in the supply of modern text-books, enabling them to acquire, under the Maxwell Budget Plan, all the books and other educational aids they need at the beginning of their full- or part-time studies and to pay for them by easy monthly instalments.

A unique, free bibliographical service for research workers and managers will help scientific research, industry and commerce to keep up to date with the latest professional publications throughout the world. Maxwell's free bibliographical service, based on co-operation with more than 1,000 of the most important publishers and institutions at home and abroad, brings users—on individual I.C.T. punch cards—accurate weekly advance information about all significant new publications on subjects of their own choice. The service is available in 435 subject classifications, ranging from medicine to packaging and marketing, to high-energy physics, plastics, building materials and teaching techniques.

A unique new feature of Maxwell's is the information systems, office equipment and stationery department. This will provide expert advice through its information and office-efficiency specialists—in consultation with each individual firm—on the best procedures, forms, facilities and equipment to be used to ensure the improvement or establishment of efficient and effective low-priced information systems, libraries and office methods in their firms.

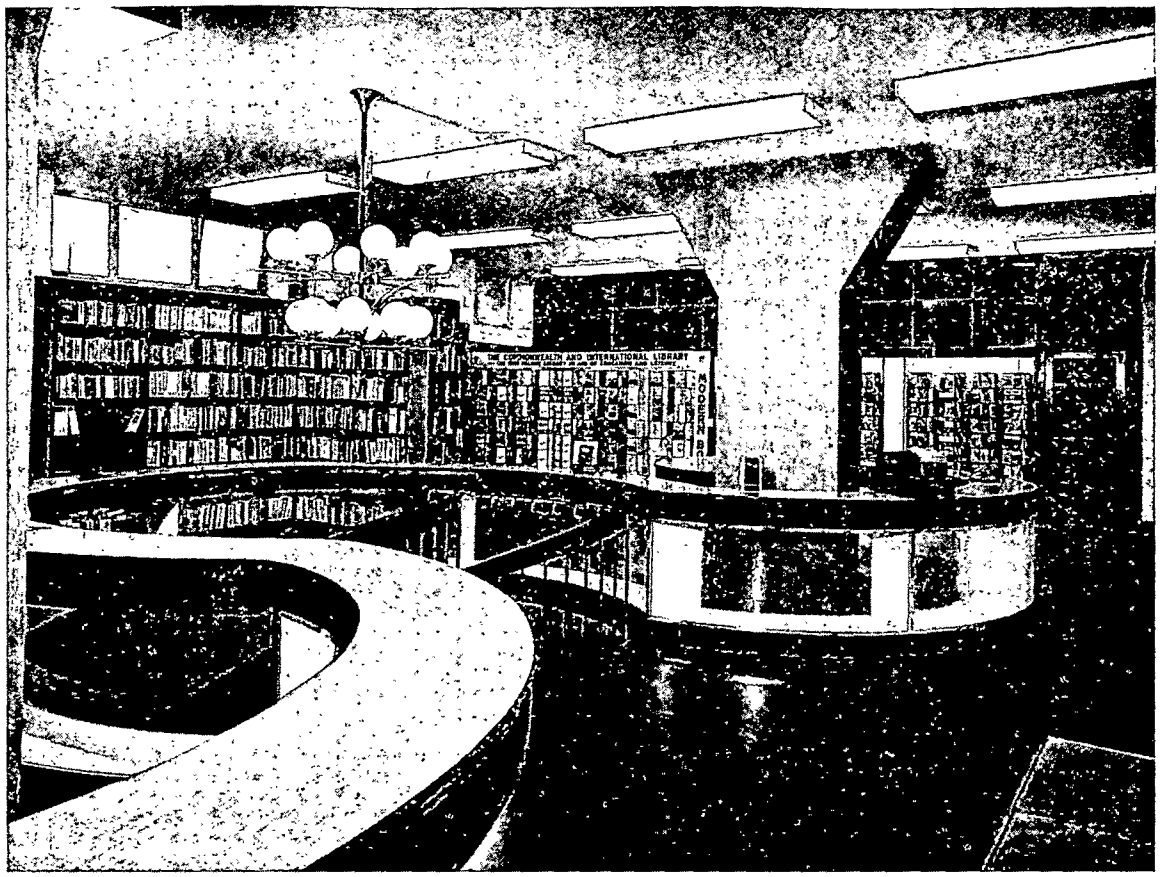


Fig. 1. Showcase and paperback book and journal section of the Maxwell bookshop in Magdalen College, Oxford

A further facility of interest to scientists is the stocking of a substantial range of scientific and medical books in German, French and Russian. There is also a 'teachers and librarians only' room; in this room are displayed the latest text-books and teaching aids.

Response from visitors from overseas suggests that Maxwell's will become the prototype of a chain of 'twentieth-century bookshops for university towns' all over the world. Following his recent tour of Maxwell's, for example, Dr. J. A. Matheson, vice-chancellor of the rapidly expanding Monash University, Victoria, Australia, invited Maxwell's to establish a bookshop on the University grounds at Clayton. A professor interested in plans for new buildings at Grenoble University has invited Maxwell's to participate. Long before these developments come to fruition, however, a second Maxwell's will be in operation in September opposite the University Medical School in Edinburgh.

OBITUARIES

Prof. Sidney Russ, C.B.E.

Sidney Russ was born in London on December 2, 1879, and died on July 27, 1963. He started his career as a physicist in London, graduating from University College in 1905, after which he went to Manchester as demonstrator in physics. Here he spent five years in the exciting atmosphere of Rutherford's laboratories when new discoveries in radioactivity were almost a weekly occurrence. From this came his life-long interest in radioactivity, which led in 1910 to a most unusual step for a physicist to take. Having been awarded a Beit Memorial Fellowship, he decided to take it up in the new Cancer Research Laboratories of the Middlesex Hospital. This was followed in 1913 by his appointment as physicist to the Hospital, almost certainly the first appointment of its kind in the world and perhaps the starting point of a new branch of applied physics. In 1919 Russ transferred from Hospital to Medical School, and in 1920 was appointed to the newly instituted Joel chair of physics, which he held until his retirement in 1946.

During his early years at the Middlesex Hospital, Russ pioneered vigorously the application of precise physical principles and techniques to the use of radium and X-rays in medicine, a task for which his Manchester years well fitted him. Later his interests turned more to the biological and biophysical aspects of ionizing radiations, his greatest contribution probably lying in the field of radiation protection. His work with Gladys Scott on the biological effects of X- and gamma-rays at low dose-levels, which seemed rather naïve and crude at the time, was clearly pointing the way to the enormous range of investigations in this field carried out in recent years.

In 1919 there was growing concern about the hazards of the uncontrolled use of ionizing radiations. Russ became the first secretary of the British X-ray and Radium Protection Committee, the first recommendations of which were published in 1920. This slim document, produced before there was even an accepted unit of radiation dose, was quickly recognized throughout the world and became the pattern for future codes of practice. In its style it clearly shows the imprint of the hand of Russ.

Russ was scientific secretary of the National Radium Commission, secretary of the Medical Research Council Radiology Committee, president of the Röentgen Society, active in the affairs of the King Edward's Hospital Fund and the British Empire Cancer Campaign, and was awarded a C.B.E. in 1931.

Large numbers of doctors will remember Sidney Russ as a teacher of both undergraduate medical students and radiologists. In many parts of the world, I have met ex-students who remember his concise, slightly pedantic lectures and his firm, but always kindly, demeanour in the examination room or laboratory. In the laboratory he was a hard taskmaster, given to flights of fancy but always firmly down to earth when results were produced. Many a humble research worker, both medical and non-medical, struggling in the borderline field between physics and biology, has good reason to remember his kindly help and understanding.

The passing of Sidney Russ breaks one of the few remaining links with those exciting days when the new science of atomic physics invaded and revolutionized a major branch of the art of medicine.

J. E. Roberts

Prof. G. M. Petropoulos

Prof. George M. Petropoulos, director of the Laboratories of Electrical Measurements and High Voltage Engineering of the Technical University of Athens, died suddenly in Oslo on June 24 at the age of fifty-four. He had been attending a meeting of Committee No. 8 (Lightning and Surge Phenomena) of the Conférence Internationale des Grands Réseaux Électriques in Trondheim and was on his way back to Athens when he suffered a heart attack and died in his hotel.

After graduating in the Technical University of Athens in 1931, George Petropoulos joined the Piraeus Electricity Company and in 1937 went to the Technical University of Berlin for two years, where he began his researches on high-voltage phenomena for which he was so well known. He returned to the Piraeus Company for a few years and was appointed reader in 1946 and later professor in the Technical University, during which time he also acted as adviser to the Public Power Corporation. His main scientific contributions have been on transient electrical phenomena on transmission line systems, and on breakdown of air in uniform and diverging electrical fields, and it was on work on this latter subject that he was reporting in Trondheim. He had developed a vigorous research school in Athens and had become an authority on electrical discharges in gases.

His colleagues from many countries represented on the Conférence Internationale des Grands Réseaux Électriques will remember his quiet penetrating analysis of problems studied in committee, and his wise counsel will be greatly missed. He visited Britain many times, and was attracted to the English way of life; British scientists were always made very welcome when visiting him in Athens. He leaves a widow, one son and a daughter.

T. E. Allibone

NEWS and VIEWS

Pure Mathematics at Leeds: Prof. A. W. Goldie

Mr. A. W. Goldie, reader in algebra in King's College (University of Durham), Newcastle upon Tyne (renamed the University of Newcastle upon Tyne as from August 1) has been appointed to the newly established second chair of pure mathematics at the University of Leeds. Mr. Goldie took Part II of the Mathematical Tripos at Cambridge in 1941, and then spent the rest of the war years in the Armament Research Department, Ministry of Supply. After two years as an assistant lecturer in the University of Nottingham, he was appointed in 1948 to a lectureship at Newcastle, where he was promoted to senior lecturer in 1959 and to reader in 1960. During the academic year 1960–61 he was a visiting research associate at Yale University. Apart from an excursion into functional analysis with two joint papers on Banach algebras, his research work has been entirely in algebra, with the theory of rings as his main field. A group of papers on non-commutative rings with maximum condition were of special importance and have aroused widespread interest. The break-through with which this work began was a complete structure theory for prime rings with maximum condition.

U.S. National Bureau of Standards; New Appointments

Dr. R. D. Huntoon, at present deputy director, has been made responsible for managing the Standard Reference Data Program as deputy director for basic standards and services. Dr. Huntoon will continue to manage the Bureau's programmes associated with the development of basic measurement standards and for calibration services associated with the dissemination of the basic measurement standards. Dr. I. C. Schoonover, at present associate director, has been appointed to the newly created position of deputy director for technological standards and services. He will be responsible for all programmes involving the development of industrial standards and test methods, which provide a common basis for the exchange of technological products and services in industry and commerce. Associated with these changes, the Commodity Standards Division of the Office of Technical Services, U.S. Department of Commerce, has been transferred to the National Bureau of Standards to become part of the Bureau's increased and concentrated effort on technological standards. Dr. A. T. McPherson, formerly an associate director of the Bureau, and more recently associate director of the Office of Technical Services, is returning to the Bureau as special assistant to the director for international standards. R. B. Scott, at present acting director of the Bureau's Boulder Laboratories (Colorado), is appointed to the new position of manager of the Boulder Laboratories.

Dr. R. D. Huntoon

Dr. Robert D. Huntoon has been a deputy director of the National Bureau of Standards for the past five years. For more than twenty years he has organized and directed fundamental research at the Bureau on atomic physics, electronic digital computers, electron physics, electronic instrumentation, and electronic ordnance devices, including guided missiles. Dr. Huntoon has been the recipient of many awards, including the Presidential Certificate of Merit and Department of Commerce Exceptional Service Award for his work on V. T. fuses, the Washington Academy of Sciences Award in Physical Sciences, etc. He joined the Bureau staff in 1941, and played an important part in the development of V. T. fuses. From 1944 until 1946, Dr. Huntoon acted as consultant to the Secretary of War; in Europe, during this period, he assisted in the evaluation of V. T. fuses and electronic aids to bombing. In 1946, returning to the Bureau, he served in a succession of increasingly responsible posts, becoming chief of the Atomic and Radiation

Physics Division; in 1951, he went to Corona, California, to serve for two years as director of the then newly established Corona Laboratories. Dr. Huntoon was appointed associate director for physics at the Bureau in 1953, and deputy director in 1958. He was born in Waterloo, Iowa, in 1909, studied at Iowa State Teachers College, and took his Ph.D. degree in experimental nuclear physics at the State University of Iowa in 1938.

Dr. J. C. Schoonover

Dr. Irl C. Schoonover has for the past five years been an associate director of the National Bureau of Standards, serving as principal staff adviser to the director on programme development, co-ordination and evaluation. Dr. Schoonover's thirty-year association with the Bureau has included the organization and direction of basic research programmes in the properties of materials, particularly at extremely high temperatures. He is well known for his direction of a research programme on dental materials, and was a pioneer in research on polymer structure at the Bureau. His work and leadership provided an integrated approach to studies of the chemical and physical properties of long-chain molecules in relation to structure. Dr. Schoonover received the Department of Commerce Superior Accomplishment Award in 1946 for his work on refractory materials, undertaken during his secondment to Los Alamos, and in 1961 he was awarded the Department of Commerce Gold Medal for Exceptional Service, being cited for "extraordinary versatility as a scientist-executive, and . . . exceptional leadership". Dr. Schoonover was born in Belington, West Virginia, and studied at Davis and Elkins College and George Washington University. He gained M.A. and Ph.D. degrees from Princeton University.

Dr. A. T. McPherson

Dr. A. T. McPherson has been associate director of the Office of Technical Services of the Department of Commerce for the past sixteen months. He worked there with Government agencies and national standards organizations in developing a new programme of inter-American co-operation on standards. Prior to this, Dr. McPherson had for ten years been an associate director of the National Bureau of Standards, co-ordinating committee activities on codes and specifications, and directing calibration and testing services throughout the Bureau. His investigations at the Bureau have included work in the fields of gas chemistry, electrical insulation, natural and synthetic rubber, and high polymers. From 1943 until 1951 he was chief of the Organic and Fibrous Materials Division, when the major part of his own scientific work was concentrated on rubber research. During the Second World War he served as a technical adviser on the Rubber Survey Committee, and was instrumental in the application of numerous new techniques in the making of both natural and synthetic rubbers. Dr. McPherson was born in Marceline, Missouri, and studied at Trinity University and the University of Texas. He was awarded a Ph.D. in chemistry by the University of Chicago in 1923.

R. B. Scott

Russell B. Scott has for the past ten months served as acting director of the Boulder Laboratories (Colorado) of the National Bureau of Standards. He was chief of the Cryogenic Engineering Laboratory at Boulder from its establishment in 1954 until his appointment as acting director. The Cryogenic Laboratory is one of three which comprise the National Bureau of Standards Laboratories at Boulder, the others being the Central Radio Propagation Laboratory and the Radio Standards Laboratory. Mr. Scott is a veteran of thirty-five years service in the cryogenic field with the Bureau, beginning with a post in the low-temperature section in Washington Laboratories in 1928. During the Second World War he was involved in the work of determining properties of uranium compounds, in support of the Manhattan Project. In 1948 he was appointed chief of the Cryogenic Physics Section in Washington. Mr. Scott was born in Ludlow, Kentucky, in 1902, and studied at the University of Cincinnati and the University of Kentucky. He was awarded an M.S. degree in physics by the University of Kentucky in 1928.

Research Contracts Awarded by the International Atomic Energy Agency

The two-hundredth research contract awarded by the International Atomic Energy Agency since this programme, intended to promote the peaceful uses of nuclear energy, began in 1958, was recently given to the Lovanium University at Leopoldville, Congo. In addition, 165 contracts were renewed during that period. The cost of the programme so far amounts to 2,923,035 dollars (1,770,469 dollars for the original 200 contracts and 1,152,566 dollars for 162 renewals). Out of this total 2,447,292 dollars were paid out of the Agency's own funds and 475,743 dollars were provided by the United States under an agreement concluded in 1960 between the Agency and the United States Atomic Energy Commission. The following is a breakdown of research contracts awarded by the Agency:

Subject category	Number of contracts placed or renewed	Amount allocated in U.S. dollars
Radioactive waste management and environmental research	56	508,158
Health physics and radiation protection	90	573,382
Radiobiology	81	697,697
Safeguards methods	14	235,520
Investigations involving the use of reactors	11	171,685
Radioisotope applications in agriculture	58	307,650
Radioisotope applications in hydrology	9	67,185
Radioisotope application in medicine	41	335,158
Miscellaneous	5	26,600
Total	365	2,923,035

The research to be carried out in Leopoldville at the *TRICO* reactor Centre should help to elucidate various affections of bone frequently found in inhabitants of that region, and caused by leprosy, smallpox and other diseases or by nutritional deficiencies. The Agency will provide radioisotopes as well as various other supplies and equipment worth 7,750 dollars.

Science in Sixth-form General Education

Part 2 of the Science and Education Report *Science in Sixth-Form General Education*, issued by the Association for Science Education, complements the Policy Statement previously issued by outlining the proposals of the Panel for courses which aim to give some understanding of the methods of science and appreciation of the effects of science on everyday life (Pp. 62. London: John Murray. Published for the Association for Science Education, 1963. 2*s*. net). Besides indicating the general principles of such courses, the Report presents detailed suggestions for courses on the nature of scientific thought; cosmology; energy; matter; life; behaviour; environment; and science and society. They are not intended to provide a syllabus to be studied in full, but rather to offer a broad field of suggestions which may assist teachers to devise their own courses. For the first course, for example, a historical and methodological approach and a logical and philosophical approach are outlined, with adequate bibliographies, and these bibliographies are frequently supplemented by lists of film-strips and films. The Report is intended to be read in conjunction with the Policy Statement and with the other three sections of Part 1, dealing with physics, biology and chemistry, respectively, for grammar schools. It is an admirable piece of work on

which the Association as well as the compilers are to be warmly congratulated. If it is widely and wisely read it could do a great deal to remove the weaknesses in education which have been the subject of present-day complaints, and it behoves the universities to make sure that no obsolete entrance requirements are allowed to frustrate the adoption in the schools of courses of the type herein proposed.

Measurements of Standard Frequency Transmissions

Measurements of the deviations of standard frequency transmissions have been made for a number of years by the Standards Division of the National Physical Laboratory, Teddington. Publication of these values, which related to Rugby transmitters, *MSF* 60 kc/s and *GBR* 16 kc/s, and the Droitwich 200-kc/s transmitter of the British Broadcasting Corporation, were discontinued in July 1962 when the journal in which they had appeared ceased to exist. Arrangements have now been made between the Director of the National Physical Laboratory and the Council of the British Institution of Radio Engineers for these standard frequencies to be published each month in the Institution's journal, *The Radio and Electronic Engineer*. The first set of values relating to the month of June 1963 were published in the July 1963 issue. A short communication from the National Physical Laboratory explaining the methods of measurements appears in *The Radio and Electronic Engineer* for July.

Studies in Density Difference Phenomena

The Department of Scientific and Industrial Research has granted £38,000 to Prof. W. Frazer and Dr. D. I. H. Barr, of the Civil Engineering Department of the Royal College of Science and Technology, Glasgow, in aid of integrated field and laboratory studies in density difference phenomena. The grant will allow for the extension of the present laboratory apparatus in which basic studies of the phenomena are carried out, and will enable the College to purchase and man a vessel with which intensive field studies can be initiated.

Density currents are set up in bodies of water when a flow differing in density from the receiving body is introduced. This can occur in many ways—by the fresh water from a river entering an estuary or the sea; by hot circulating water from the cooling system of a thermal power station being discharged into the sea or a river; and by the discharge of a sewage outfall. Mixing does not occur immediately and in many cases the introduced water can be traced for considerable distances. A striking example is the fact that the fresh-water flow from the River Amazon can be traced for hundreds of miles from the mouth of the river. The economic importance of the study of the phenomena is obvious; the currents set up in an estuary can play a very large part in the movement of sediment, and the Clyde Navigation Trust has made a grant of £8,000 to intensify work on this aspect of the subject in the River Clyde. In a thermal power station it is important that the hot circulating water issuing is not taken back into the system. This latter problem has been the subject of investigation at the College for six years, and studies have been carried out on two large hydraulic scale-models of the circulating water systems of Methil and Cockenzie Generating Stations by the Civil Engineering Department on behalf of the South of Scotland Electricity Board. At present, Prof. Frazer is advising on the similar problem which may arise in connexion with the proposed 2,400-MW station at Longannet in Fife. This will involve the construction by the South of Scotland Electricity Board of a very large hydraulic scale model of the Forth Estuary.

Television in the Medical School

An edited version of the verbatim transcript of the proceedings of the symposium *Television in the Medical School*, arranged by the Association for the Study of Medical Education and the Scientific Film Association at the London Hospital Medical College, May 10–11, 1961, has now been issued (Pp. v+73. Edited by Charles Engel and John Ellis. London: Association for the Study of Medical Education, 1963). It includes the papers presented at the session on television in research, dealing with basic theory, radiology, microscopy, endoscopy–ophthalmoscopy, and behaviour study, and the subsequent discussions; those presented at the sessions on television in teaching, dealing with present-day applications and investigations of television in American medical and dental schools; the application of television in preclinical teaching; in teaching histology; in teaching psychiatry; television endoscopy in clinical teaching; and clinical applications in German universities, as well as the ensuing discussions. Finally, it includes the papers presented on installations and on minimum basic equipment for television, and the replies from a panel answering questions on equipment and staff given at the session on practical requirements and cost of television in the medical school.

Soil Survey of Great Britain

Chief credit for the early steps in Britain on the examination of soils in the field, based on Russian, and later American, concepts of pedogenic processes and profile development, must be attributed to a small group of soil scientists including the late Prof. G. W. Robinson, Mr. Morley Davies, Sir William Ogg and Prof. T. Wallace. Meetings were held in various parts of the country to compare notes, discuss classification and initiate mapping. It was not until after the War, however, that the survey of soils was undertaken on a serious scale; it became one of the responsibilities of the Agricultural Research Council with the formation of the Soil Survey Research Board and an annual report of the work completed and in progress is issued; the present one provides the reader with an excellent account of the rapidly increased volume of information available on the soils of Great Britain (Report No. 15, 1962. Pp. vi+48. London: Agricultural Research Council. Obtainable from H.M.S.O., 1963. 5*s*. net). The scientific staff of the Soil Survey now numbers more than fifty; about two-thirds are employed in England and Wales with headquarters at Rothamsted, the remainder being in Scotland with headquarters at the Macaulay Institute. Frequent exchanges of views among surveyors are designed to maintain agreement in classification and nomenclature, and memoirs are published to explain and supplement the information on the maps. It will take many years to complete the soil map of the whole country; rapid reconnaissance is helpful, but detailed surveying is essential, and the effort spent has already been justified in the successful application of the results in agricultural and forestry problems. In addition to the regular mapping programme, assistance is frequently given in special surveys for such activities as Nature reserves, restoration of open-cast sites, archæological excavations, husbandry farms and field experimentation. An important contribution has been made in the production of a soil map of Europe, and officers are from time to time seconded to start or advance surveys of economic importance abroad.

Bibliography of Seismology

The Bibliography of Seismology continues its valuable work. It is compiled by J. H. Hodgson and F. E. Langill and published by the Dominion Observatory, Ottawa, for the Canadian Department of Mines and Technical Surveys. Nos. 5–10, Volume 22, which have been recently published, deal with work published during the years 1959, 1960 and 1961 and together contain 2,954 items. Seismic prospecting provides 533 items, studies of crustal structure 140 entries, and studies of surface waves 166 notes. The

origin of earthquakes, including fault plane mechanism, occupies 146 items, and there are 119 titles of publications on seismic instruments. An important group concerns 28 papers on Antarctic seismicity.

A Special Mechanism for some Antarctic Earthquakes

T. HATHERTON and F. F. Evison have written on this question in the *New Zealand Journal of Geology and Geophysics*, 5, No. 5, 864; December 5962. Frequent small earthquakes recorded at Scott Base constitute the main seismic activity so far discovered within Antarctica. Prominent in many Scott Base seismograms is a sinusoidal wave of period 1·5–2 sec, with an onset velocity of about 650 m/sec; this has been interpreted as an air-coupled wave associated with flexural waves in the Ross Ice Shelf. Other unusual features of the earthquakes also suggest that they are caused by fractures in the ice near the Shelf edge, leading to the calving of icebergs.

"Interchange Reactions of Disulphides"

THE following references were omitted from the communication under the above title, by Dr. E. R. Cole, which appeared on p. 1083 of the June 15 issue of *Nature*:

[1] Kharasch, M. S., Nudenberg, W., and Meltzer, T. H., *J. Org. Chem.*, 18, 1233 (1953).

[2] Pierson, R. M., Costanza, A. J., and Weinstein, A. H., *J. Polymer Sci.*, 17, 221 (1955).

[3] Davis, R. E., and Perrin, C., *J. Amer. Chem. Soc.*, 82, 1590 (1960).

[4] Schaafsma, Y., Bickel, A. F., and Kooyman, E. C., *Tetrahedron*, 10, 76 (1960).

[5] Barnard, D., and Cole, E. R., *Anal. Chim. Acta*, 20, 540 (1959).

The Society for Applied Bacteriology

AT the annual meeting of the Society for Applied Bacteriology, held in Aberdeen on July 16, the following were elected officers of the Society: *Honorary President*, Dr. T. Gibson; *Honorary Secretary*, Dr. Ella M. Barnes; *Honorary Treasurer*, Mr. G. Elis Jones; *Honorary Editors*, Dr. D. J. Jayne-Williams and Mr. G. Sykes; *Honorary Publications Manager*, Mr. A. H. Walters; *Honorary Advertising Manager*, Mr. E. J. Mann; *Members of Committee*, Dr. Christina M. Cousins, Dr. Elizabeth M. Harper, Dr. A. Hurst, Dr. J. G. Murray, Dr. Agnes A. Nichols, Dr. Patricia M. Scholes, Dr. A. Seaman, Dr. F. A. Skinner, Dr. Joan Taylor. Dr. J. R. Norris, was co-opted as an *Honorary Assistant Secretary*.

Courses at Sir John Cass College, London

THE following courses will be held at Sir John Cass College, London, during the academic year 1963–64: statistical methods in scientific and industrial research (twenty lectures on Monday evenings, commencing September 23); medical laboratory techniques (commencing September 23); domestic and industrial fuel, part 1 (twelve lectures on Tuesday evenings, commencing September 24); spectrochemical analysis (twelve lectures on Wednesday evenings, commencing September 25); general technology of petroleum (twenty-two lectures on Thursday afternoons, commencing September 26); molecular spectroscopy (fifteen lectures on Friday evenings, commencing September 27); solid-state physics (commencing October 1); domestic and industrial fuel, part 2 (fifteen lectures on Wednesday evenings, commencing October 2); fundamentals of microbiology (thirty lectures on Wednesday evenings, commencing October 2); fuel plant technology (twenty-one lectures on Wednesday evenings, commencing October 2); applied microbiology (thirty lectures on Thursday evenings, commencing October 3); solid fuels (seven lectures on Thursday evenings, commencing October 3); electronic computing systems, part 1 (twelve lectures on Wednesday evenings, commencing October 23); liquid fuels (seven lectures on Monday evenings, commencing January 13); patents and industrial design protection (eight lectures on Thursday evenings, commencing January 16); distillation (eight lectures on Friday evenings, commencing January 17); electronic computing systems, part 2 (twelve lectures on Wednesday evenings, commencing January 15); vibrational spectroscopy (sixteen lectures on Wednesday evenings, commencing February 26); trade marks (four lectures on Thursday evenings, commencing April 30); advanced mathematics four-year course for full-time or part-time students); petroleum and petroleum products (two-year evening course); mathematics (two-year evening course to Advanced Level General Certificate of Education standard). Further information can be obtained from the Secretary, Sir John Cass College, Jewry Street, Aldgate, London, E.C.3.

Announcements

SIR HANS A. KREBS, Whitley professor of biochemistry in the University of Oxford and 1953 Nobel Prizewinner for Physiology and Medicine, has been appointed official visitor to the British Baking Industries Research Association for the next five years. Dr. T. F. Macrae, who has been an official visitor to the Research Association for the past five years, has accepted re-appointment for a further three years.

THE annual open day of the Water Research Association will be held at its new Research Station at Medmenham, Bucks (see *Nature*, 192, 803; 1961), on September 18. Further information can be obtained from the Water Research Association, Medmenham, Marlow, Bucks.

A CONFERENCE on "Diamond Abrasives and Tools", sponsored by De Beers Consolidated Mines, Ltd., will be held in Milan on October 7, during the eighth European Machine Tool Exhibition. Further information can be obtained from John Burls, Diamond Abrasive and Tool Conference, 40 Holborn Viaduct, London, E.C.1.

THE seventh international symposium of the Society of European Nematologists will be held at the West of Scotland Agricultural College, Auchincruive, Ayr, during September 9–13. The symposium will be concerned largely with the practical control of eelworm diseases of crop plants. Further information can be obtained from Dr. John Grainger, West of Scotland Agricultural College, Auchincruive, Ayr.

A CONFERENCE on "Management, Productivity and Industrial Relations", arranged by the Institute of Personnel Management, will be held at Harrogate during October 10–12. Four one-day courses are being arranged to precede the conference on October 10, dealing with the following subjects: the economics of personnel management; Trade Union membership and the non-manual worker; fringe benefits: philosophy, practice and dangers; recruitment, selection and training of selling staff. Further information can be obtained from the Institute of Personnel Management, Management House, 80 Fetter Lane, London, E.C.4.

AN ordinary meeting of the Midlands Section of the Society for Analytical Chemistry, on the subject of "Contributions to Analytical Chemistry by Younger Members of the Profession", will be held in the Chemistry Department of the University of Birmingham on September 18. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

THE second Croxson Memorial Lecture of the Society of Non-Destructive Examination on "A Lifetime's Experience of Non-Destructive Testing in the Steel Industry" will be delivered by Mr. E. W. Colbeck, deputy chairman of Hadfields, Ltd., Sheffield, at the Conference Centre, Church House, London, on September 11. Further information and tickets can be obtained from the Conference Secretary, Fourth International Conference of Non-Destructive Testing, Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1.

THE ROYAL SOCIETY CLUB OF EDINBURGH

FROM the seventeenth and eighteenth centuries onwards, dining clubs have flourished in the United Kingdom. Shakespeare, Ben Jonson, Marlowe, Donne, Dekker, Dr. Johnson, Boswell and Sir Joshua Reynolds were all men who relished good food and conversation around a tavern table. This taste was shared by leaders of scientific progress; and out of the meetings of twelve or twenty enthusiasts, who met at the Bull-Head Tavern in Cheapside in 1650 to eat and drink, to experiment and to discuss science, was born the Royal Society of London. The Royal Society Club, unofficially known as the "Royal Philosophers", dates its minutes from 1743, but, probably, became a regular dining club about 1725.

Dr. Douglas Guthrie, in a recently published volume, ably records the leading features of an equally select club*. He begins with a brief account of the Royal Society of Edinburgh, which was founded in 1783. This arose from the amalgamation of a Society for the Improvement of Medical Knowledge, dating from 1731, and a Philosophical Society (for Science and Literature) which had existed from 1739.

The Royal Society Club was founded in 1820 "with the view of promoting the objects of the Royal Society". Membership was limited to forty-six Fellows of the Society elected by ballot. Since 1950 the number has been raised to sixty. The Club met at various Edinburgh hotels up to 1909, but now normally meets in the rooms of the Royal Society in George Street. Dinners have also been held in or near Edinburgh.

* *A Short History of the Royal Society Club of Edinburgh* 1820 *to* 1962. By Douglas Guthrie, with a foreword by Prof. E. L. Hirst, president of the Royal Society of Edinburgh. Pp. 40, illustrated. Published privately by the Royal Society Club, Edinburgh, 1962.

Interesting sketches are given of distinguished members of the Club. Sir Walter Scott was the third president of the Royal Society of Edinburgh (1820–32) and the only 'literary' president. He was an original member of the Club, and was præses (chairman) at the dinners of March 6, 1820, February 5, 1827, December 3, 1827, and January 18, 1830. He attended on other dates and was elected an honorary member in 1831. Other eminent members are mentioned, for example, James Skene, the first secretary, antiquary and close friend of Sir Walter; Lord Meadowbank, the judge; Sir Archibald Geikie, geologist; the versatile Prof. John Stuart Blackie; Sir Robert Christison; Lord Playfair; Lord Kelvin; and coming to later times, Dr. G. A. Gibson, Sir D'Arcy Thompson and the great bacteriologist, Sir Robert Muir. In the nineteenth century music and song added to the conviviality of the dinners, and some of the witty songs sung were composed by the members. The Club has entertained many distinguished guests, including the Crown Prince of Sweden, the Prince of Monaco, Captain Peary, Fridtjof Nansen, Dr. Sven Hedin, Sir J. J. Thomson, the physicist, Lord Balfour, statesman and philosopher, and Sir Arthur Keith. An account is given of two other clubs (now extinct) for more junior Fellows, namely, the Royal Society New Club and the Royal Society Supper Club. The book is illustrated by portraits of certain members.

The Club has been fortunate in persuading Dr. Guthrie, who has been for many years a devoted member both of the Royal Society of Edinburgh and of the Royal Society Club, to write its interesting history.

ARTHUR MACNALTY

RESEARCH INTO EDUCATION IN BRITAIN

IN a debate in the House of Commons on July 24 on the Schools Council for Curriculum and Examinations which was opened by Mr. J. Boyden, who expressed some concern about the establishment of such a council, Mr. F. Wiley referred to proposals made by the universities to the Minister for Science for the establishment of an educational research council. Mr. Wiley stressed the need for conditions of support which preserved for the individual worker the intellectual initiative essential for creative work. The universities, he said, pointed out that an educational research council should be independent of the executive departments of the Government, and directly responsible, not advisory, for the expansion of research, including research into problems of primary, secondary and tertiary education in Britain as a whole. They suggested a council of about 12 members, chosen for their distinction and bringing together wide experience in research. They would not be representative, and this body was the antithesis in character of the council of 58 or 59 which the Minister of Education was proposing to establish.

In reply, the Minister of Education, Sir Edward Boyle, said that he had convened a meeting on July 19 to discuss a memorandum which he had circulated widely, and which was inspired by the central thought that curriculum development on the scale now required demanded the willing and enthusiastic co-operation of all partners in Britain's educational service. The discussion was of extremely high quality, and at the end of the meeting a resolution was passed which (*a*) noted that there was wide support for the proposal to establish co-operative machinery in the fields of school curriculum and examination; (*b*) appointed a working party, with Sir John Lockwood as chairman, comprising one representative of each of the bodies present at the meeting, together with assessors and a secretariat appointed by the Minister for Education, to consider how effect could best be given to the matters discussed and to make recommendations; (*c*) agreed to reconvene to consider and reach conclusions on the working party's recommendations.

On the general issue of research, Sir Edward Boyle said that while the sum allotted in this year's Estimate was only about £70,000, the total cost of all the projects being undertaken would, when completed, be between £200,000 and £250,000. One of the difficulties in this field, besides isolating the specific fields, was to find teams capable of doing full justice to them. Sir Edward assured the House that he was fully aware of the great importance of the research work in progress and for the future he thought that team work on curriculum and methods should proceed in a context which ensured the exchange of ideas between different teams. He pointed out that the curriculum study groups and the inspectorate were not entirely separate, the former including many of the ablest inspectors. He had discussed these ideas with a representative meeting of inspectors and there was no intention to diminish the role of the inspectorate, which was even more important in the educational system than many people realized. There was, however, a danger that some central bodies could be pushed into

oxtending their proper terms of reference, and he did not wish the establishment of the curriculum study groups to lead towards centralized control of the curriculum or towards misdirected central influence on it. If a schools council were set up, however, he would wish it to be more than a purely advisory body and that it should possess complete autonomy in all matters which did not fall within the Minister's statutory powers and responsibilities. With regard to the size of any such council, Sir Edward insisted that education in Britain was a shared service, and accordingly such a council must bring together the Ministry and local authorities and the teachers. He hoped that the council would also include not only representatives of those concerned with the day-to-day work of education in the schools, but also some who could point out lines of research on issues which might be overlooked by others, members who were competent to challenge the Ministry and Britain's traditional day-to-day approaches to the kind of research which was most needed.

The proposals mentioned by Mr. Wiley were sent to the Minister for Science by the heads of departments and institutes of education of universities in England and Wales. They refer to the danger of a patchwork of short-term projects and base their proposals on the organization of the existing Research Councils responsible to Lord Hailsham.

TRANSLATIONS OF LITERATURE ON NUCLEAR ENERGY

THE European Atomic Energy Community (Euratom), the United States Atomic Energy Commission, and the United Kingdom Atomic Energy Authority have agreed to pool their efforts in order to collect and disseminate information on translations of nuclear literature, in particular of documents originally written in languages unfamiliar to the Western reader. An information office 'Transatom' has been set up at the headquarters of Euratom in Brussels for the collection of data concerning translations of interest, and a monthly guide to nuclear literature in translation, *Transatom Bulletin*, compiled by Euratom is published by the Elsevier Publishing Company, Amsterdam. The *Bulletin* is in English and provides a unique survey of translations into English, French, German and Italian, of articles, monographs, books, reports, etc., in the field of nuclear science and technology originally published in the non-Western world. Precise bibliographical data of the original Russian, East European, Japanese and Chinese documents are included in the *Bulletin*, together with the addresses of the translation agencies which can supply copies of the translations listed and the conditions of availability of the translations. Certain journals receive cover-to-cover translation and a separate list of these is printed in every issue of the *Bulletin*. The various entries in the *Bulletin* are classified under fourteen headings, covering general and specialized areas of science and technology in which nuclear energy is either fundamental or has found some application.

A list of translations in preparation, but yet not completed, which may be of special value to many organizations so as to avoid duplication of effort and unnecessary expense, is an added feature of the *Bulletin*. Both author and source indexes are given in every issue of the *Bulletin*, and cumulative quarterly indexes are included in the Nos. 3, 6 and 9 issues, with an annual cumulative author and source index in the final (No. 12) issue of the year and volume. The *Bulletin* is now in its third year of publication.

A new publication of Euratom, *Euratom-Information* *, is to be published bi-monthly. The research undertaken by Euratom which is now five years old is beginning to give rise to a rapidly increasing number of reports, at present at about one a day, and the new publication will act primarily as a periodical catalogue of the reports. The contents of the first issue of *Euratom-Information* is divided into five sections. The first contains a list of 104 scientific and technical publications; the second 33 patents; the third the research contracts signed by the Euratom Commission up to December 31, 1962; the fourth details of the research activities which the Commission plans to carry out as from January 1, 1963, and in which individuals and organizations within Euratom can collaborate; and finally, a statement of the Euratom–United States of America joint research and development programme. In the first section, where the publication referred to is not in English, the foreign language summary together with the translation of both the title and summary are given. Details of how to obtain copies of the reports and publications listed are included.

S. WEINTROUB

* *Euratom-Information*, Vol. 1, No. 1 (May 1963). (A publication of the European Atomic Energy Community.) Pp. 1–81. Published at two-monthly intervals. (Düsseldorf: Handelsblatt, G.m.b.H., Kreuze-Strasse 21, 1963.) Annual subscription 15 dollars.

REFLECTED LIGHT MICROSCOPY

THE quantitative methods which can be applied to the study of opaque minerals (natural and synthetic) under the polarizing microscope are the measurement of reflectivity, of rotation properties, and of micro-indentation hardness. Recent advances in these methods have brought them to the point where it is believed that they can be generally applied in a way similar to the use of optical methods in the identification of transparent minerals.

With the view of disseminating knowledge of these methods as widely as possible, a small committee of British mineralogists was set up to organize an international Summer School. With the aid of a generous grant from the Scientific Affairs Division of the North Atlantic Treaty Organization, an advanced study course was held during June 23–July 2 in the Department of Mineralogy and Petrology, Cambridge. There were thirty-one members, including eight observers, drawn from twenty-three different countries; all except two of those in the observer category were mineralogists engaged in academic work.

The basic techniques and theory were covered in seven lectures and practical demonstrations, after which the members divided into two sections (ore minerals and coal petrology) for a further six lectures followed by practical work in either section. Each member had a total of thirty hours of practical work, and in addition there were three colloquia. Throughout the course emphasis was placed on the practical aspects of quantitative measurements, and this was made possible by the generous loan of equipment by several firms. Much of the apparatus, which included reflecting-polarizing microscopes, monochromators, photometers and micro-indentation hardness testers, was of recent design, and the opportunity was taken of obtaining comments from the members on the equipment.

Further information about this Summer School can be obtained from the secretary, Dr. N. F. M. Henry, Department of Mineralogy and Petrology, Cambridge.

ASIA AND OCEANIA CONGRESS OF ENDOCRINOLOGY

THE second Asia and Oceania Congress of Endocrinology was held in Sydney during May 28–June 3 under the auspices of the Endocrine Society of Australia. The president of the Congress was Prof. C. W. Emmens, and Prof. T. Miyake (Japan), Dr. H. D. Purves (New Zealand) and Prof. V. M. Trikojus (Australia) as vice-presidents.

One hundred and eighty delegates participated in the Congress, including fifteen from Japan, six from New Zealand, five from the Philippines, four from both Great Britain and the United States, two from India, one from Hong Kong and one from Italy. Eighty papers were read from 120 offered.

Prof. A. S. Parkes (Physiological Laboratory, Cambridge) opened a symposium on "Regulation of Fertility", and discussed climatic, social and nutritional factors. Rapid-acting and neural effects were differentiated from the slower-acting responses mediated via the anterior pituitary. The importance of smell as an exteroceptive factor working through slowly acting neurohumoral mechanisms was discussed. Related papers, including a number from workers in Japan and from a group in Sydney on various aspects of œstrogenic and anti-œstrogenic effects, followed the opening paper.

Dr. H. D. Purves (Endocrinology Research Department, Medical Research Council of New Zealand) opened a symposium on "Control of Thyroid Secretion" and discussed aspects of the feed-back mechanism in relation to control of thyroid function. He was followed by Prof. T. Miyake (Kyoto, Japan), who reported on clinical studies of thyroid hormone metabolism, and a series of papers from groups working in New Zealand, in Melbourne and in Adelaide were presented.

A symposium on "Hormonal Response to Environment" was led by Prof. E. C. Amoroso (Royal Veterinary College, London), who spoke on "The Endocrine Environment of the Fœtus" and discussed aspects of the complicated hormonal environment in which the developing mammalian organism grows pre-natally. This was followed by papers from a group in Melbourne on electrolyte balance in ruminants, and by a paper on the suckling stimulus in marsupials from workers in Canberra, and by two papers from Indian workers.

Dr. M. S. Raben (Boston, United States) opened a symposium on "Protein Hormones in Growth", and reported investigations of metabolic and clinical effects of human growth hormone. Problems concerned with the production of a satisfactory preparation were summarized and details given of metabolic work on selected patientf receiving these preparations. The characterization os pituitary growth hormones from various species was described by a group working in Sydney.

In addition to these symposia a number of papers on adrenal physiology, comparative endocrinology, steroid hormones and hormone assay were presented in concurrent sessions.

The interest and prominence of a session on hormone assay, particularly of steroid hormones, indicated the concern of many endocrinologists with details of basic methods which will enable reliable results to be obtained by workers in different laboratories and thus lead to comparisons which have some significance.

On the final day a group of papers on clinical endocrinology was presented, and during the afternoon a plenary session was held with The Royal Australasian College of Physicians, which was celebrating its Jubilee in the week following the Congress. The session was opened by Dr. R. F. Escamilla (San Fransisco), who spoke on "Stimulation of Growth in Short Children: Experiments with Human Growth Hormone", and papers were presented from the United States, Japan, India and Australia on various clinical aspects of human growth hormone, anabolic steroids, idiopathic hirsutism, corticosteroid treatment and diabetes in pregnancy.

It is apparent from the general interest of the participants and the quality of the papers presented that the study of endocrinology is being actively pursued in a number of centres in the Asia and Oceania region, and that a high standard is being maintained.

A factor in the success of the Congress was associated with the opportunities afforded delegates to make new, and renew old, acquaintances. The site of the Congress, a section of the Chemistry School, University of Sydney, and the limited number attending were both conducive to a free circulation of members during lunch and tea periods. The spirit of informality pervaded the lecture rooms and led to that most important aspect in the success of a Congress, lively and friendly discussion.

Acknowledgement should be made to the Executive Committee for their organization and to Dr. P. J. Claringbold (C.S.I.R.O. Division of Animal Genetics), the secretary to the Congress, for his excellent administration, which contributed largely to its success.

The third congress will be held in Manila in January 1967.

I. G. Jarrett

VOLTA BASIN RESEARCH PROJECT

By Prof. G. W. LAWSON
Department of Botany, University of Ghana, Legon

WHEN the Volta Dam in Ghana is completed next year a lake will begin to form which will eventually cover an area of 3,275 square miles. This will be the largest man-made stretch of water in the world. The water-level will begin to rise in about September after the rainy season of 1964 and will reach the 200-ft. contour in the first year, but the final level will not be reached until some years later. The Volta Scheme will primarily provide hydro-electric power for an aluminium smelter at the new port of Tema which will ultimately produce 120,000 long tons of aluminium per annum, mostly for export. Ghana is and has always been an agricultural country relying mainly on the export of cocoa for the foreign exchange needed to buy manufactured goods. The availability for the first time of cheap electricity means that conditions will be created for a rapid industrial revolution with an inevitable rise in standards of living throughout the country.

Additional benefits from the scheme will include a large freshwater fishery and a cheap inland water transport system. It is also planned to grow crops, especially rice and sugar cane under irrigation. Undoubtedly the impact on Ghana's economic life will be tremendous.

The creation of the lake will also, however, have many important biological implications, the most important and immediate of these being the removal and rehabilitation of more than 70,000 people from the area to be flooded. This work is being undertaken by the Volta

River Authority, a Government body set up to control all aspects of this far-reaching scheme. The transplantation of these people, consisting of many different tribal, language and religious groups, and about who there exists little written information, creates far-reaching sociological problems.

As with every major development project, it is necessary, by careful advance study and planning, to safeguard against certain dangers which so large a change in the environment is capable of provoking. One such danger, in this case, is that certain water-borne diseases such as bilharzia and onchocerciasis (river blindness), already serious in Ghana, will become accentuated. There is also the undoubted possibility that aquatic weeds may become a serious problem as they did at Kariba Lake in Central Africa, where more than 200 square miles of water is covered by a dense mat of *Salvinia auriculata* adversely affecting fisheries and making lake transport difficult or impossible.

Apart from these intensely practical problems many more academic but highly interesting phenomena require investigation. A biological and physical survey needs to be undertaken now to ascertain the populations of fish and other animals that at present inhabit the Volta River and its tributaries and the conditions under which they exist. Likewise the phytoplankton will undergo a unique series of changes as the lake forms. On the human side there is much information to be gained about such subjects as the distribution of language groups and the social organization of the inhabitants of the Volta Basin area before they are moved. Several archæological sites need to be investigated. They do not compare in importance, of course, with the monuments in the Sudan which may be lost by the building of the high Aswan Dam, but in view of the relatively little that is known of the pre-history of West Africa they are of distinct academic interest.

Not only the Basin area but the area below the Dam will be affected both biologically and economically. The present seasonal flooding enriches both the land used for agriculture and the numerous creeks used for fishing by the riverine population. During the dry season, when the river is low, collection of river clams (*Egeria radiata*) is carried out. This flourishing industry, which employs several thousand women and is worth about £100,000 a year, will be threatened by the eventual steady flow which will be too fast throughout the year for collection to take place and which may also affect the delicate balance of saline and fresh water in which the clams thrive. Transplanting of the clams to more favourable areas may be possible. Such problems must be investigated and possible solutions to them need to be attempted before it is too late.

These are just some of the things that cry out to be done in the little more than a year that remains before flooding, and in view of the urgency that exists the University of Ghana has decided to launch a Volta Basin Research Project. Work by existing staff has already commenced on several aspects of research connected with the project and it is intended to recruit Research Fellows to undertake specific problems. The Project Committee will welcome any outside assistance and will endeavour to give every possible help to individuals or groups who wish to be associated with it.

The initial financing of the project is being provided by the University, and the project has the support and approval of the Volta River Authority and the United States A.I.D., who, it is hoped, will be able to contribute to its development both as regards equipment and personnel.

ANALYSIS OF THE LIFE-CYCLE IN MAMMALIAN CELLS

UNDER this title Defendi and Manson[1] have recently published results obtained with a few normal and aberrant types of mammalian cells, and from these results, and from a selection of information taken from the literature, they have generalized "that most mammalian cells of normal and tumour origin, *in vivo* or *in vitro*, pass a similar period of time in DNA synthesis and in preparing for mitosis irrespective of the generation time". It is possible that this conclusion may ultimately prove to be justified, but at the moment the available evidence scarcely appears to be adequate, and a more appropriate conclusion might be that for most mammalian cells of normal and tumour origin no information exists at all.

They further conclude that "the *primum movens* of cell division has to be searched for not at the initiation of mitosis but farther back in the cycle at the time of initiation of DNA synthesis". This has, of course, been obvious for a long time, although the important proviso must be added that the actual point at which the cell commits itself to prepare for division may well be earlier than the point of initiation of DNA synthesis. The beginning of DNA synthesis is merely the earliest point that can be observed with present-day techniques.

Finally, they quote the conclusion of Gelfant[2] that adult mammalian epidermis is an exceptional tissue in that it contains a unique population of cells which have completed DNA synthesis but which remain quiescent for "an indefinite period of time, acting as a reservoir of cells immediately ready to undergo mitosis". Unfortunately, for a variety of reasons Gelfant's conclusions cannot be accepted; it is sufficient to note that his published results are statistically inadequate. However, as is shown here, there may well be a half-truth in this particular conclusion.

In view of the confusion which may be created it seems necessary to set out the most significant of the evidence now available regarding the mitotic cycle in adult mammalian cells. However, before doing this it is necessary to analyse, name and define the phases through which a regularly dividing cell passes (Fig. 1). The literature contains a varied and sometimes contradictory terminology.

Cycle of cell growth and mitosis. The generation time is the time taken by a cell to pass through the whole cycle, for example, from prophase to succeeding prophase. There is a large literature on the generation times of various tissues and cell types, and this has been well reviewed[3,4].

On present knowledge the whole cycle of cell growth and mitosis can be subdivided as follows:

(1) *Early interphase:* the period between the end of telophase and the time when the cell 'decides' whether to differentiate or to prepare for yet another mitosis (in the literature this phase is variously called "G 1", the "first growth phase", the "first gap", or the "presynthetic resting phase").

(2) *Dichophase:* a new name derived from διχο (expressing doubt between two ways ahead) and here used to define what may be the most interesting and important phase in the whole cell cycle, the "phase of decision" when within the cell a 'choice' is made between synthesis leading to differentiation and synthesis leading to division. The duration of this phase is unknown.

(3) *Prosphase:* a new name derived from προσ (expresing movement towards) and here used to define the whole

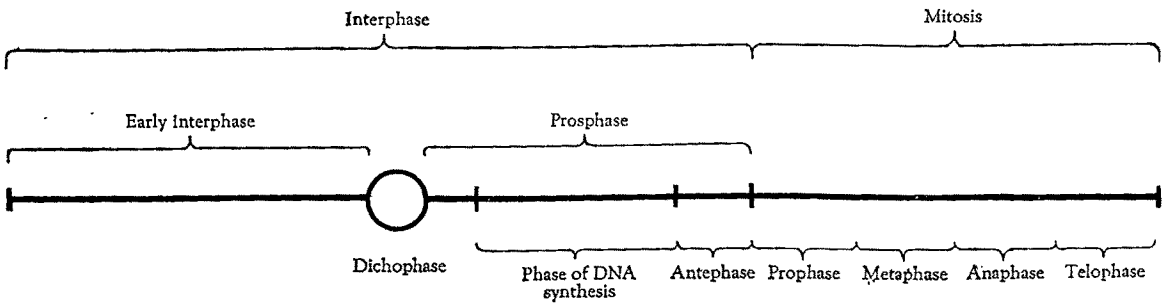


Fig. 1. The sequence of phases in the cycle of cell growth and mitosis. In dichophase a cell may be diverted to differentiate

period of preparation for mitosis. It extends from the end of the dichophase to the beginning of mitosis. All that is at present known is that during the prosphase the DNA content of the chromosomes is doubled, the spindle protein is at least partially synthesized, and an energy store is established for the use of the cell during mitosis[5]. The prosphase can be subdivided, but so far only two subdivisions have been clearly defined:

(*a*) *Phase of DNA synthesis:* this may begin at the same time as, or later than, the beginning of the prosphase, but no information is available on the point (in the literature it is called either "S" or the "synthesis stage", which are, in fact, general terms which should strictly be related to the whole of the prosphase). The duration of this phase has been accurately determined in several tissues by the use of tritiated thymidine.

(*b*) *Antephase:* this extends from the end of the phase of DNA synthesis to the onset of mitosis[5] (it is also called "G 2", the "second gap", or the "second growth phase", though no growth is evident, or alternatively the "predivision resting phase", though the cell is probably not resting). The antephase was originally defined as that stage which immediately precedes the visible prophase and in which an energy store is established[6]. There has since been some argument as to the time of establishment of this energy store, but in the case of mouse epidermis the evidence still favours the antephase as being most important in this connexion[7].

(4) *Mitosis*: the visible stages of cell division from prophase to telophase (in the literature it is often simply called "M"). The subdivisions of mitosis are too well known to need repetition.

In considering the time relations of these various phases within the whole cycle of cell growth and mitosis, it is immediately obvious that they must vary widely between the various cell types found in an adult mammal. Broadly and artificially speaking, three categories can be recognized: those with a short generation time of hours, those with a moderate generation time of days, and those with a long generation time of weeks, months or even years. A fourth category can also be defined to include cells that have suffered some pathological change.

Cells with a short generation time. Most of the attempts which have been made to time the durations of the various phases of cell growth and mitosis in the adult mammal have concerned tissues with a high mitotic rate, and this is understandable since with such tissues answers are more easily and rapidly obtained. However, it must be remembered that, in the adult mammal, tissues with a naturally high mitotic rate are the exception rather than the rule.

Examples of the results obtained with such tissues (Table 1) are taken from the precise analyses of the mitotic cycle in the duodenal crypts of Lieberkühn by Lesher *et al.*[8], and from the study of hair roots by Cattaneo *et al.*[9]. Descriptions of the techniques used have been given, for example, by Fry, Lesher and Kohn[10,11].

Table 1. Approximate Durations of the Phases of Cell Growth and Mitosis in Two Tissues with a High Mitotic Rate

Type of tissue	Early interphase	DNA synthesis	Antephase	Mitosis	Authors
Crypt of Lieberkühn	5 h	5 h	less than 2 h		8
Active hair bulb	3 h	7 h	1 h	1 h	9

It will be noted that the cells of both these tissues have a generation time of roughly 11–12 h. Since no shorter generation times are known, the cells of these tissues may be multiplying at the maximum speed that is possible in a normal adult mammal. If this were so, then it should follow that each of the separate phases of cell growth and mitosis is also being completed at the maximum possible speed.

Cells with a moderate generation time. Only one complete analysis of the phase times of normal cells with a generation time of days rather than hours appears to have been published to date, and this relates to mouse ear epidermis[12] (Table 2). However, since this was published, new information has been obtained which indicates that a full analysis of the behaviour of epidermal cells is likely to reveal a complex situation[7]. Setting aside the fact that the generation time of the basal epidermal cells may vary in different body regions, and even between different strains of mice[7], it is important to note that epidermis shows at least two remarkable differences from the pattern seen in Table 1. In the first place, it has been found that the duration of epidermal mitosis is very variable[7]. This has been determined using mouse ear epidermis *in vivo* and *in vitro*, and a brief selection of the kind of results obtained are shown in Table 3. Full accounts of this work will be published elsewhere, but the following points may be briefly noted: that the range of variation in the duration of mitosis in mouse ear epidermis *in vivo* and *in vitro* so far discovered is 1·5–5·3 h; and that in normal epidermis the duration of mitosis is proportional to the adrenaline concentration. In Table 3 the mice which were asleep or adrenalectomized had epidermis with a low adrenaline content, and so, too, did the control epidermis *in vitro*; the epidermis of the mice which were awake, like the epidermis *in vitro* with added adrenaline, had a higher content of this hormone.

Table 2. Examples of the Varying Duration of Mitosis in the Ear Epidermis of Adult Male Mice (ref. 7)

In vivo condition of mice			*In vitro* with 0·02 M glucose and oxygen	
Awake	Asleep	Adrenal-ectomized	Control	With 0·025 μg adrenaline/ml.
3·8 h	2·6 h	2·7 h	2·8 h	4·6 h

Table 3. Approximate Durations of the Phases of Cell Growth and Mitosis in the Ear Epidermis of Adult Male Mice

Early interphase	DNA synthesis	Antephase	Mitosis	Authors
483 h	30 h	4·6 h	3·8 h	12
—	—	as long as mouse remains awake or stressed	2·6–3·8 h	7, 13

The other important difference shown by epidermal cells is their behaviour in antephase[13]. While adrenaline can only slow down the process of mitosis, it can completely block the passage of a cell in antephase. It is the imposi-

tion of this blockage in a mouse when awake and its release in a mouse when asleep that gives rise to the well-known diurnal mitotic rhythm. The mitoses that develop during sleep are partly those of cells which at that time complete their preparations and partly those of cells which had completed their preparations previously while the animal was awake. It has been shown that the longer a mouse is forced to remain awake the higher is the number of mitoses developing during subsequent sleep[13]. It can now also be added that the higher the number of mitoses obtained in this way the faster each one passes to completion[7], but this is a phenomenon that will be analysed elsewhere.

It is important to notice that although a higher adrenaline concentration, found in animals when awake, causes an antephase inhibition and slows the passage of mitosis in the epidermal cells, these effects are not seen in the cells of active hair roots, which are epidermal derivatives[14]. Indeed, it appears probable that whereas tissues with a moderate mitotic rate may all be adrenaline sensitive, since so many of them are known to show a diurnal mitotic rhythm, tissues with a high mitotic rate may all be insensitive to adrenaline, since they evidently show no diurnal rhythm. It is significant that, after wounding, those epidermal cells that develop a high mitotic rate also lose their sensitivity to adrenaline[13].

In generalizing in this way it may, of course, be necessary to make an exception of such tissues as the vaginal and uterine lining epithelia, in which mitotic activity is hormone dependent.

In mouse epidermis the situation has been further complicated by the discovery that inhibition in antephase and during mitosis is due not to adrenaline itself but to an unstable complex formed between the adrenaline and some substance secreted within the epidermal cells. This substance is probably epidermis-specific, and it may be a protein. It has been proposed that tissue-specific substances of this kind, which may be common in the body, should be called chalones[15]. The existence of at least three separate chalones in the skin has so far been inferred[16].

This new information provides some explanation for what evidently happens when the epidermal mitotic rate rises: in sleep, as the rate of adrenaline secretion falls, the chalone–adrenaline complex breaks down and the mitotic inhibition is therefore removed; *in vitro*, as the adrenaline washes out from the epidermis (which it may do in a matter of minutes[17]) the same situation arises (this, incidentally, may explain the curious results reported to be obtained after cutting the skin and culturing *in vitro*[18]); while after wounding, it appears that it is the chalone itself that is lost from the adjacent cells so that adrenaline can no longer act as a co-factor to interfere with mitosis, and there is therefore no diurnal rhythm[19]. This last observation could lead to a suggestion that in tissues with a naturally high mitotic rate there may either be a lack of chalone with which adrenaline could combine to form a mitotic inhibitor, or alternatively that there may be a lack of the enzyme necessary to catalyse their combination.

To return to the main argument, it is now evident that in epidermis the higher the concentration of the unstable chalone–adrenaline complex the longer the antephase will last and the slower each mitosis will proceed. From results with mice stressed continuously it seems that the antephase may be prolonged for days[13], but in a normal mouse in a well-kept animal house it may not usually exceed the few hours of the normal waking period.

It is evidently only in this limited sense that in the words of Defendi and Manson[1] there exists "a special class of cells of the mouse epidermis . . . acting as a reservoir of cells immediately ready to undergo mitosis". However, it is possible that in a poorly organized animal house, where the mice are unusually stressed, there might sometimes be an unusually large number of cells arrested in antephase.

The release of cells from antephase blockage, whether *in vivo* or *in vitro*, must not be confused with the normal wounding reaction of damaged epidermis. This is a reaction which begins not in antephase but usually in already differentiated cells. The mitotic activity of wounded ear epidermis *in vivo* does not begin to rise until after some 36–48 h (ref. 19), which might be regarded as the time taken by the adjacent cells to revert to the undifferentiated condition and to pass through the prosphase to mitosis.

It is also possible that this reversion may itself be the result of the loss of chalone, although at the moment the evidence for this is only indirect.

Cells with a long generation time. In an adult mammal the various cell types can be arranged in a graded series from those with a short generation time to those with a generation time so long that most cells may never be replaced during the whole of life. Most connective tissues and most well-protected internal organs such as liver and kidney belong to this last group, and indeed, they give the impression that being fully differentiated they are all mitotically inert.

However, it is interesting to note that most of these tissues resemble epidermis in their ability to develop high mitotic activity after wounding, and in such a situation the cells dedifferentiate. There seem to be good grounds for suggesting that these tissues do not differ fundamentally from epidermis[15]; they may merely be under some tighter control mechanism which, if it is like that of the epidermis, may be based on a stable rather than an unstable chalone–adrenaline complex. However, at the moment this is mere hypothesis.

Abnormal cells. It is only in the light of the normal cycle of cell growth and mitosis, and of the mechanisms by which it is controlled, that any real understanding can be obtained of conditions in pathological growth. From time to time, and for one reason or another, normal tissues may produce within themselves abnormal cells which develop such an unusually high mitotic rate that the usual precise balance between cell wastage and cell gain is disturbed. However, such abnormal cells evidently show wide variations in their degree of independence from the normal tissue control mechanisms, and also possibly in the actual manner in which this independence has been achieved.

They are also evidently very varied in their generation times, and Bertalanffy[20] gives figures ranging from about 12 h to several days. It is tempting to speculate whether the generation time may be a function of the degree of independence achieved.

From an inadequate literature it would also seem that tumour cells may vary in their reactions to adrenaline since some have been reported to show a diurnal mitotic rhythm[21] while others have not[20]. Again, it seems possible that the presence or absence of the rhythm may depend on the degree of independence achieved by the cells. Thus the existence of a diurnal rhythm in a tumour could be taken to suggest that the cells may still retain some significant part of their normal chalone–adrenaline control mechanism, and such cells, like normal epidermis, might show an antephase and a mitotic phase of variable length.

Conversely, the absence of a diurnal rhythm in a tumour might mean that the cells no longer retain any significant part of their normal control mechanism. Such cells, like normal hair roots, might show phases of cell growth and mitosis which are both short and of relatively constant duration.

Conclusions. Perhaps the only conclusion to which no exception can be taken is that it is still too early to discern more than a vague outline of the type of mitotic control mechanism which must exist in the tissues of an adult mammal and which must determine both the onset and the duration of the various phases within the cycle of cell growth and mitosis. However, although what little is known relates to only one or two tissues, it would cer-

tainly be surprising if it were discovered that there is no common basis to the various control mechanisms of the various tissues. Mitosis itself is such a uniform process wherever it is found that the pattern of its control may be equally uniform, although, of course, it may be expected that each tissue will show its own specific variation within that pattern.

In the cycle of cell-growth and mitosis there is one period of outstanding interest and importance, and it is unfortunately the one about which least is known. This is the period here defined as the dichophase, the phase of unknown duration within which the decision is reached whether the cell's synthetic machinery shall be directed towards differentiation or towards preparations for yet another division.

The only slight information that can be deduced about the dichophase and about differentiation itself comes from the results of experiments on wounding and on 'compensatory hypertrophy'. In such experiments cells which have previously committed themselves to differentiate are seen to reverse this decision and to prepare once more for mitosis. The impression is gained that most cells that are not so highly differentiated that they can never again divide are in fact normally held in the differentiated state by some factor which, when it is withdrawn, releases the cells to revert back through the dichophase to their basic activities of growth and mitosis. After wounding and in 'compensatory hypertrophy' such a withdrawal is only temporary, and there is some evidence to support a hypothesis that the factor withdrawn may in fact be the chalone which is specific to the tissue in question[5,15,19]. On this argument the chalone may prove to be a basic part of the mechanisms both of differentiation and of mitosis inhibition, if indeed these mechanisms can be separated. Also on this argument differentiation may be regarded as an unstable state.

It may be noted incidentally that some indication of the speed with which a differentiated cell can return to the dichophase has been recorded for rabbit lens epithelium by Harding and Srinivasan[22], who have found that thymidine-incorporating cells first appear in the vicinity of a wound about 14 h after injury. This period may be roughly similar to that in epidermis, but other tissues, such as mouse hypodermis and mouse ear cartilage[7], probably take much longer. Whether such information gives any indication of the time normally taken to pass in the reverse direction from dichophase to the fully differentiated condition is, of course, debatable.

In a tumour, the undifferentiated condition shown by the cells is permanent. Theoretically this sort of change might occur in several ways: on the chalone hypothesis it could be due either to a failure to produce sufficient chalone or to a failure to react to what chalone is present. In either case, the failure might be one of degree only: the more complete it is, the more cells might be expected to dedifferentiate, the higher might be the mitotic rate, and the shorter might be the various phases of the cycle of cell growth and mitosis.

However, until a much clearer understanding has been obtained in normal mammalian tissues of the manner of control of the various cycle phases, of the factors that determine the choice made in the dichophase, and of the manner in which a differentiated cell is maintained in that condition, it is of little value to speculate on the conditions that may exist within cells that have suffered a pathological change.

Note added in proof. Since this article was written an important discovery has been reported by Pilgrim, Erb and Maurer (following article, p. 863). They have provided the first indication that in certain tissues not only may there be a diurnal rhythm in the number of cells in mitosis but also a similar rhythm in the number of cells in the phase of DNA synthesis. However, the available evidence is against their conclusion that the diurnal mitotic rhythm may be the delayed consequence of the diurnal rhythm in DNA synthesis. It appears more probable that there may exist two highly sensitive points in the mitotic cycle, the first during the transition from dichophase to prosphase (or to DNA synthesis) and the second during the transition from antephase to mitosis. It is now extremely important to discover whether both these points are sensitive to the inhibitory action of the same mitotic control mechanism.

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Without making detailed comments on Prof. Bullough's communication, or on the very general scheme he proposes, we would like to rectify some of his statements presented in reference to our own communications.

(1) Prof. Bullough's statement in the initial paragraph is incorrect in minimizing the basis of our conclusions since, in addition to four tumour cell lines, we have tested three strains of human diploid cells which, by any criteria we can consider, are neither "aberrant" nor "pathological".

(2) While, of course, little is known about all the various factors involved in the preparation for mitosis of tumour and normal cells, it is still true that the wealth of information which has been collected during the past few years concerning the periodicity and length of DNA synthesis certainly permits some general statement.

(3) It is quite true that the *primum movens* of cell division must occur before, and not at the time of, initiation of DNA synthesis. The time of action and the nature of this *movens*, notwithstanding the elegant names and speculations of Prof. Bullough, are still completely unknown. Since we were concerned with what we were able to determine and measure, we chose the occurrence of certain biological events leading to the beginning of DNA synthesis as the time at which the decision to enter replication is irrevocably taken by a cell under normal conditions.

(4) Our communication was not intended to be a review, and the results in the table were selected with that in mind. Since results in numerous other publications dealing also with 'long generation time' cells have not contradicted our hypothesis, it seemed useless to list a long series of references. The case of the mouse skin was purposely omitted since there are conflicting reports on this 'complex situation' which cannot be resolved at the present time without the further work of interested workers.

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DIURNAL FLUCTUATIONS IN THE NUMBERS OF DNA SYNTHESIZING NUCLEI IN VARIOUS MOUSE TISSUES

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IT has often been shown that the mitotic rates reveal marked diurnal fluctuations in various tissues of many organisms. In rats and mice the mitotic rates often seem to reach a maximum at night and a minimum during the day. Bullough has given two reviews of this subject and has discussed, in terms of waking and sleeping, the possible causes of these waves of activity[1,2].

This investigation was undertaken to discover whether the number of nuclei in DNA synthesis (S-phase) is also subject to diurnal fluctuations. Mice were injected intraperitoneally with tritiated thymidine (0·7 μc./g) at intervals throughout the day. Before and during the experiments the animals were kept under constant conditions and were fed *ad libitum*. Forty min after the injection the animals were killed by decapitation. Autoradiographs were prepared (Ilford $G5$ emulsion) from 3-μ thick paraffin sections of various tissues. Then the percentage of labelled nuclei (tritium index) and the percentage of cells in mitosis (mitotic index) were estimated. In addition, the mean grain number was counted in nuclei which had been cut approximately centrally. The results of one series of experiments are shown in Table 1. The figures are the average values from two animals, which showed little variation from the mean. In addition, the results were confirmed by a second series of experiments.

Table 1. TRITIUM INDEX, MITOTIC INDEX AND MEAN GRAIN COUNT PER NUCLEUS AT DIFFERENT TIMES OF DAY

Tissue	Hour of day	Tritium index (%)	Mitotic index (%)	Grains per nucleus
Œsophagus strat. bas.	5.30	1·5	0·83	9·2
	11.30	1·0	0·14	9·5
	17.30	6·4	0·19	9·7
	23.30	3·7	0·14	9·5
Fore-stomach strat. bas.	5.30	3·9	1·18	9·9
	11.30	3·0	0·38	9·5
	17.30	5·5	0·35	9·6
	23.30	3·8	0·42	9·6
Abdominal skin epidermis	5.30	1·7	0·47	9·1
	11.30	1·0	0·18	9·5
	17.30	4·5	0·21	8·9
	23.30	2·7	0·19	10·2
Tongue strat. bas	5.30	4·9	1·01	9·7
	11.30	4·3	0·17	9·9
	17.30	20·7	0·25	9·6
	23.30	9·9	0·59	9·6
Jejunum crypt. epith.	5.30	43	4·8	9·6
	11.30	42	4·7	9·2
	17.30	44	3·8	9·6
	23.30	41	4·7	9·8
Kidney convol. tub.	5.30	1·2	—	9·8
	11.30	1·2	—	9·9
	17.30	1·2	—	9·2
	23.30	1·1	—	9·1

It will be seen that no diurnal fluctuations in the mitotic index were present in the epithelia of the jejunal crypts. However, in the other epithelia of the digestive tract (tongue, œsophagus, forestomach) and in the abdominal epidermis diurnal fluctuations were obvious, a finding which agrees with the results of other authors[3]. It will also be seen that the tritium index shows similarly large fluctuations. Again, however, an exception is provided by the intestinal epithelium and also, in this case, by the tubuli contorti of the kidney. Thus, depending on the time of day, a partial synchronization is apparent in the S-phase nuclei precisely as can be seen in the mitoses.

The observed fluctuations in the tritium-index could be caused by diurnal fluctuations of the duration of DNA synthesis since an increase in the duration of DNA synthesis would result in an increase of the tritium index. However, according to Table 1, the mean grain number per nucleus does not show any diurnal fluctuation suggesting that the rate of synthesis, and therefore also the duration of synthesis, is constant. Thus the most probable explanation for the variations in the tritium index is that the number of nuclei entering S-phase from G_1-phase per unit time depends on the time of day.

From many of the papers previously published[2] the impression may be gained that the diurnal fluctuations in the mitotic index are directly due to parallel fluctuations in the triggering process of mitosis. In this case one would expect later on, during the following generation-cycle (that is, after the end of G_1-phase), to find a certain accumulation of S-phase nuclei. But it is very improbable that the observed fluctuations of the tritium index can be explained this way. For the rather sharp maximum of the tritium index would be difficult to understand since the G_1-phase requires several days in the cell types studied here and is certainly subject to additional individual variations. Furthermore, the fact that the time-interval between the tritium and the mitotic index is of similar size in all examined cell types would not fit into this theory.

It is much more likely that the primary process consists of a synchronization of S-phases. Consequently, one would expect an increase in the mitotic figures some hours later. The time-interval between the two maxima should be equal to = 1/2 S-phase + G_2 + 1/2 duration of mitosis. Unfortunately only incomplete data concerning the G_2-phase of the examined cell types are available. In the epithelia of the œsophagus and the forestomach (S-phase = about 8 h (ref. 4); G_2-phase = several h (ref. 5) the actually observed intervals of 12 h would be understandable in this way.

Furthermore, the experiments show (Table 1) that the duration of the period during which the mitotic index is at a maximum is approximately equal to the period during which the tritium index is at a maximum. (In some cell types the length of the mitotic index maximum might be somewhat shorter than that of the tritium index). In addition, the degree of fluctuation is about the same in both cases. This, too, is understandable if it is assumed that the length of the synchronization wave is approximately equal to the S-phase duration.

Equally great fluctuations of the tritium index were found in the ear epidermis. The interval between the maxima of the indices, however, was only a few hours (possibly plus any multiple of 24 h). These cells are also exceptional in that the duration of their S-phase is clearly longer than that in other tissues[6]. Further work on these cells might prove to be of special significance for the explanation of the phenomena of synchronization.

The experiments indicate that the causes of the fluctuation in the mitotic rate are obviously more complex than has been hitherto assumed. They can probably be interpreted, at least partially, as the result of a partial synchronization of S-phases.

In several publications the duration of the generation cycle has been deduced from the tritium index or from the mitotic index[7]. However, this is only possible in the case of a completely asynchronous cell population, that is when the cells are distributed uniformly throughout the different phases of the generation-cycle. The diurnal fluctuations in the number of cells in the S-phase described here show that this assumption may be unwarranted.

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MOLECULAR GEOMETRY OF LANOSTENYL IODOACETATE

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THE crystal structure of lanostenyl iodoacetate, $C_{32}H_{53}IO_2$ (Fig. 1), was determined by Fridrichsons and Mathieson in 1953[1] by use of 'heavy atom' single crystal X-ray diffraction analysis. The unit cell is orthorhombic (space group $P2_12_12_1$) with cell constants of $a_0 = 7{\cdot}6$ Å, $b_0 = 10{\cdot}9$ Å, and $c_0 = 38{\cdot}6$ Å, and contains four molecules. The density is 1·24–1·25 g/cm³. The atomic parameters obtained by Fridrichsons and Mathieson[1] have been used to calculate: (*a*) a series of 'best' plane equations for the entire molecule and various sub-portions thereof (Table 1) using the method of Schomaker *et al.*[2]; (*b*) the root mean square distances of atoms from the various 'best' planes (Table 2); (*c*) intra- and inter-ring dihedral angles (Table 3); (*d*) the perpendicular distances of individual atoms from one or more of the 'best' planes (Table 4). After consideration of these various calculations, the following details of the molecular geometry of lanostenyl iodoacetate are apparent:

(1) The 32 carbon atoms of the molecule have a non-planar spatial distribution. The 'best' plane passing through these atoms has the equation $0{\cdot}00x + 0{\cdot}85y + 0{\cdot}52z = 4{\cdot}68$. The root mean square distance of atoms from this plane is 0·75 Å.

(2) The steroid nucleus is also non-planar. The 'best' plane passing through the atoms of the steroid nucleus (C_1–C_{17}) has the equation $0{\cdot}04x + 0{\cdot}80y + 0{\cdot}60z = 5{\cdot}07$. (The coefficients of x, y and z are based on a Cartesian co-ordinate system in Å.) The root mean square distance in this case is 0·29 Å.

(3) Rings *A*, *B*, *C* and *D* have root mean square distances of 0·23, 0·24, 0·24 and 0·16 Å respectively and are non-planar, although ring *D* has a higher degree of planarity than rings *A*, *B* and *C*. The dihedral angles between the 'best' planes of these rings were calculated in order to visualize the overall trend of the lanostenyl iodoacetate molecule. It is apparent that *B* and *D* are nearly parallel, since $A \wedge B$ equals 162°, $B \wedge C$ equals 175°, and $C \wedge D$ equals 165°.

(4) The smaller sub-portions of the ring system are all planar, with the exception of *B*2 the atoms of which have

Table 1. Values of α, β, γ, d in the Equations $\alpha x + \beta y + \gamma z = d$ of the 'Best' Planes of Lanostenyl Iodoacetate

	α	β	γ	d
Entire molecule (32 carbons)	0·00	0·85	0·52	4·68
Steroid nucleus (C_1–C_{17})	0·04	0·80	0·60	5·07
A	0·13	0·89	0·44	4·52
B	0·00	0·73	0·68	4·81
C	−0·10	0·74	0·66	4·42
D	−0·14	0·89	0·44	4·58
*A*1	0·12	0·98	−0·17	0·52
*A*2	0·11	0·73	0·67	5·60
*A*3	0·14	0·98	0·14	3·40
*B*1	0·14	0·98	0·14	3·42
*B*2	−0·02	0·67	0·74	4·69
*B*3	−0·17	0·45	0·88	3·70
*C*1	−0·07	0·36	0·93	3·95
*C*2	−0·11	0·71	0·69	4·50
*C*3	−0·09	0·99	−0·08	4·64
*D*1	−0·08	0·99	−0·09	4·64
*D*2	−0·14	0·81	0·56	4·15
*A*3*B*1	0·14	0·98	0·14	3·42
*B*3*C*1	−0·12	0·41	0·90	3·91
*C*3*D*1	−0·09	0·99	−0·08	4·59

α, β, γ = direction cosines of plane; d = perpendicular distance from origin.

Table 2. Root Mean Square Distances (R.M.S.) of the Atoms of Lanostenyl Iodoacetate from the 'Best' Planes

'Best' planes	R.M.S. distances (Å)
Entire molecule (32 carbons)	0·75
Steroid nucleus (C_1–C_{17})	0·29
A	0·23
*A*1	0·00
*A*2	0·01
*A*3	0·00
B	0·24
*B*1	0·00
*B*2	0·17
*B*3	0·00
C	0·24
*C*1	0·00
*C*2	0·03
*C*3	0·00
D	0·16
*D*1	0·00
*D*2	0·03
*A*3*B*1	0·01
*B*3*C*1	0·05
*C*3*D*1	0·02

Table 3. Dihedral Angles of the 'Best' Planes of Lanostenyl Iodoacetate

Planes	Angle (deg.)
$A \wedge B$	162
$B \wedge C$	175
$C \wedge D$	165
*A*1 ∧ A2	128
*A*2 ∧ *A*3	145
*A*3 ∧ *B*1	177
*B*1 ∧ *B*2	140
*B*2 ∧ *B*3	163
*B*3 ∧ *C*1	172
*C*1 ∧ *C*2	155
*C*2 ∧ *C*3	131
*C*3 ∧ *D*1	178
*D*1 ∧ *D*2	145

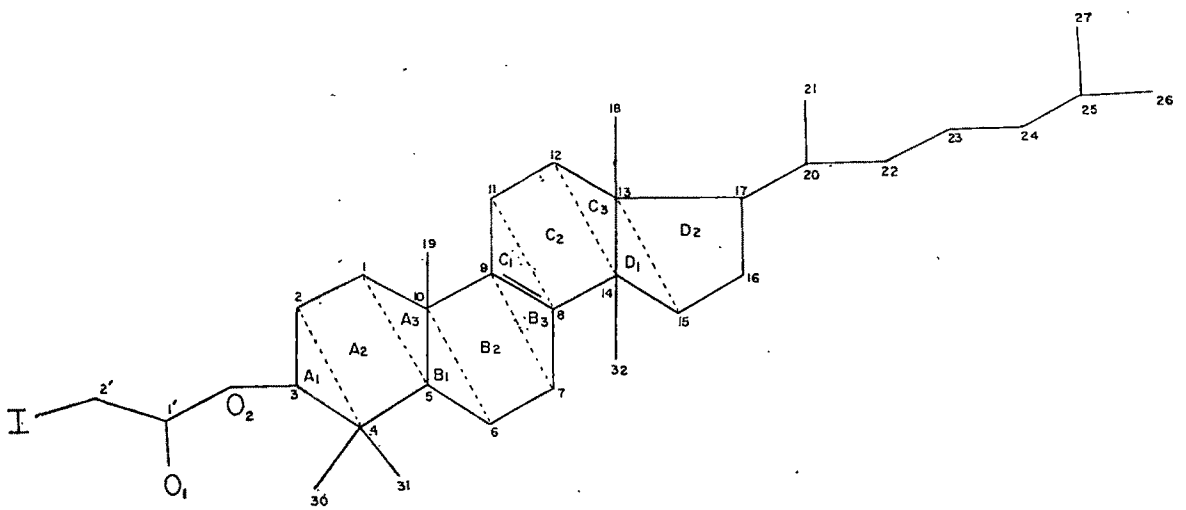


Fig. 1. Lanostenyl iodoacetate

Table 4. PERPENDICULAR DISTANCE (Å) OF INDIVIDUAL ATOMS FROM ONE OR MORE OF THE 'BEST' PLANES OF LANOSTENYL IODOACETATE

	Entire molecule (32 carbons)	Steroid nucleus (C_1–C_{17})	*A*	*A*1	*A*2	*A*3	*B*	*B*1	*B*2	*B*3	*C*	*C*1	*C*2	*C*3	*D*	*D*1	*D*2
C_1	+0·15	+0·16	+0·25		−0·01	0·00											
C_2	−0·12	+0·00	−0·15	0·00	+0·01												
C_3	+0·32	+0·61	+0·38	0·00													
C_4	−0·43	−0·02	−0·14	0·00	−0·01												
C_5	−0·15	−0·03	+0·26		+0·01	0·00	+0·34	0·00									
C_6	−0·68	−0·56					−0·35	0·00	−0·16								
C_7	+0·06	+0·05					+0·10		+0·16	0·00							
C_8	+0·35	+0·20					+0·21			0·00	+0·11	0·00	−0·03				
C_9	−0·27	−0·40					−0·26		−0·17	0·00	−0·28	0·00					
C_{10}	−0·35	−0·34	0·00			0·00	−0·04	0·00	+0·17								
C_{11}	+0·15	−0·11									+0·15	0·00	+0·03				
C_{12}	+0·49	+0·06									+0·17		−0·03	0·00			
C_{13}	+0·16	−0·27									−0·40			0·00	−0·18	0·00	−0·02
C_{14}	+0·81	+0·50									+0·24		+0·03	0·00	+0·23	0·00	
C_{15}	+0·56	+0·27													−0·16	0·00	+0·02
C_{16}	+0·57	+0·09													+0·02		−0·03
C_{17}	+0·40	−0·15													+0·08		+0·03
C_{18}	−1·36	−1·78									−1·93			−1·21	−1·71	−1·20	−1·56
C_{19}	−1·75	−1·77	−1·39			−1·19	−1·47	−1·19	−1·26								
C_{20}	−0·27	−0·98													−0·39		−0·62
C_{21}	+0·19	−0·59													+0·26		+0·04
C_{22}	+0·32	−0·47													+0·16		−0·24
C_{23}	−0·31	−1·26													−0·28		−0·86
C_{24}	+0·03	−1·00													+0·04		−0·70
C_{25}	−0·61	−1·76													−0·47		−1·39
C_{26}	−0·14	−1·38													−0·03		−1·31
C_{27}	−0·12	−1·42													+0·25		−0·77
C_{30}	+0·06	+0·54	+0·50	+0·07	+0·82												
C_{31}	−1·84	−1·49	−1·57	−1·39	−1·26												
C_{32}	+2·13	+1·86									+1·62	−0·57	+1·42	+0·80	+1·51	+0·79	
I	−0·40	+0·12	−1·01	−3·20	+0·40												
O_1	+2·26	+2·72	+2·06	+0·04	+2·96												
O_2	+0·22	+0·62	+0·13	−0·98	+0·85												
*C_1'	+1·09	+1·54	+0·88	−0·83	+1·78												
*C_2'	+0·50	+1·09	+0·14	−2·22	+1·45												
*O_1'	Acetate carbons																
*O_2'																	

a root mean square distance of 0·17 Å. The atoms of the planar portions *A*2, *C*2 and *D*2 have root mean square distances of 0·01, 0·03 and 0·03 Å respectively.

(5) Combinations of the four-carbon-atom sub-divisions which have two common carbons are planar, namely, *A*3*B*1, *B*3*C*1 and *C*3*D*1, as can be seen from the root mean square distances of the relevant carbon atoms (0·01, 0·05 and 0·02 Å respectively). This planarity is also verified by the dihedral angles $A3 \wedge B1$ (177°), $B3 \wedge C1$ (172°), and $C3 \wedge D1$ (178°).

(6) The angular methyl groups C_{18} and C_{19} are at the same distance from the plane of the steroid nucleus (1·77 Å and 1·78 Å respectively).

(7) One of the two methyl groups attached to C_4, the C_{31} methyl, is bonded almost perpendicularly to the plane of the steroid nucleus as indicated by the near correspondence of the perpendicular distance of C_{31} from the plane of the steroid nucleus (1·49 Å) and the C_4–C_{31} bond-length (1·51 Å) (ref. 1). The other C_4 methyl group (C_{30}) is equatorial. Its perpendicular distance from the 'best' plane of the steroid nucleus is 0·54 Å, and its angular separation ($C_{30}C_4C_{31}$) from the C_{31} methyl group is 97°.

(8) The perpendicular distance of the methyl group (C_{32}) attached to C_{14} is 1·86 Å from the plane of the steroid nucleus. This approximately corresponds to the sum of the perpendicular distance of C_{14} from the 'best' plane of the steroid nucleus (0·27) plus the C_{14}–C_{32} bond length (1·54) (ref. 1), thereby indicating that the C_{32} angular methyl group is axial.

In conclusion, it may be said that the lanostenyl iodoacetate molecule is similar to the molecules of other steroids the molecular details of which have been worked out (namely, cholestery liodide[3,4], 4-bromoœstrone[5], and 4-bromoœstradiol[6]) in that neither the entire molecule, the steroid nucleus, nor the rings *A*, *B*, *C*, *D* are planar. The only exception to this is the instance wherein planarity of rings *A*, *B*, *C* and *D* is forced by the presence of three double bonds such as in the œstrogens. In all steroids thus far studied, true planarity is encountered only in sub-divisions of the molecule which are representative of the transjoined chair arrangement, that is, *A*1, *A*2, *A*3, etc.

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STRUCTURE AND FUNCTION OF *E. COLI* ERGOSOMES

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FROM our previous investigations on the structure and function of rat liver ergosomes[1–3] we concluded that protein synthesis requires the movement of messenger RNA (*m*RNA) through a series of ribosomal condensing sites at each of which the genetic message is transcribed nto the co-linear structure of a growing peptide chain. Thus, a cycle of protein synthesis is thought to begin with the successive attachment of 73 *S* ribosomes to one end of a messenger strand and to be terminated when these ribosomes have all been successively released at the other end, each of them with its finished polypeptide chain.

This 'tape-mechanism', that is the movement of *m*RNA through a ribosomal site in the synthesis of protein, appears to have been first advanced by Gilbert[4] and by Gierer[5]. Evidence for the idea that aggregates of several ribosomes, linked by one *m*RNA strand, are the functional units ('polysomes', 'ergosomes') was presented independently and almost simultaneously by Gierer[5], Warner, *et al.*[6,7] and Wettstein *et al*[1,2]. This interpretation

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implies that during the lifetime of a messenger RNA molecule *in vivo*, ribosomes are continuously attached and released so that the entire *m*RNA length is occupied by closely spaced ribosomes engaged in protein synthesis. Since *m*RNA is turning over *in vivo*, not all these ergosomes would be expected to be in a steady state in which an intact *m*RNA strand would be kept occupied by attachment and release of monomers, but some aggregates might be at any moment in a state of breakdown and others in process of assembly. Thus, the fraction of ergosomes present in the state of assembly and breakdown, and hence the size distribution of the ergosome population would depend on the average time required for assembly and breakdown relative to the average lifetime of *m*RNA. Since in this concept assembly and breakdown are coupled to protein synthesis by the movement of *m*RNA through the ribosomal condensing sites, we may estimate the proportion of ergosomes engaged in assembly and breakdown from the average number of protein molecules synthesized during the average lifetime of the messenger. In exponentially growing *E. coli*, a mean decay time of about 2 min has been estimated for *m*RNA (ref. 8) and a value of about 5 sec for the average time required to synthesize a polypeptide chain of 20,000 molecular weight[9]. Assuming an average ergosome size of $(70\ S)_7$, we calculate from these values that each *m*RNA molecule makes on the average about 100 polypeptide chains of mol. wt. 20,000. Hence the fraction of ergosomes engaged in assembly and breakdown should be small.

Contrary to this expectation, investigations with *E. coli* reported here indicate that bacterial extracts, even when prepared by particularly gentle disruption of the cells, contained predominantly 70 *S* monomers* and small oligomers. This might indicate that our isolation procedures resulted in the loss or breakdown of a substantial portion of the ergosomes. Nevertheless, by concentration methods which eliminate the bulk of the large population of small particles, it was possible to isolate ergosomes consisting of more than ten 70 *S* ribosomes. Examination of the structural features of bacterial ergosomes by sedimentation analysis and electronmicroscopy as well as studies on the kinetics of amino-acid incorporation *in vitro* gave results that are analogous to our previous findings with the rat liver system.

Preparation of ribosomes and soluble enzyme fraction. An unpiliated mutant of *E. coli* B_{am} (ref. 27) was grown with aeration in nutrient broth at 37° C to a density of approximately 5×10^8 bacteria per ml. and gathered by centrifugation after chilling with crushed ice. The cells were washed once with buffered salt solution (0·05 M hydrochloric acid–*tris* pH 7·6, 0·025 M potassium chloride, 0·01 M magnesium chloride and 0·005 M 2-mercaptoethanol) and disrupted by grinding for 10–15 min in a porcelain mortar with a mixture of powdered dry ice and glass beads. After the dry ice had evaporated, buffered salt solution was added to the paste (3 ml./g wet cells) and the suspension centrifuged at 18,000*g* for 10 min to remove unbroken cells and debris. The supernatant fluid (crude extract) was added in 5·5-ml. portions to 13-ml. centrifuge tubes that had been previously filled with 3·5 ml. of 0·5 M sucrose layered over 3 ml. of 2 M sucrose solution. The sucrose solutions were buffered by dissolving the required amount of sucrose in buffered salt solution. After centrifugation for 4 h at 40,000 r.p.m. and 0°–2° C in a Spinco model *L* ultracentrifuge, the upper 3·5–4 ml. of the supernatant fluid, which was free of ribosomal particles and DNA, was removed and kept frozen at −60° C. This fraction, designated 'post-ribosomal supernatant', contained the soluble enzymes and transfer RNA used for amino-acid incorporation *in vitro*. The interface between the 2 M and 0·5 M sucrose solutions which contained a gelatinous layer of DNA was discarded. The colourless translucent ribosomal pellet ('*C* ribosomes') was rapidly rinsed with 2 ml. buffered salt solution, resuspended to a concentration of about 3 mg RNA per ml. and stored at −60° C.

Zone centrifugation analysis. Centrifugation was carried out in a Spinco *SW* 25 rotor at temperatures close to 0° C as previously described[1,2,10]. The fluid was collected into an automatic fraction collector after passing through a continuous-flow ultra-violet absorption cell attached to a strip chart recorder. The fraction collector and strip chart recorder were synchronized with a precision pump which delivered the gradient fluid at a constant flow rate of 0·5 ml./min. Since the cylindrical absorption cell of 3-mm diameter gives a non-linear absorbance response, the recordings were corrected with the aid of a calibration curve and the scale was converted to read absorbancy units based on a 10 mm light path. The Mg^{++} concentration of the buffered salt solution used in the sucrose gradients was reduced to 0·005 M, in order to prevent the dimerization of free 70 *S* ribosomes observed at higher ratios of divalent to monovalent cations.

Amino-acid incorporation in vitro. The standard incorporation mixture contained the following reagents in μmoles per millilitre: hydrochoric acid–*tris* buffer *p*H 7·6 (50·0), magnesium chloride (8·6–9·0), ATP (1·0), GTP (0·4), potassium phosphoenolpyruvate (10·0), crystalline pyruvate kinase (10 μg), 2-mercaptoethanol (3·0). Ribosomes, post-ribosomal supernatant and ^{14}C-leucine were added as specified for each experiment.

For measurement of ^{14}C-leucine incorporated into protein, the samples were treated with perchloric acid, the precipitates collected on filter pads, washed and prepared for radioassay in a Packard liquid scintillation counter as described previously[1]. Counting efficiency was about 50 per cent.

Preparation of samples for electronmicroscopy. Purified ribosomes were prepared for electronmicroscopy at 0°–4° C by the collodion agar filtration method of Kellenberger[11], using agar which contained the same buffered salt solution concentration as the ribosomal solution. The filtered specimens were fixed with formaldehyde vapour for 7 min and the grids shadowed with 3/4-in. of 0·008-in. thick platinum wire at 41 amp for 40 sec at an angle of 4 : 1. The shadowed grids were examined with an R.C.A. electronmicroscope model *EMU–3F*.

Experimental Results. Representative sedimentation patterns of *E. coli* ribosomes, obtained after zone centrifugation of two different batches of crude extracts from exponentially growing cultures are reproduced in Fig. 1. In addition to the major proportion of 30 *S*, 50 *S* and 70 *S* particles, the presence of ergosomes is clearly revealed by a sequence of 4 distinct peaks corresponding to aggregates of the composition $(70\ S)_2$–$(70\ S)_5$, and by an unresolved band of even larger aggregates. Disregarding the 30 *S* and 50 *S* particles which might be nascent ribosomes, the aggregates accounted for about 18 per cent of the population of ribosomes and ribosomal aggregates (solid line) under the conditions used for disrupting the cells. This fraction was further increased to about 25 per cent when the cells were cooled as rapidly as possible to 0° C before gathering and extraction. Extracts from cells collected after slow cooling, on the other hand, were almost devoid of ergosomes.

For further investigations the ribosomes were purified by centrifugation through a double layer consisting of 0·5 M and 2·0 M sucrose solution. This procedure results in the selective concentration of aggregates, '*C* ribosomes'[1], as evident from the reduced proportion of 70 *S* monomers in the sedimentation patterns of the re-suspended pellets obtained from different crude extracts (Figs. 2*A*, 4*A*, 5*A*).

In subsequent experiments we obtained additional evidence that the bacterial ergosomes are closely analogous in their structural and functional properties to the mam-

* Following convention, we assign a 70 *S* value to the *E. coli* ribosome particle unit. To rat liver ribosomes we have assigned a value of 73 *S*. These values are not meant to reflect the true relative sedimentation rates of the particles from the two different sources.

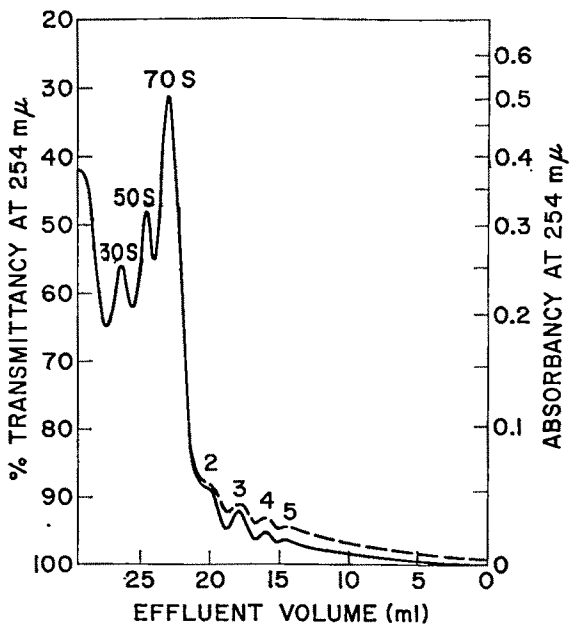


Fig. 1. Zone centrifugation of crude extract. Input corresponding to 0·5–0·6 mg RNA. Centrifugation for 3 h at 25,000 r.p.m. The solid and dashed lines represent two different extracts, normalized for equal input. The extract corresponding to the dashed line was prepared after more rapid chilling of the cells

malian ergosomes studied previously[1–3]. Thus, incubation of the bacterial ergosomes with traces of crystalline pancreatic ribonuclease (10^{-6} g/ml.) at 37° C for 5 min caused complete breakdown of the aggregates into 70 *S* monomers (Fig. 2*B*). A similar sensitivity to pancreatic RNase is exhibited by rat liver ergosomes which have been shown to break down by random cleavage of exposed *m*RNA bonds within aggregates[3]. Electronmicrographs of *E. coli* ergosomes show all the characteristic features first observed with ribosomal aggregates isolated from reticulocytes[6]. The preparations shown in the electronmicrographs in Fig. 3 were isolated from different regions of the sucrose gradient indicated in Fig. 4*A*, that is, from the monomer (*C*) and pentamer (70 *S*)$_5$ (*B*) peak, and from a cut in the unresolved region corresponding in position to aggregates ranging from (70 *S*)$_8$ to (70 *S*)$_{11}$ (*A*). General agreement of the aggregate sizes determined by sedimentation analysis or electronmicroscopy is evident from these data. In a number of aggregates, a filament-like structure, 10–20 Å thick, is visible, which appears to connect the ribosomal particles of 200–250 Å diameter (insert Fig. 3*A*). It seems reasonable to identify this structure with messenger RNA in view of other evidence supporting this conclusion[1–3,5–7,12–15].

In our previous work on rat liver ergosomes, we found that amino-acid incorporation *in vitro* is coupled to the successive release of single 73 *S* particles from the ribosomal aggregates engaged in protein synthesis. The evidence derived from a kinetic analysis of these two processes is compatible only with the tape mechanism of protein synthesis[3]. Several characteristic features of this mechanism are susceptible to experimental analysis by kinetic tests: (1) Each time the messenger has traversed a distance equal to the distance between two ribosomal condensing sites, the terminal 70 *S* particle is released from the aggregate together with a finished polypeptide chain. Thus, protein synthesis is accompanied by ergosome breakdown which proceeds through the stepwise release of monomers from one end of each aggregate. This can be tested experimentally by following in a sucrose gradient the breakdown pattern of an ergosome population resulting from protein synthesis. (2) The average amount of amino-acid incorporated per 70 *S* particle is proportional to the length of *m*RNA that, in the average, has passed through the condensing site of each ribosome. (*a*) If the time of protein synthesis is short, only a few amino-acids will be added to the growing end of the polypeptide chain present on each 70 *S* particle of an aggregate. Hence, if in a sucrose gradient we analyse a ribosome population of mixed aggregate sizes that had been exposed to a short incorporation pulse, the amount of radioactive amino-acid incorporated per unit of ribosomal mass should be independent of aggregate size. (*b*) If, on the other hand, ergosomes of different aggregate sizes are incubated separately for a long period until amino-acid incorporation ceases, the smaller ergosomes will have exhausted the stretch of *m*RNA available to them before the termination of the experiment, whereas the largest ergosomes will still be potentially active. The amount of radioactive amino-acid incorporated per unit of ribosomal mass should therefore be proportional to the initial aggregate size of each class of ergosomes, as with increasing aggregate size a proportionately longer piece of *m*RNA will in the average have passed through the ribosomal condensing sites. The following experiments were designed to test these predictions in the *E. coli* ribosome system.

The progressive breakdown of ergosomes in a population of mixed aggregate sizes (*C* ribosomes) during amino-acid incorporation *in vitro* is illustrated in Fig. 4, which shows the size distribution of *C* ribosomes in a sucrose gradient after incubation for 0 (*A*), 1 (*B*) and 6 (*C*) min. As already described previously[3], the increase in the absorbancy of the monomer peak indicates that the observed aggregate breakdown occurs through the stepwise release of 70 *S* particles as predicted by the tape mechanism. We have shown this pattern to be clearly distinguishable from that produced by random cleavage with pancreatic RNase[3].

The effect of short incorporation pulses on the distribution of nascent protein with respect to aggregate size was investigated in the same experiment. The distribution

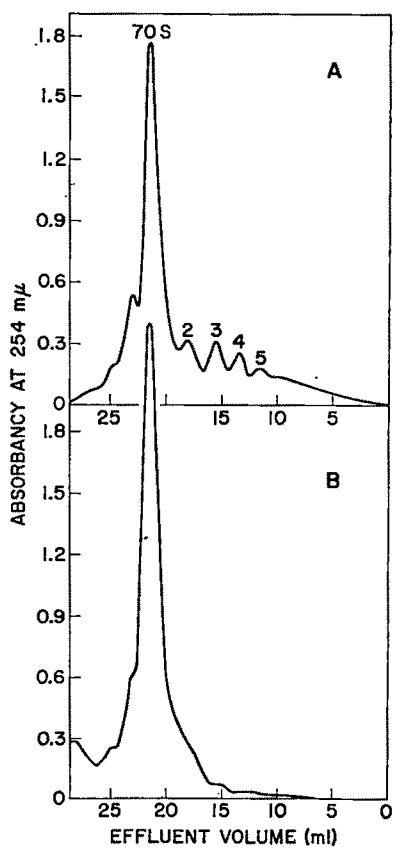


Fig. 2. Sedimentation pattern of *C* ribosomes, (*A*) before and (*B*) after treatment with RNase (10^{-6} g/ml.) at 37° C for 5 min. Input and centrifugation as indicated under Fig. 1

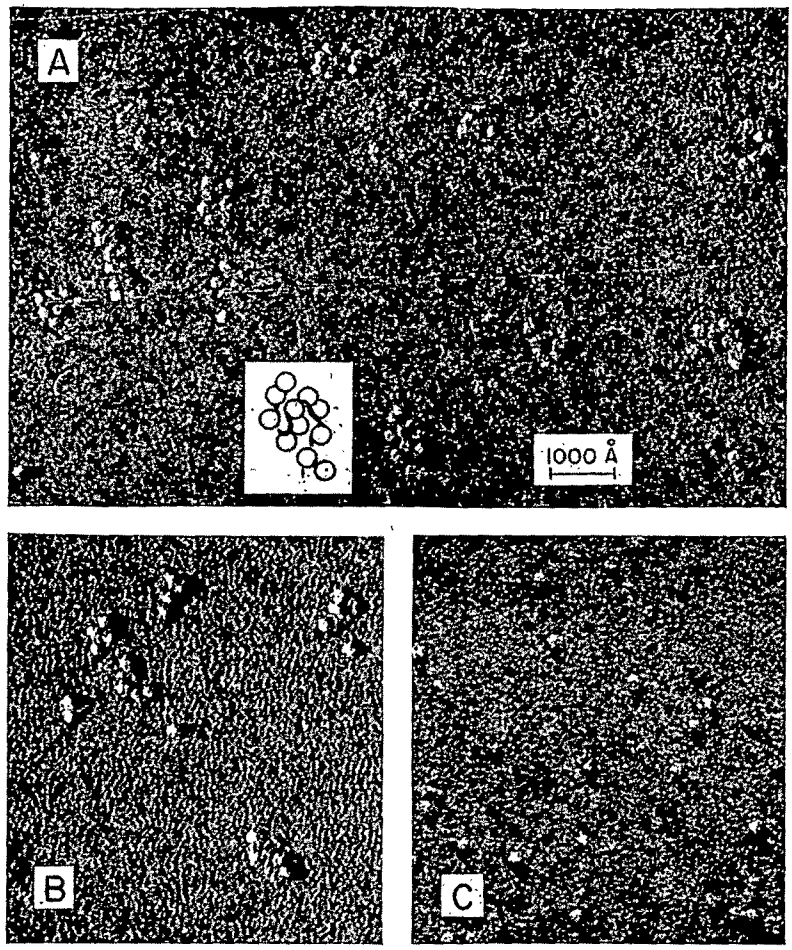


Fig. 3. Electronmicrographs of ribosomes isolated from different regions (*A*, *B*, *C*) of sucrose gradient shown in Fig. 4*A*. Insert in *A* shows enlarged drawing of the ergosome at right to suggest course of presumed *m*RNA-thread connecting eleven 70 *S* particles, as apparent on original plate

of labelled protein, synthesized during a 60 sec interval after addition of ^{14}C-leucine to the incorporation mixture, is shown in Fig. 4. In one sample the label was added at zero time (*B*), in another after 5 min of incorporation in the presence of ^{12}C-leucine (*C*). When the amount of labelled protein associated with different aggregate sizes was computed per unit of ribosomal mass, a constant value was obtained (Fig. 4, bottom graph). This indicates that the rate at which a polypeptide chain is synthesized at the condensing site of each 70 *S* particle in an aggregate is independent of aggregate size as expected from the tape mechanism. The radioactivity associated with the monomer peak is another manifestation of the breakdown process. For the monomers to which the labelled nascent protein is attached must originate from aggregates engaged in protein synthesis, since ribosomes in the monomer state are inactive, as shown in the subsequent experiment. In computing for different aggregate sizes the amount of radioactivity incorporated per absorbancy unit, a correction was made for the overlap of the monomer peak with the adjacent peaks of dimers $(70\ S)_2$ and trimers $(70\ S)_3$ as indicated by the dashed lines in Fig. 4. Fig. 4 also shows that, after 5 min of incubation, the incorporation rate has decreased to about 45 per cent of the initial value, indicating inactivation or depletion of rate-limiting enzymes or substrate components.

In order to test the postulated proportionality between aggregate size and the amount of protein synthesized per aggregate mass during long incorporation periods, *C* ribosomes were fractionated according to particle size by sedimentation in a sucrose gradient and later assayed for amino-acid incorporation during a 45-min incubation period. The sucrose gradient was cut into 29 1·0 ml. fractions, each of which was assayed separately. The sedimentation pattern, reflecting the size distribution of ribosomes before incubation, is reproduced in Fig 5*A* together with the distribution of the labelled protein that had been synthesized during the incorporation period. The results confirm our previous findings with rat liver ergosomes[1,3] and the results of Marks *et al.*[16] and of Gierer[5] with the reticulocyte system. The fractions containing mostly single 73 *S* particles were completely inactive. Moreover, the plot in Fig. 5*B* of specific activity (c.p.m. ^{14}C-leucine incorporated per 0·1 mg RNA) versus aggregate size shows a linear increase up to the heptamers $(73\ S)_7$; the curve then slopes off and reaches a maximum near $(73\ S)_{10}$. The apparent decline in the region of aggregates heavier than $(73\ S)_{10}$ might be due to contamination with unspecific aggregates or heavy cell components that had not been completely removed during the preceding centrifugations.

Discussion. The results of experiments on the structure and function of *E. coli* ribosomes described in this article support our previous conclusions, derived from studies of the rat liver system[3] that ribosomes become functional by attachment to *m*RNA and subsequent reading of the message by the tape mechanism as postulated by Gierer[5] and by Gilbert[4]. This process could explain the formation of ergosomes, the size of which would be determined both by the length and lifetime of *m*RNA, as previously discussed in detail[3].

Much recent debate has centred around the question of the nature of the active complex in *E. coli*, mainly because published data seem to conflict with respect to the *in vitro* incorporation activity of single 70 *S* monomers[17]. However, in the present concept these difficulties might be resolved by the following considerations. Available evidence suggests that a ribosome population obtained after breaking the cells contains at least four classes of potentially functional 70 *S* particles which reflect the different phases of ergosome assembly and breakdown.

(I) 'Vacant monomers'. These particles, which are released from ergosomes during protein synthesis *in vivo* and show a characteristic tendency for dimerization, make up the steady-state pool in the cell and are capable of accepting *m*RNA[2,4,5,18,19].

(II) Monomers to which a finished peptide chain is attached. This class of monomers accumulates during protein synthesis in cell-free systems that are limiting in release factor. On incubation with release factor, they are converted into class I monomers which are now competent to accept *m*RNA[5,20,21].

(III) Monomers containing both an unfinished peptide chain and a fragment of *m*RNA. These monomers result during the isolation procedures from cleavages by RNase of bonds within the exposed stretches of *m*RNA that connect the 70 *S* particles in ergosomes[1,5,7]. The piece of *m*RNA that remains attached to these monomers probably contains less than 50–60 nucleotides[3,19].

(IV) Monomers attached to the end of a nascent *m*RNA strand, a portion of which may still be combined with polymerase and the DNA template. It is tempting to speculate that such a hypothetical complex might represent the initial step in the assembly of ergosomes *in vivo*[3]. This complex would be expected to sediment faster than

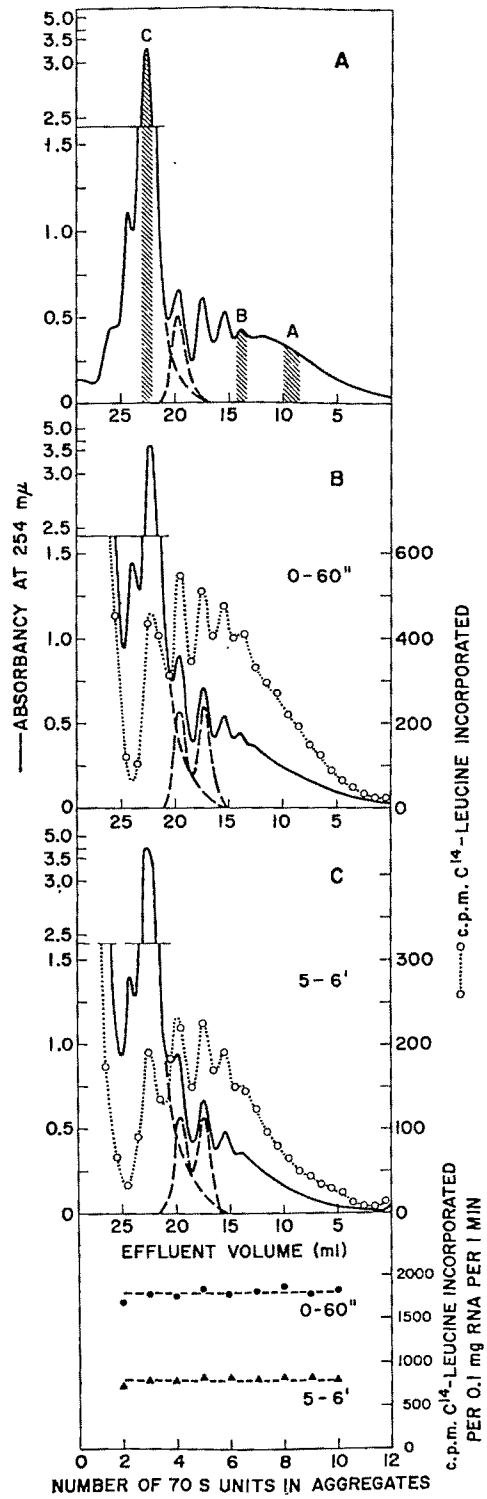


Fig. 4. Distribution of absorbancy and nascent protein associated with *C* ribosomes after incorporation pulses *in vitro*. (*A*) Sedimentation pattern of *C* ribosomes before incubation. (*B*) Sedimentation pattern of ribosomes and nascent protein labelled during 60-sec period at beginning of incubation period and (*C*) at end of 5-min incorporation in presence of non-radioactive amino-acids. The incubation mixtures (1·0 ml.) contained in addition to standard reagents: *C* ribosomes (0·8 mg RNA) post-ribosomal supernatant (0·3 ml.), and uniformly labelled ^{14}C-L-leucine (0·008 μmoles; 246 mc./mmole) which was added at zero time (*B*) or after 5 min (*C*). Reaction at 30° C was terminated by chilling, and mixtures centrifuged for 3 h at 25,000 r.p.m. The gradient fluid was collected in 1·0-ml. fractions and acid-insoluble material precipitated after adding 1·5 mg bovine albumin as carrier. The acid-insoluble radioactivity at top of gradient consists of *s*RNA-bound ^{14}C-leucine. The dashed lines show the absorbancies of the dimer and trimer peaks after correction for the estimated amount of contamination with monomers

the other classes of 70 *S* particles and might account for the active 'heavy 70 *S* ribosomes' sedimenting between 70 and 100 *S* (refs. 22, 23). The occurrence of such a complex could perhaps explain the origin of the large 12 *S* *m*RNA liberated from the 70–100 *S* region at low Mg^{++} concentrations[22].

In our experiments with purified ribosomes, and with an incorporation system from which most of the DNA, and hence the DNA-bound RNA polymerase[24], had been removed, the monomers were inactive. However, amino-acid incorporation by class III monomers expected to be present in this fraction cannot be ruled out, since the addition of only 1–2 leucine residues per growing peptide chain, permitted by the short *m*RNA piece of these particles, would have escaped detection. Monomers isolated from crude extracts, on the other hand, have been shown to synthesize protein, even in the presence of DNase[4]. This must be due to either the presence of class IV monomers or the attachment of class I monomers to *m*RNA generated by the polymerase reaction. This interpretation is supported by the findings of Doerfler *et al.*[24], who showed that the DNA–RNA–polymerase complex is relatively resistant to DNase and continues to function at DNase concentrations which cause rapid degradation of free DNA.

According to the tape mechanism, assembly of 70 *S* ribosomes into ergosomes is coupled to protein synthesis. The heavy complexes between 70 *S* ribosomes and poly U (refs. 4, 13–15, 17) or viral RNA (ref. 17), which may form in the cold and in the absence of the components required for protein synthesis, may result from fortuitous

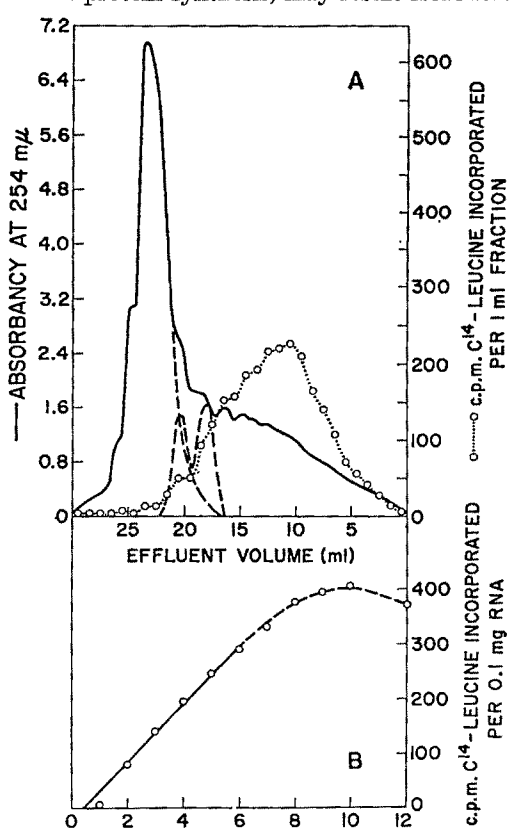


Fig. 5. Amount of protein synthesized after long incubation periods by fractions of different aggregate sizes. (*A*) Sedimentation pattern of *C* ribosomes (3 h at 25,000 r.p.m.; 3·2 mg RNA input) before incubation and total radioactivity incorporated as alkali-stable acid-insoluble counts during 45 min at 37° C by each 1·0-ml. fraction collected from gradient. (*B*) Specific activity (c.p.m. ^{14}C-leucine/0·1 mg RNA) as a function of aggregate size. The incubation mixtures (1·3 ml.) contained in addition to standard reagents: ribosomes (0·7 ml. from each gradient fraction), post-ribosomal supernatant (0·3 ml.), and DL-leucine-1-^{14}C (0·06 μmoles; 21·5 mc./mmole). *s*RNA-bound radioactivity was removed by incubating samples for 1 h at 25° C with 1 ml. 1 N sodium hydroxide before precipitation with acid

aggregation[17]. Thus, under ionic conditions which prohibit this secondary aggregation, Haselkorn *et al.*[17] found that at saturation about 10 per cent of their 70 *S* particles combined with about one virus RNA molecule in the cold and that the mixture synthesized protein on subsequent incubation in the complete system. Barondes and Nirenberg, on the other hand, observed that assembly of 70 *S* particles by TMV–RNA required conditions of protein synthesis[13]. Moreover, poly U-directed synthesis of polyphenylalanine usually proceeds with a characteristic lag, regardless of whether or not incorporation has been preceded by aggregation of ribosomes with poly U in the cold[13,25,26]. So long as in each of these experiments aggregation by fortuitous attachment of *m*RNA to ribosomes cannot be rigorously excluded, all these seemingly contradictory results in no way invalidate the view that the orderly assembly of 70 *S* ribosomes into functional ergosomes by sequential attachment of the particles to the cognate end of the messenger is coupled to protein synthesis.

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GENETIC AND DEVELOPMENTAL STUDIES OF A QUANTITATIVE CHARACTER

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THODAY[1] has described techniques which can be used to locate some of the individual genes responsible for a heritable difference affecting a quantitative character. He pointed out that the location of such genes is a prerequisite to understanding their nature and their individual effects. This article describes the morphological effects of the components that have been isolated from such a polygenic complex.

The quantitative difference studied is that distinguishing the *Drosophila melanogaster* line vg 4 of Thoday and Boam[2] (which has a mean of about 37 chætæ) from the standard inbred wild-type strain Oregon (with a mean of about 20). The two differ at three loci[3,4] at each of which there are high chæta number factors in vg 4. Two high factors (III*a* and *b*) are at approximately 28·5 and 32·5 centimorgans in chromosome III, and the third (II) is at approximately 44 centimorgans in chromosome II. In the absence of II, III*a* and *b* have equal effects and they do not interact. II has only slight effect alone but II and III*a* interact, together increasing chæta number by more than the sum of their individual effects. There is no interaction between II and III*b*.

Regional effects of the different genes. The effects of these three genes on the arrangement of sternopleural chætæ were studied in a series of stocks differing at the three loci, much of the rest of their genomes being derived from inbred Oregon. A cavity slide was prepared which made it possible to orientate the flies in a standard way so that they might be drawn with the aid of an Abbé *camera lucida*. The sternopleurites were drawn and the positions of all the chætæ noted. The drawings were then scanned with a grid which divided the sternopleurite into several regions as illustrated in Fig. 1.

Drawings were made of both sides of 50 male and 50 female flies in each of the stocks. Statistical analyses of the frequency of chætæ in the different regions (details of which will be given in a fuller publication) show that there are no significant differences between sexes or between sides, but there are differences between genotypes. The chæta numbers in each region for each genotype are given in Table 1, and the increases (compared with Oregon) are given in Table 2.

It can be seen that III*a* increases chætæ in all parts of the sternopleurite though in region *A* the effect is slight and not demonstrably significant. The other factors appear to exert more local effects. III*a* also increases the number of chætæ on other parts of the fly (Table 3) whereas III*b* and II do not. III*a* therefore has a general effect on the number of chætæ that the flies bear, although region *A* is not affected so much as the other regions are.

Both this evidence that III*a* has a general effect, whereas that of III*b* is local, and that they differ in their

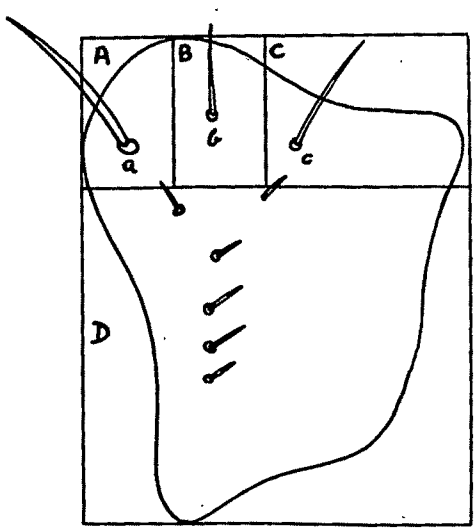


Fig. 1. Sternopleurite and chætæ divided into regions

* Victoria Woodhull Fellow of The Royal Institution.

Table 1. Number of Chætæ in Each Region of the Sternopleurite

Genotype	Region of sternopleurite A	B	C	D	Total Chæta No.
Oregon inbred	204	322	286	1,197	2,009
IIIa IIIb+ II+ / IIIa IIIb+ II+	224	375	343	1,406	2,348
IIIa+ IIIb II+ / IIIa+ IIIb II+	208	344	306	1,516	2,364
IIIa+ IIIb+ II / IIIa+ IIIb+ II	212	474	288	1,204	2,173
IIIa IIIb II+ / IIIa IIIb II+	220	366	363	1,713	2,692
IIIa IIIb+ II / IIIa IIIb+ II	228	762	350	1,412	2,752
IIIa+ IIIb II / IIIa+ IIIb II	214	498	295	1,515	2,522
IIIa IIIb II / IIIa IIIb II	228	746	355	1,718	3,047

Table 2. Percentage Increase in Chætæ in Each Region compared with Oregon Inbred

Genotype	A	B	C	D
IIIa IIIb+ II+ / IIIa IIIb+ II+	9·8	16·5	19·9	17·5
IIIa+ IIIb II+ / IIIa+ IIIb II+	2·0	10·7	7·0	26·7
IIIa+ IIIb+ II / IIIa+ IIIb+ II	3·9	47·2	−1·0	00·6
IIIa IIIb II+ / IIIa IIIb II+	7·8	13·7	26·9	43·1
IIIa IIIb+ II / IIIa IIIb+ II	11·8	136·6	22·4	18·0
IIIa+ IIIb II / IIIa+ IIIb II	4·9	54·7	3·1	26·6
IIIa IIIb II / IIIa IIIb II	11·8	131·7	24·1	43·5

Table 3. Mean Chæta Numbers in Various Parts of IIIa and Oregon Flies

Area	Genotype IIIa	Oregon	Significance level of differences
Sternopleurite	30·6	19·8	$p<0·001$
Coxæ first pair of legs	26·6	22·4	$p<0·001$
Coxæ second pair of legs	29·6	23·4	$p<0·001$
Fourth and fifth abdominal sternites	45	41	$p<0·001$

interactions with II, show that the linked pair of polygenes on chromosome III function in quite different ways, though their effects on total sternopleural chæta number are quantitatively equal and additive. The interaction between IIIa and II is also localized.

Size of fly and number of chætæ. It is known that number of chætæ is sometimes correlated with size of fly[5] and it was expected that IIIa, as its effect is general, would prove to increase the size of the fly. Flies with IIIa and that section of the vg 4 chromosome III to the left of IIIa were therefore weighed, but there was no difference in weight distinguishing them from Oregon. However, further genetic analysis has shown that this is because there is in the vg 4 chromosome an additional factor that decreases fly size without affecting chæta number.

The method of chromosome analysis of the left arm of chromosome III is described by Thoday, Gibson and Spickett[3] and need not be detailed here. The technique (which is derived from that of Breese and Mather[6]) makes it possible to build up chromosomes of which a known part comes from the stock under test, the remainder coming from Oregon. The marker stock *ve, h, eyg, cp* was used to make such 'synthetic' chromosomes. If the left arm of chromosome III or Oregon is represented as L L L L, each L being that part of the chromosome in the region of a marker, the left arm of vg 4 may be similarly designated H H H H. Using this notation we may, for example, designate as L L H H the various types of synthetic chromosome combining *ve* and *h* regions from Oregon with the *eyg* and *cp* regions from vg 4. The probability that a factor, located between *h* and *eyg* and which distinguishes vg 4 from Oregon, will be found in the L L H H chromosome will depend on its position on the linkage map between *h* and *eyg*. The results obtained by weighing flies from several stocks of each type of 'synthetic' chromosome are shown in Fig. 2. It is clear that there are in vg 4 a weight diminishing factor (designated IIIw) to the left of the locus *h* (it is at about 13 cM) and a weight-increasing factor between the loci *h* and *eyg* (which is at about 29 cM, that is, very close to, if not at, the same locus as IIIa). In addition, all the H H L L stocks containing IIIa that were derived by Thoday, Gibson and Spickett[3] were similar in weight to Oregon, but those without IIIa were lighter than Oregon. Their L L H H stocks were also of two weight classes. Those which carry IIIa are heavier than Oregon. Those that do not carry IIIa are lighter than Oregon. The presence or absence of IIIb had no detected effect on weight. There is therefore considerable evidence in favour of the chæta increasing factor IIIa and the weight increasing factor being very closely linked if not the same. At least in the experiments reported here they were inherited as one.

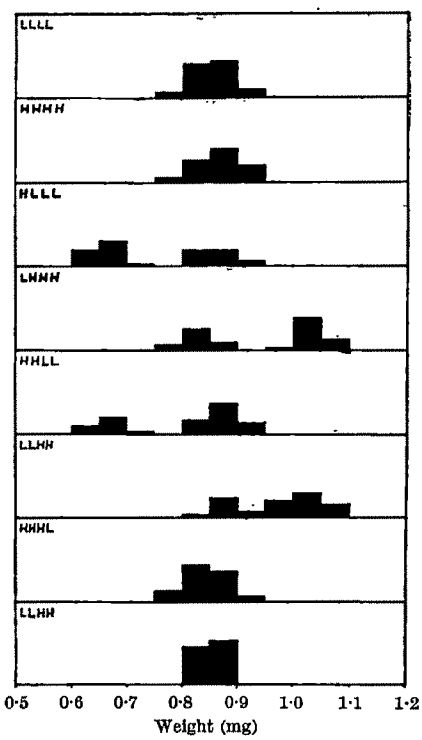


Fig. 2. Chromosome analysis of vg 4 for factors affecting fly weight. Data based on thirty stocks of each type of synthetic chromosome. Twenty male flies were weighed from each stock

As the locus IIIw has no effect on sternopleural chæta number whereas IIIa affects both weight and chæta number, it is clear that different polygenic systems affecting different characters may have loci in common. The situation provides an example of the type of complex discussed by Mather[7] and demonstrated by Breese and Mather[8] which involves interlocking of different polygenic systems.

Size and number of cells. Following this demonstration that the different chæta number loci have different morphological effects, attention was concentrated on variation in the hypodermis of the sternopleurite as the larger flies tend to have larger sternopleural areas[5]. The hypodermis was removed from the sternopleural region, rapidly stained in hæmatoxylin and mounted in water under a coverslip. All the nuclei in a standard area were counted and drawn with the aid of a *camera lucida* using standard magnification. This was done for stocks carrying IIIa and for stocks carrying IIIw (both on Oregon backgrounds) and for Oregon itself. The results (Table 4) show that IIIw flies have more nuclei in the standard area

Table 4. Number of Nuclei in a Standard Field, Relative Area of Sternopleurite and Ratio of Proportionate Cell Numbers in Sternopleural Hypodermis

	Genotype		
	Oregon	III*a*	III*w*
No. of nuclei in a standard field (x_n)	449	462	518
Relative sternopleural area (y_n)	86	98	79
Ratio of proportionate cell Nos. in sternopleural hypodermis $\frac{x_n}{x_{Or}} \times \frac{y_n}{y_{Or}}$	1·00	1·17	1·06

than III*a* or Oregon and they therefore have smaller cells. At the same time III*w* has a smaller sternopleurite area than Oregon, and III*a* a larger area than Oregon. Estimates from these data of the relative number of cells in the sternopleurite show that III*w* and Oregon have similar numbers of cells whereas III*a* flies have more cells.

Wigglesworth[9,10] has shown that in *Rhodnius prolixus* body size may be altered either by changing cell number or by changing cell size, and that variation in cell size does not affect the number of epidermal plaques that develop whereas variation in cell number does. The present results suggest that the same is true of *Drosophila melanogaster*, for III*w*, which affects cell size, does not seem to influence the differentiation pattern whereas III*a*, which increases cell number, generally increases chæta number.

*Local effects of II and III*b. In considering the local effects of the other two genes (II and III*b*) we have to take into account the development of chætæ in *Drosophila melanogaster*, which has been described by Lees and Waddington[11]. During its development, the chæta initial cell undergoes a transient but considerable enlargement. Such cells in this condition are easy to see in the sternopleural hypodermis if it is stripped, during the white puparium stage, and viewed from the inner side after brushing with indian ink. In low chæta number flies and in all flies examined, except those carrying II, the earliest enlarged cells were in positions similar to those of the three large chætæ *a*, *b*, *c* (see Fig. 1). The other large cells develop later. However, in flies carrying II only two large cells were seen in the younger hypodermis, in positions *a* and *c*; later several large cells develop in the region of *b*, which is, in such flies, occupied by several smaller chætæ. It may therefore be concluded that large chætæ develop earlier than small chætæ and that II brings about its effect by delaying the initiation of chætæ in region *B*.

No differences in region *D* were observed in the time of chæta initiation. However, measurements of the chætæ showed that they were shorter in flies with III*b* than in other flies. Moreover, chætæ were found over a relatively larger area in III*b* flies and this found expression in the tendency to form a second row of chætæ in region *D* (Fig. 3).

Conclusions. The activities of the factors may be summarized as follows:

III*a*. Increases the size of the fly by increasing cell number, and as a consequence produces a general increase in chæta number.

III*b*. Increases the number of chætæ in region *D*, extending their distribution and reducing their size.

II. Retards the development of large chætæ in region *B* with the result that several smaller chætæ develop in this region.

III*w*. Decreases fly size by reducing cell size and in consequence compensates for the size effect of III*a* without altering III*a*'s effect on chæta number.

This knowledge makes it possible to propose a model for the development of chæta pattern in the sternopleurite.

Stern[12] has argued that, in *Drosophila melanogaster*, there are regions or 'pre-patterns' within the bounds of which any competent tissue may form chætæ. Such a pre-pattern may be the expression of the distribution of some substance essential to the development of chætæ from hypodermal cells. The competence of the hypodermal cells may depend on another substance. Variation in chæta pattern could be caused by changes in the overall distribution and concentrations of either of these two substances. The peaks of concentration in regions *A*, *B* and *C* must be both high and sharp so that a single cell starts to differentiate well in advance of the others and hence takes up essential substrates to such an extent that it excludes the possibility of differentiation of other chætæ near to it. These peaks may be lowered or dispersed by the action of a gene (for example, II in region *B*) so that no single cell shows precocious differentiation but instead several cells start to differentiate at the same time and several smaller chætæ develop instead of one large chæta. If the number of cells in the element is increased the intensity of suppression of new differentiation between two differentiating centres would diminish. Thus the effect of II in causing later, but more frequent, differentiation centres between *a* and *b* is enhanced by III*a*, which causes more cells to intervene between *a* and *b*. Redistribution of the pre-pattern over a larger area would result in the formation of more chætæ, but if the amount of chæta differentiating substrates were the same the chætæ would be smaller. This appears to be the effect of III*b*.

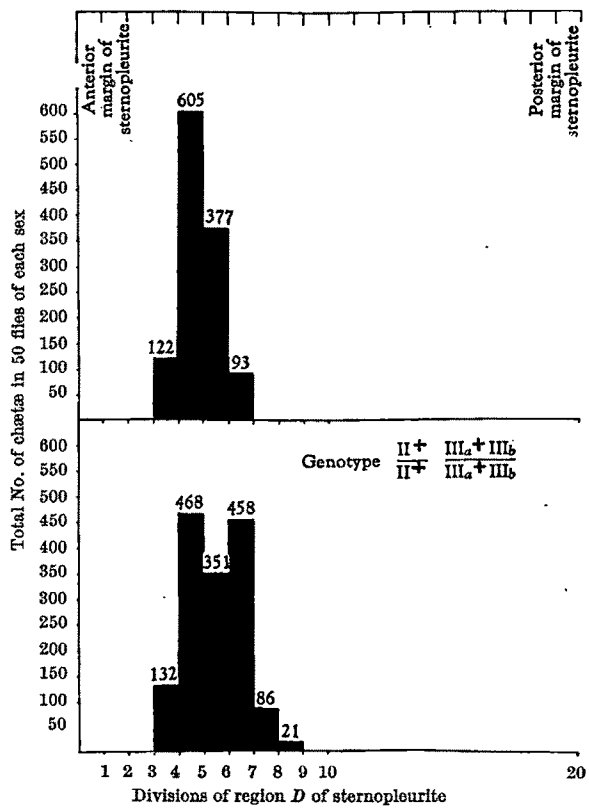


Fig. 3. Distribution of chætæ in region *D* of sternopleurite of Oregon and III*b* flies. The region was divided into twenty sectors from anterior to the posterior margin and the number of chætæ in each sector of fifty flies of each sex of each kind is plotted in the figure

That different parts of a single pattern may be separately affected by genetic variation as has been shown previously by Smith and Sondhi[13], and Reeve[14] has shown that the sternopleural chæta pattern of *Drosophila melanogaster* may be changed specifically by selection. The present report shows how such observations may be extended when the different genes can be handled more precisely in breeding experiments, and also shows how the separate effects of individual polygenes (which in this example are quite different) may then be investigated, in a way similar to that which has been used by previous workers, for example, Waddington[15], who studied the effect of major gene differences with the view of aiding understanding of development.

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IMMUNOLOGICAL COMPETENCE OF THYMUS CELLS AFTER TRANSFER TO THYMECTOMIZED RECIPIENTS

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THERE is now much evidence that the thymus controls the origin and multiplication of lymphocytes and the development of the immune response. In embryonic life the lymphocytes of both thymus and spleen arise only from the thymic epithelium[1]. After birth, the presence of the thymus is necessary for the development of the lymphoid tissues and of immunological responsiveness[2]. In the adult it is required for the recovery of responsiveness after irradiation[3,12]. The means by which the thymus influences lymphopoiesis is still in doubt. In neonatally thymectomized mice grafted at one week of age with cytologically marked neonatal thymus, Miller[5] found that by the time the mice had reached adulthood, responsiveness had been restored, and donor mitoses were present in the spleen. This observation gave substance to the hypothesis, proposed by many authors, that the thymus is the source of lymphocytes, and that during development these are 'seeded out' to the other lymphoid tissues, and become the competent cells of the adult. Further investigations showed that the thymus graft itself becomes largely re-colonized by host cells, while results obtained with the use of Simonsen's discriminant assay revealed that the cells responsible for competence in the spleen were mainly of host origin[16]. This suggested that some type of cell (perhaps of thymic origin in the first place[1]) may enter the thymus, divide therein, and then pass out to the other lymphoid tissues. There is evidence that this cell resides principally in the bone marrow[4,18].

The cell responsible for competence is probably a small lymphocyte[7]. In view of this, and the foregoing evidence that some competent cells come from the thymus, it is of interest that the thymus itself, which consists largely of lymphocytes, does not respond to most antigenic stimulations[8]. Moreover, suspensions of thymus cells have only a very low ability to transfer secondary responsiveness to protein antigens[9], and their performance in the graft-versus-host reaction is poor[10]. Evidence summarized here shows that thymus cells are nevertheless capable of acquiring a degree of competence comparable with that of lymph node cells.

The aim of the experiment (Fig. 1) was to test the ability of various lymphoid tissue inocula to restore responsiveness to thymectomized mice, and then to determine what proportion of the competent cells had been provided by these inocula. *CBA* mice were thymectomized at 2–3 days old. Thymectomy at this age in *CBA* mice leads to a mild form of wasting disease, but rarely results in the premature death seen after thymectomy earlier in life[5,6]. However, it greatly depresses the capacity of *CBA* mice to produce antibody against bovine serum albumin (BSA) and impairs to some extent the ability of their lymphoid cells to cause a graft-versus-host reaction in F_1 hybrid hosts[12]. Thirty male mice were thymectomized and six sham-operated. Seven weeks later the thymectomized mice were divided into 5 groups of 6 and were treated with various lymphoid tissue inocula as detailed in Table 1. The donors were of both sexes and were aged either 2–3 days ('neonatal') or 2–3 months ('adult'). Three of the transfers in each group were taken from mice co-isogenic with *CBA* but bearing the T6 marker chromosome (*CBA/T6*), and the other three from normal *CBA* mice. After a further seven weeks the mice were injected with BSA in Freund's adjuvant[13], and then bled 35 days later. The titres of antibody in the sera were measured by Farr's antigen-binding-capacity method[14].

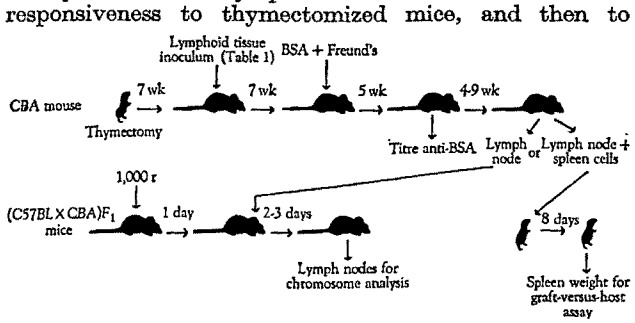


Fig. 1

Table 1

	Group	Lymphoid tissue inoculum	Arithmetic mean anti-gen-binding capacity of serum at 1 μg BSA/ml. (μg/ml.)	Graft-versus-host responsiveness relative to sham-operated controls (%)	Mean	No. *T6*/No. counted	Mean % *T6*
	I	50 million normal lymph node cells	33 ± 27*	14		15/18	
				137		10/14	
				27	59	23/39	71
	II	50 million normal adult thymus cells	31 ± 28	NR		14/27	
				NR		7/22	
				NR	—	10/18	47
Thymectomized	III	Graft of neonatal thymus	32 ± 12	132		15/46	
				115		4/32	
				360	202	10/45†	23
	IV	Graft of adult thymus	2·9 ± 1·1	86		21/58	
				187		10/43	
				NR	136	NR	30
	V	No transfer	3·8 ± 4·5	0·5		—	
				53		—	
				4·4		—	
				NR		—	
				NR		—	
				NR	19	—	—
Sham-operated	VI	No transfer	64 ± 37				

* Standard deviations.
† In the thymus graft of this mouse 17/17 mitoses were of host origin.
NR, no result due to death or other accident.

An attempt was then made to estimate, by chromosome analysis, the proportion of donor-type cells among the competent cells of the mice which had received grafts or cell transfers from the *CBA/T6* donors. Each experimental mouse was killed and a cell suspension made from its lymph nodes. This was injected intravenously into a lethally irradiated (1,000 r.) (*C57BL*) × *CBA*)F_1 mouse. Two to three days later the hybrid recipient was injected with 4 μg/g body-weight of 'Colcemid' (Ciba) and chromosome preparations were made from its lymph nodes

(mod. of 15). A chromosome analysis (without cell transfer) was also made on one of the neonatal thymus grafts, after exposure to 'Colcemid' *in vitro*. The proportion of mitoses in normal lymphoid tissues which belong to immunologically competent cells is unknown, and may be quite small. The purpose of the foregoing procedure was to amplify this proportion. A reconstruction experiment[12] has indicated that the majority of mitoses in the irradiated hybrid recipients probably represent donor cells engaged in a graft-versus-host reaction. Thus the proportions of *T*6 mitoses recorded in Table 1 reflect the proportions of cells, among those competent to react to *C57BL* antigens, which had arisen from the original *CBA/T*6 inocula.

The remaining mice in the thymectomized groups (I–V) were used in graft-versus-host assays. In these the potency of lymphoid cell suspensions (spleen and lymph nodes) from the thymectomized mice in causing spleen enlargement in $(C57BL \times CBA)F_1$ recipients was compared with that of the sham-operated controls. A standard 4-point assay procedure was used. For details of this see ref. 17. The potency was then multiplied by the factor:

$$\frac{\text{No. of cells in thymectomized mouse}}{\text{No. of cells in sham-operated mouse}}$$

to obtain the 'responsiveness' of the thymectomized mouse relative to the sham-operated control. Referring to Table 1, Groups I and II, it can be seen that adult thymus cells had been as effective as lymph node cells in the restoration of responsiveness to BSA, and that both had provided a substantial proportion of the cells competent to perform a graft-versus-host reaction.

Antibody production in the thymus may normally be prevented by the existence of a blood-thymus barrier which is impermeable to antigens, as suggested by Marshall and White[11]; but this does not explain the low activity of thymus cells in the graft-versus-host reaction[10,12]. In this situation, however, they are of necessity exposed to antigen immediately after transfer, whereas in the experiment just described a period of 7 weeks was allowed to elapse between cell-transfer and antigenic stimulation. Further experiments have since shown that the competence of thymus cells remains low even up to 1 week after release from the gland, but is well developed by 6 weeks[12]. Presumably these cells require to undergo some process of maturation before they can become competent. The results obtained with the mice which received thymus grafts (Groups II and IV of Table 1) confirm those of Miller[16] mentioned in the introductory paragraph, while using chromosome analysis rather than Simonsen's discriminant assay to determine the origin of the competent cells. It can be seen that by the time of challenge with BSA, responsiveness to this antigen had been restored in the mice receiving neonatal thymus grafts, but not in those receiving adult thymus grafts. However, by the time the graft-versus-host assays were made Group IV had also acquired responsiveness—at least to tissue antigens—and indeed the graft-versus-host responsiveness in both Groups III and IV was higher than in Group 1. The results of the graft-versus-host assays were also borne out in the chromosome analyses by observation of the mitotic density. Although no measurements were made, this was obviously higher in Groups III and IV than it was in Groups I and II. Thus, while being at least equal in competence to Groups I and II, the mice receiving thymus grafts show, among their competent cells, a smaller proportion of donor origin, as can be seen from the chromosome analyses. It is therefore evident that, besides providing competent donor cells, the thymus grafts had caused an increase in the number of competent host cells. While it seems very probable that the grafts brought about this result partly by stimulating host-cell division within themselves, they might also have stimulated lymphopoiesis in other sites by humoral means.

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DORMANCY REGULATORS IN WOODY PLANTS

Experimental Induction of Dormancy in *Betula pubescens*

HEMBERG[1] first proposed the theory that growth-inhibiting substances play a part in the regulation of bud dormancy in woody plants. The theory is mainly supported by the fact that a correlation can be shown to exist between seasonal changes in the state of dormancy of the buds of certain species and corresponding variations in the levels of the endogenous inhibitors within them. Thus, it has been shown for several woody species that the gradual reduction of bud dormancy during the winter is associated with a decrease in the level of endogenous growth-inhibitors[1–5]. Moreover, it has been shown that short-day conditions, which induce dormancy of woody plants, result in increased levels of inhibitors in the leaves and buds[6–8]. The inhibitory effect on the growth of buds exerted by leaves maintained under short-day conditions strongly suggests the transmission of inhibitory substances from the leaves to the buds[9,10]. Nevertheless, the crucial test of whether dormancy can be induced in actively growing seedlings of woody plants by application of inhibitors extracted from the same species has apparently not been carried out; the present report describes the successful induction of dormancy in seedlings of *Betula pubescens* by such treatment.

The inhibitor solution for these experiments was obtained from the leaves of *Betula pubescens* by extraction with 80 per cent aqueous methanol, and subsequent partition into ether. The ether fraction was further partitioned by paper chromatography with isopropanol/aqueous ammonia (sp. gr. 0·880 × 1/100) (4:1 v/v) as solvent, and the zone at R_F 0·65–0·95 was eluted in water and reduced to a convenient volume. In preliminary experiments it was found that the inhibitor significantly reduced the hypocotyl extension of birch seedlings grown on it. Since the inhibitor was obtained from the leaves, it seemed reasonable to re-apply it to the leaves. The inhibitor solution was applied to the leaves of actively growing birch plants maintained under a 14·5-h photoperiod, by immersing a leaf of each plant in an aqueous solution of the inhibitor contained in a small tube. The solution was renewed regularly during the experiment which lasted

28 days. Measurements of stem extension were recorded, and it was clear that the water-treated control plants were growing actively throughout the experiment, whereas the apical growth of the inhibitor-treated plants was completely arrested.

The compact structure of the arrested apices was in marked contrast to that of the actively growing apices and in this respect closely resembled the apices of plants rendered dormant by short-days. These results suggested that the inhibitor had induced a state of dormancy, a conclusion that gained further support from the results of extractions of the arrested and actively growing apices. Higher levels of the inhibitor were detected in the apices of the inhibitor-treated plants as determined by the coleoptile-section test. The possibility remained that this condition may have arisen by the action of a toxic substance. This possibility was tested in a subsequent experiment where the plants were treated with the inhibitor solution for 28 days, by which time the apical growth was arrested, and then gibberellic acid was applied to the buds. The buds rapidly expanded and the resulting growth showed no evidence of toxicity or abnormality. These results would seem to provide strong evidence in support of the inhibitor hypothesis of dormancy.

On the other hand, the discovery that gibberellic acid overcomes the dormancy of many tree buds raises the question as to whether endogenous gibberellins are also involved in the regulation of dormancy. It has been mentioned that the resting-buds formed in response to applied inhibitor readily resumed growth, indicating that gibberellic acid can overcome the effect of the inhibitor. This conclusion was supported by the results of an experiment in which shoots of birch, collected in mid-March, were placed with the basal end in inhibitor solution in conjunction with various concentrations of gibberellic acid. Bud-break was greatly reduced in the presence of inhibitor, but at gibberellic acid concentrations of greater than 1 mg/l. the effect of the inhibitor was overcome. Thus, the effect of winter-chilling in overcoming the dormancy of buds may be due to an increase in the levels of endogenous gibberellins rather than to a reduction in the inhibitors. This hypothesis is suggested by observations on potato tubers[11] and hazel seeds[12], in which an increase in endogenous gibberellins occurred in response to chilling. Further evidence is provided by our own observations that there are significant changes in the levels of endogenous gibberellins in the buds of *Acer pseudoplatanus* during December–April, certain fractions increasing and others decreasing during this period. Thus, the emergence of buds from dormancy may be regulated by a balance between growth-inhibitors and endogenous gibberellins.

It is clear that a large number of substances occurring in plant-tissues are likely to be inhibitory to growth, but the existence of growth-inhibitory activity does not, in itself, indicate that such substances are important in dormancy. However, the demonstration that the growth-inhibiting fraction extracted from birch leaves can induce dormancy in seedlings of that species strongly suggests the existence of endogenous dormancy-regulating substances, and it is proposed that the term 'dormin' should be used for substances which appear to function as endogenous dormancy-inducers.

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Isolation of the Inhibitor varying with Photoperiod in *Acer pseudoplatanus*

Seedlings of *Acer pseudoplatanus* show marked photoperiodic responses in relation to dormancy[1]. Under short days the seedlings rapidly cease extension-growth and form resting-buds, whereas under long days extension-growth is more prolonged. The possibility that the dormancy of buds and other resting-organs results from the presence of specific growth-inhibitors[2] suggests that the induction of dormancy by short days may be due to the greater production of inhibitors under these conditions as compared with long days. Evidence in support of this hypothesis was obtained by Phillips and Wareing[3], who found that transfer of *A. pseudoplatanus* from long-day to short-day conditions resulted in greater production of a substance which inhibited extension-growth in sections of wheat coleoptiles. Further work to isolate and determine the chemical nature of these growth-inhibitory fractions in *A. pseudoplatanus* is now described. For the work described in this paper, growth activity was assayed by the 'straight-growth' method with 1 cm sections of wheat coleoptiles, but it is significant that the substance was also effective as an inhibitor of elongation in sections of hypocotyls from *A. pseudoplatanus* and inhibited expansion of leaf disks cut from this species. When extracts of *A. pseudoplatanus* were chromatographed on paper with isopropanol/aqueous ammonia/water as solvent the inhibitor was detected at R_F 0·6–0·8, corresponding to the location of the 'inhibitor β' of other workers.

Large batches of leaves from *A. pseudoplatanus* have been extracted in order to identify the inhibitor which is varying with photoperiod. The leaves were extracted with aqueous methanol (80 per cent v/v) and after removal of the methanol by vacuum distillation the aqueous extract was shaken with diethyl ether. The ether phase was separated into acid and neutral fractions and the acid fraction, which contained the inhibitor, was chromatographed on paper with isopropanol/aqueous ammonia (sp. gr. 0·880)/water (10:1:1 v/v/v) as solvent. The inhibitor was located at R_F 0·6–0·8 and the eluate was re-chromatographed on paper with *n*-butanol/aqueous ammonia (sp. gr. 0·880 × 1/100) in the proportion 5:1 (v/v) as solvent. The inhibitor ran at R_F 0·4–0·6 and was separated from several non-inhibitory phenolic compounds which ran at the same R_F as the inhibitor in isopropanol/aqueous ammonia/water.

The inhibitor was eluted from the chromatograms, developed with *n*-butanol/aqueous ammonia and reduced in volume until dry. The residue was distributed between ether and 0·1 M sodium phosphate buffer (*p*H 5·8) for 50 transfers in a Craig apparatus. Material in the aqueous layers was recovered by extraction into ether after acidification to *p*H 3. On bioassay, one fraction completely inhibited extension-growth of coleoptile sections when assayed at 50 p.p.m. and in this respect was as active as coumarin. This fraction was non-crystalline, colourless and had a distinctive odour. Ultra-violet and infra-red examinations of the fraction indicated the presence of —OH, —CO—, —CH_2— and/or —CH_3 groups, but there was no evidence of an aromatic structure. The fraction formed a precipitate when added to 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid and showed some reducing power towards hot Fehling's solution. The inhibitor was found to be unstable to acid, alkali and heat at 100° C. Biological activity was also destroyed by treatment with sodium borohydride but not by treatment with sodium periodate. Thus, there is strong evidence for the presence of an aldehyde or ketone group in the inhibitor, which could possibly be a β-hydroxy acid.

Thus it appears that inhibitor β in *A. pseudoplatanus* could be a single active compound and not an accumulation of several weakly inhibitory substances which have similar R_F values, as has been found in some other species.

Also, the fact that the inhibitor does not appear to be an aromatic compound conflicts with the suggested nature of inhibitor β in several other species of plants and plant organs. Varga[4] has extracted growth-inhibitors from several species of fleshy fruits and concludes that inhibitor β is composed of several aromatic compounds and, in particular, phenolic compounds. In *Citrus medica* L., Varga has identified three phenolic compounds which are present in eluates of inhibitor β, namely, salicylic acid, *o*-coumaric acid and either *p*-coumaric acid or ferulic acid. Ether extracts of *C. medica* L. made at Aberystwyth showed, in agreement with the results of Varga, that the inhibitor β is located at R_F 0·6–0·8 when chromatographed on paper with isopropanol/aqueous ammonia/water as solvent. After chromatography of the eluate with *n*-butanol/aqueous ammonia as solvent the inhibitor was located at R_F 0·4–0·6. This corresponds with the position of the inhibitor from *A. pseudoplatanus* in this solvent system. There were no reactions indicative of phenolic compounds in this region when chromatograms were sprayed with detecting reagents. Pure samples of the phenolic compounds mentioned by Varga were co-chromatographed on paper in *n*-butanol/aqueous ammonia with the eluate from R_F 0·6–0·8 of chromatograms of the ether extract from *C. medica* L. developed with isopropanol/aqueous ammonia/water. The R_F of the phenolic compounds did not correspond with the zone R_F 0·4–0·6 where inhibitor was detected by bioassay. Thus, the phenolic compounds mentioned by Varga are apparently not the major source of inhibition of extension-growth in coleoptile sections grown in the eluate of inhibitor β from *C. media* L.; the correspondence in the R_F values in two different solvent systems suggests that possibly the β-inhibitor in this species is related to the inhibitor of *A. pseudoplatanus*.

We thank Dr. J. W. Cornforth of 'Shell' Research, Ltd., for his helpful advice and collaboration on the chemical aspects of the work.

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FUNCTIONAL COUPLING BETWEEN CELLS IN THE VISUAL CORTEX OF THE UNRESTRAINED CAT

By Dr. J. S. GRIFFITH and Dr. G. HORN
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THE discharge of cells in the visual cortex of two conscious, dark-adapted cats was recorded[1]. The depth at which the first unit showing a resting discharge was encountered by the microelectrode was taken as zero, and all recordings were made from units between this point and another lying not more than 3 mm deeper in the line of penetration. Spikes were amplified, monitored on an oscilloscope and recorded on magnetic tape. When an experiment was completed, the material stored on the tape was displayed on a cathode ray tube. Occasionally the electrode recorded the simultaneous discharge of two or three cells (cells 1, 2 and 3). When it was possible, on the basis of differences in polarity, amplitude and/or shape for the experimenter to discriminate reliably between the two or three sets of action potentials on the oscilloscope, the discharges were filmed. Records of 8 such pairs of cells were analysed together with one record which contained the action potentials of three cells. Each length of film was carefully examined manually, and each spike was classified as belonging to cell 1, 2 or 3 and marked accordingly.

The positions of spikes on the film were then measured in the following way. A length of film was laid across a screen over which would be drawn a cursor line moving from left to right over a distance of 10 in. Movement of the cursor line was measured automatically and the distances from zero at left automatically set up on a counter. The cursor line was placed over a spike and by pressing a button the reading on the counter was punched on five level paper tape. The cursor line was moved to the next spike and the procedure repeated. Spikes were identified on the punched paper tape by a symbol according to whether they were from cell 1, 2 or 3. The upper limit of accuracy of the measuring instrument was 0·001 in., and on no occasion was the interval between successive spikes less than this. When a 10-in. length of film had been measured in this way both film and cursor were drawn to the left, the counter set to zero and a symbol indicating overflow punched on the paper tape. The distances of spikes on the succeeding 10 in. of film were then measured from the new position. This procedure was repeated until the whole reel of film had been measured. The punched paper tape was printed out and compared in detail with the films to make sure the number and general distribution of spikes was correct and that the overflow symbols were correctly inserted. The paper tapes were then fed into a digital computer (*EDSAC II*) with a programme which gave further checks on the accuracy of the punching. Finally, the tapes were subjected on the computer to the analysis described here.

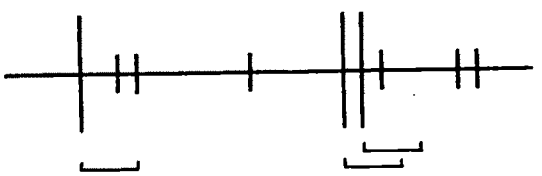


Fig. 1. Schematic diagram of a section of film containing spikes due to two cells. The square brackets show 5-msec intervals following cell 1 spikes

Statistical procedures. The object of the analysis was to see whether the firing of one of a pair of cells influenced the probability of discharge of the other. It seemed reasonable to suppose that this influence, if it were present, would last for a period of 10 or perhaps 100 msec. Accordingly we proceeded in the following way. Each time there was a spike due to cell 1 the number x_2 (0, 5) of spikes due to cell 2 in the next 5 msec was recorded. All these numbers were added together to give the total number X_2 (0, 5) of spikes of cell 2 which occurred within 5 msec after a spike of cell 1. In Fig. 1 three cell 1 spikes are shown together with their associated 5 msec intervals. The three corresponding values of x_2 (0, 5) are 2, 1 and 1 so that X_2 (0, 5) = 4. It will be noticed that the cell 2 spike which occurs in both the two overlapping intervals gives two contributions to X_2 (0, 5). Calculating the totals in this way is convenient because it then becomes easy to calculate the expected value of X_2 (0, 5).

(*Continued on page 893*)

BRITISH ASSOCIATION MEETING IN ABERDEEN

INVESTMENT IN MAN

By SIR ERIC ASHBY, F.R.S.
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President of the British Association

1. Introduction

TWO hundred and seventy years ago James Blair, a graduate from Aberdeen and Edinburgh, secured a royal charter for the College of William and Mary in Virginia. Apart from Harvard, it was the only other place of higher education to be founded in North America in the seventeenth century. Blair became its first president. Its curriculum and statutes embodied the Scots tradition. This is but one example of the deep and lasting influence which Aberdeen and its University have had on the advancement of learning. Not only Virginia, but Pennsylvania, too, is in debt to Aberdeen for a heritage of higher education, for another Aberdeen graduate, William Smith, made an enduring contribution to the New World when he introduced in Philadelphia, as long ago as 1756, a curriculum comprising one-third classical studies, one-third science and technology, and one-third social sciences: a mixture which would nowadays be considered novel, but which was to be found in Marischal College two centuries ago. And here in Britain science and scholarship have been greatly enriched by men who have taught or studied in Aberdeen: physics by Clerk Maxwell, medicine by Patrick Manson, mathematics by George Chrystal, biology by Arthur Thomson and Chalmers Mitchell. There is no corner of the Commonwealth which has not been illuminated by the intellectual life of this City of Aberdeen.

This is the fourth occasion on which the City and University have acted as hosts to the British Association, and it is a great pleasure to begin—as my distinguished predecessors in Aberdeen began—by thanking the Lord Provost and the Vice-Chancellor, and all who have helped them, for inviting us here and for making such masterly arrangements for this meeting. For months people in this City have given up their energy and leisure to prepare for this week; at times they must have wondered whether these annual meetings are still necessary, and have asked themselves whether the aims of the British Association were not accomplished, and its mission completed, a generation ago. Anyone still assailed by such doubts as these should turn to the presidential address given here in 1885 by Lyon Playfair. Indeed, you would be well served on this occasion if I were simply to read Playfair's speech to you. Most of it is still as apposite as when it was written and it is composed with a wit and eloquence rarely equalled in presidential addresses to this Association. His mordant criticisms of the failure of Parliament to invest in science and education; his piercing comparisons between the financial support given to universities by governments in Britain and abroad; his advocacy of non-vocational teaching (illuminated by an epigram from Epictetus: "though sheep eat grass, it is wool which grows upon their backs"); his plea for research in universities; and his stirring conclusion ("the competition of the world has become a competition of intellect"): all these things still need to be said and have never been better said.

2. Investment in Man

But it is my duty to spin you something new, and I have a clear lead from last year's presidential address. Sir John Cockcroft spoke of "Investment in Science". The prerequisites for investment in science are, first, scientists, and second, a public opinion sufficiently well informed and enlightened to give financial support to scientists. Both these prerequisites are acquired through education. To invest in science you have first to invest in man.

Alfred Marshall once wrote that the most valuable of all capital is that invested in human beings. This is my theme. The advancement of science has its roots in the educational process. We have abundant evidence that our present investment in science is restricted by our educational policies over the past generation: you have only to study the pages of advertised vacancies for scientists and technologists to realize the extent of the restriction. Regarded in its lowest terms, investment in man brings high economic rewards; and in its highest terms, investment in man is a moral obligation which the State owes to its citizens. More than a century ago, in 1858, the Newcastle Commission on the State of Popular Education in England was told that "it is quite possible to teach a child soundly and thoroughly . . . all that is necessary for him to possess in the shape of intellectual attainment, by the time he is ten years old". We now invest more wisely than that in the young people of Britain but still not wisely enough.

Investment in man covers all kinds of education from primary school to the training of research workers. Even if we confine ourselves, as is proper on this occasion, to investment in man for the advancement of science, we are still left with too large a canvas. For you cannot isolate science. It is part of the seamless fabric of civilization. The advancement of science depends not only on the training of professional scientists; it depends also on the public image of science, and this is a product of the machinery which weaves science into contemporary culture; scientific journalism, science fiction, and communication of ideas about science through radio and film and television.

3. Resources of Ability

Having spread this scene before you I now ask you to follow me into one corner of it, to study it in more detail. Let us consider investment in man at the level of higher education alone. Within these arbitrary and narrow

limits, I shall discuss three questions: (1) What are the resources of ability for this sector of investment in man? (2) What policies and principles guide investment in these human resources? (3) What is the state of our knowledge about the social institutions we use as instruments for this sector of investment?

In 1962 some 113,000 boys and girls in Britain completed a course of secondary education with sufficient success to qualify them for some form of full-time higher education. They represent only about 14·5 per cent of the age-group, and of these only about half meet the requirements for entering a university. What has happened to the rest? Some 26,000 of them embarked on a grammar school course but left before taking the General Certificate of Education examination at the Ordinary Level or the Scottish Leaving Certificate, and some 474,000 of them left school before they reached the age of sixteen. Of course, the education of early leavers is not necessarily over. Through further education schemes, apprenticeship with day release, and the like, both government and industry continue to invest in many of them. But apart from a very few, who later on will break away from work to go to college, these young people will not receive any further full-time education.

Let us for a moment concentrate on the human resources which are available for full-time higher education. What principles guide our investment policy for these boys and girls?

The facts are these. Last autumn about 64,500 of them enrolled for the first time as full-time students in universities and various kinds of colleges (about 30,200 in universities, about 15,500 in colleges of technology, schools of art and commerce and similar places, and about 18,800 in teacher-training colleges). This is only about 55 per cent of those qualified for higher education, drawn from the already depleted human resources in this age-group in Britain. Let me put the figures into perspective in this way. Consider five children born in 1943: an American, a Canadian, an Australian, a Russian and a British child. There is about a one-in-three chance that the American is now receiving a full-time higher education. The equivalent chance for the Canadian is one-in-six, for the Australian, one in nine, for the Russian, one-in-twelve, and for the British child—but only if one includes all those taking sandwich courses—also one-in-twelve[1].

No one supposes that these ratios represent differences in resources of ability between these countries. What, then, do they represent? They represent differences in policy for investment in man. These comparative figures are often used to support indignant arguments about the inadequacy of provision for higher education in Britain. They are quite unreliable for this purpose. They omit students enrolled in part-time courses, which are an integral and valuable part of our system; they omit the population of pupils in sixth forms, who are doing work equivalent to first-year university work in Australia, Canada and the United States; and they omit any consideration of failure rates. Thus, some figures were published recently comparing university enrolments in a dozen European countries, together with Canada and the United States. When the data are calculated as enrolments per age group, Britain is eighth on the list out of twelve, and comes below such European countries as Belgium, France, Greece and Sweden. But when the data are calculated as the number of first degrees (per median age of taking a degree) Britain rises to fourth place on the list. There is a simple reason for this: it is that the 'efficiency' of a British university (in deference to dons I put the word in inverted commas) measured by the number of degrees awarded compared with the number of students who enrol in the hope of getting degrees, is about 85 per cent in Britain, whereas the 'efficiency' of most universities abroad is much lower: in Canada the corresponding percentage is 66, in the United States it is somewhere between 50 and 60, and in Australia it is less than 60 (ref. 2). This striking superiority in 'efficiency' of British universities introduces the next point in my argument, which concerns the policies and principles which guide our investment in human resources.

4. Policy for Investment

The American and British systems illustrate two fundamentally different approaches to investment in man. The Americans have an open door to higher education. Anyone who has completed a high-school course may claim admission to some university or other. The degree course is an obstacle race open to all competitors who care to enter it; the competitors can even choose whether to enter for difficult races with formidable obstacles, by going to universities of international standing, or to enter for easier races with less-formidable obstacles by going to universities of more modest prestige. There are prizes, in the form of degrees, for more than half the competitors; and the race is not always to the swift: the keen, persistent dogged tortoise, provided he surmounts the obstacles, finds himself a prize-winner. The policy of investment is to maximize the human resources put into the machinery for investment. Even so, not all American talent by any means is invested in higher education. It has been estimated that in the 1950's even at the highest level of intelligence—the top 0·1 per cent of ability-level—only two out of three Americans graduate from college[3].

In Britain we follow an entirely different policy over investment in man. By the age of twelve the door to full-time higher education is all but closed to 80 out of 100 children. The remaining 20 are selected for specialized privileged schooling which brings them to the gates of colleges and universities, but only about 8 out of the 20 get in. We rigidly select a small group of young people and sponsor this group through a heavily subsidized education of very high quality under very good conditions.

British policy for higher education is tenable only on certain assumptions. The first assumption is that the techniques for selection are efficient, reliable and satisfy social justice. The second assumption is that the numbers of young people selected each year for different kinds of full-time higher education are sufficient for the nation's needs. The third assumption is that we offer acceptable opportunities for part-time further education to those who are not selected. None of these assumptions is justified. Our methods of selection assume that our intellectual resources are limited by genetic factors, and that when we select candidates to go to grammar schools or to universities we are drawing from the population those with the innate ability to profit from these privileged kinds of education.

Of course, the intellectual resources in a population are ultimately limited by its genetic make-up. But we have abundant evidence that it is not genetics, but inequalities in our society and inadequacies in our educational system, which at present limit our investment in man; and this is true even in the most affluent nations. There is now convincing evidence that thousands of children fall out of our educational system each year due not to lack of ability, but to lack of motive, and incentive and opportunity[4]. A child who succeeds in climbing the ladder of education has responded to a challenge. If there is no challenge there can be no response; and our machinery for selection, unlike that in many countries, is in fact a machinery for distributing challenges. The trouble is that no refinement of our methods of selection will solve the problem which confronts us; for the problem we set ourselves is not to select from among those who leave school the candidates who are likely to profit from higher education, it is to select from among those likely to profit from higher education the candidates lucky enough to be offered it: which is quite a different thing.

In these circumstances it is no adequate test of our methods of selection to follow the success or failure of

those we select; we must also follow the success or failure of those we reject. On the one or two occasions when this has been done we find what indeed we might have expected: that the majority of those rejected by one university got into another, or took degrees externally, and (in such statistical studies as have been made) the chance of academic success among the rejected candidates was no worse than that among the accepted candidates[5]. There is, as one might expect, a general correlation between success at school-leaving examinations and success in degree examinations. This is useful information for statistical purposes, for example, for calculations of the potential size of the university population in Britain; but, of course, it gives no reliable guide to the decision the admissions officer has to make: shall I admit John Brown or Peter Smith? It is ironical that the one feature of the investment of man in higher education which we have studied scientifically leads merely to the conclusion that we could invest a much larger proportion of the age-group than we do.

Three facts confirm this view. In 1952, about 20,000 freshmen entered British universities to read for first degrees; 80·6 per cent of them were successful. Five years later, in 1957, the number of freshmen entering British universities increased by about a third, to 27,000. Some people even then talked of a dilution of quality among students, about 'scraping the bottom of the barrel' but the proportion of this generation of freshmen who successfully graduated did not fall: it rose to 82·8 per cent. The second fact is that the combined percentage of 'good honours degrees' (first and upper seconds) rose from 18·6 per cent in 1952 to 21·4 per cent in 1958, although numbers of students graduating over that period also rose by 6 per cent. The third fact is that over the past five years (1957–62) the number of new Department of Scientific and Industrial Research studentships awarded (for research-training in science and technology) rose by 85 per cent, from 649 to 1,194; but the percentage of 'good honours degrees' among these new award holders remained exactly the same: 81·7 per cent.

We are uneasy in our search for talent. We spend a great deal of time and energy over the problems of selection and we are tempted to believe that there must be a 'right' way to select students for admission to universities, if only we could discover it. Of course there is no right way and it is vain to seek one. It is our policy for investment in man which leads us into this Calvinism of the intellect. "Every selection", said the German philosopher Jaspers, "is in some way an injustice. We delude ourselves when we think that we can avoid such injustice through rational and determined effort".

So much for the first assumption underlying our policy for investment in man. The second assumption, that our system of higher education is on a big enough scale to meet our future needs for scientists and technologists, is untenable in the light of recent studies of manpower requirements[6]. The third assumption, that there are acceptable alternatives for those who are not selected for full-time higher education, does need comment. There is nothing inherently unsound—indeed there are great merits—in systems of part-time further education integrated with employment. The trouble about these systems in Britain is their inefficiency. We can well be satisfied with a system of university education which, through the use of external examiners, secures a common standard for all first degrees in all British universities, and an 85 per cent chance that a student will reach this common standard. The worm which gnaws at our satisfaction is that we have worked out a successful solution to this problem of quality control at the expense of a far greater problem which we have shirked: the problem of how to invest efficiently in the rest of our resources of talent.

While our wastage rate in universities is commendably low, we cannot forget the heavy casualties among young people who do not get full-time higher education and who try to qualify themselves through night classes, day release and correspondence courses. If you question this, study the examination statistics for Ordinary National Certificates (where the failure rate is said to be 50 per cent: in one London technical college 42 per cent of the evening students working for National Certificate dropped out in their first year), and the examination statistics for bodies which award professional qualifications in accountancy, surveying, insurance, and the like; even the professional qualifications offered by the Royal Colleges of Physicians and Surgeons. Wastage rates such as these are not the fault of the colleges for further education. They do their best to teach well. They are the fault of our social system which denies to the part-time student an opportunity to learn well.

When I say that our national policy for higher education is based on untenable assumptions I do not advocate, as some reformers do, a huge proliferation of universities in Britain. An indiscriminate inflation of the volume of investment in man would certainly not pay off. We need to know far more than we do about the pattern of higher education which is likely to be relevant to the lives graduates are going to live. If there were more university places in Britain, there is no doubt that more students would go to universities and that they would get degrees. The problem is whether—given our present curricula and teaching methods and pattern of degrees—this would be in the best interests either of the nation or of the students themselves. We cannot dissociate any increase in volume of investment in man from change in the pattern of investment[7].

This brings me to the central theme of this address: the university as a social instrument for investment in man. There are of course other social instruments which contribute to the same end. By singling out universities I do not imply that other instruments are less important; it is just that we penetrate more deeply if we focus on a small part of the field.

5. Ecology of Universities

The ecology of universities is remarkable: they reach back to the Middle Ages and they girdle the Earth. From the plains of Lombardy they have been transplanted to the grey climates of northern Europe, to the African bush, to American cities. They have invaded the ancient civilizations of Egypt and India and driven out institutions deeply rooted in the indigenous culture. They have adapted themselves to totalitarian and to democratic societies, to rural communities and to urban technologies. But through time and space they have preserved something resembling a genetic identity: they remain unique as instruments for investment in man. I cannot define this identity but I can describe it from its products. At their best, universities endow the men and women who pass through them with a characteristic intellectual equipment: it is the capacity to reconcile orthodoxy and dissent. Let me explain: the intellectual life demands a respect for what has gone before and acceptance of a rigorous discipline to a tradition of learning. To this extent universities are a point of stability in society: they anchor a society to its own past. But orthodoxy is celibate; it breeds no fresh ideas; unless tradition is continually re-examined, it becomes oppressive. So, in the course of their evolution, universities have learnt not only to pass on a corpus of knowledge and ideas, but also to train students to disclose errors in knowledge and to question ideas. Some words Michael Polanyi recently wrote about science can be applied to all the activities of a university:

> "The professional standard of science must impose a framework of discipline and at the same time encourage rebellion against it . . . This dual function . . . is but the logical outcome of the belief that scientific truth is an aspect of reality and that the orthodoxy of science is taught as a guide that should

enable the novice eventually to make his own contacts with this reality . . . The capacity to renew itself by evoking and assimilating opposition to itself appears to be logically inherent in the sources of the authority wielded by scientific orthodoxy"[8].

To train young people in this dialectic between orthodoxy and dissent is the unique contribution which universities make to society. Any change in the pattern of universities which endangers this function endangers the society which the university serves. It is for this reason that universities have to combine teaching with research. It is for this reason that they must endeavour to include among their students those who have the combination of temperament and intellect to profit from this particular approach to learning. But universities could not stay in business if they concentrated on nothing but this unique function; indeed this function arises as a by-product of a bread-and-butter job of training men and women for the professions. It is for their bread-and-butter service that the public is willing to invest money in them, and it is on their discharge of this service that their efficiency is judged.

6. Lack of Research on Education

The very word 'efficiency' applied to universities is anathema to some academic men. But of course there is no reason why our instruments for investment in man should be immune from the criteria of efficiency any more than our instruments for government or for the administration of justice are immune. Nor is there any reason why we should regard research into the production of educated people as any less important that research into the production of steel or artificial fibres. But we shy away from the idea of education as a technology. The techniques of education in Britain have scarcely been touched by the scientific revolution. This is particularly true in universities: although dedicated to the pursuit of knowledge, they are reluctant to pursue knowledge about themselves. Despite the fact that the annual expenditure on universities in Britain exceeds £70 millions, expenditure on studying the processes of teaching and learning at university level is negligible. Indeed, this distaste for research extends over the whole field of education. Despite recent grants by the Ministry of Education and the work being done by the National Foundation for Educational Research, and the imaginative projects being supported by the Nuffield Foundation, the fact remains that the proportion of expenditure on research in education to the annual expenditure on education in Britain is less than one-twentieth of a penny in the pound. Any industry which devoted so little to research and development would collapse.

Accordingly, when we come to ask whether our universities are appropriate instruments for investment in man, we lack the data to provide an answer. Each question we ask leads us into untested assumptions. To test whether the assumptions are valid we need to undertake a sustained programme of research on British higher education. Until this research is done the questions remain unanswered.

7. Utilization of Teachers and Buildings

The prime question concerns the future of the academic profession. Britain is already committed to a large expansion of higher education. There is already widespread criticism that the scale of expansion is far too small. For the next twenty years we know where the students are coming from and we have good reason to believe that there will be jobs awaiting them, in Britain or overseas, when they graduate. We know how to put up buildings to accommodate the increased numbers. What we do not know is where the teachers are coming from. Even on our present over-modest calculations for the universities alone (and they account for only about half the traffic in higher education) it is estimated that some 8,000 more teachers will be required, over and above replacements. Prophecy is dangerous, and I may well be proved wrong; but in some subjects—especially in mathematics, science, and technology—there is good reason to anticipate either a decline in quality of university teachers or a shortage in quantity[9].

It would seem prudent under these conditions to do some operational research on teaching and learning in universities. A convenient starting point is a phrase frequently heard nowadays: 'the burden of teaching'. You hear this more commonly than you hear doctors refer to the burden of healing or lawyers to the burden of litigation. The burden of teaching is measured by a mystic number called the staff–student ratio. This has its origin in the Talmud. Two millennia ago the Rabbi Raba established the rule "25 students are to be enrolled in one class. If there are from 25 to 40 an assistant must be obtained. Above 40, two teachers are to be engaged". One might have thought that with the advent of printing, the film, closed-circuit television, and the tape recorder, this ratio would have diminished. But in British universities it has increased to about one teacher to ten students—a higher ratio than is to be found to any other European country or in the United States. It is taboo in the academic profession to contemplate any diminution in this ratio. It must be emphasized that the cause of the higher ratio is the smallness of the denominator rather than largeness of the numerator: it is another consequence of the British policy for investment in man. So, as we change this policy by admitting larger numbers to higher education, we face a dilemma, in any event in mathematics, science and technology: Is it better to retain at all costs the quality of university teachers, even if it means less teaching per student and an increase in the staff–student ratio ? Or is it better at all costs to maintain the present staff–student ratio, even if it means the recruitment of some mediocre teachers, so that (as one American university president put it): "the teacher can communicate his mediocrity in an intimate environment" ?

The rational way to pursue this question is to anatomize the working day of a university teacher. According to estimates made by the University Grants Committee, nearly half his working time is spent in research. Let us concede straight away that this time must not be eroded; for although a lot of research is trivial and is (I suspect) done for reasons other than an enthusiasm for the advancement of knowledge, yet this is the seed-bed for creative work and it must be left undisturbed. The balance of his time spent in administration and teaching. Good management can always minimize administration. The difficult question is whether the techniques of learning and teaching in universities could be improved. There is evidence that they could. For some years the Fund for the Advancement of Education in the United States has promoted a massive and sustained programme of operational research on teaching and learning in American universities[10]. The results of this research, together with such fragments of work as have been published in Britain[11], are sufficient to dispel any complacency we may have about the efficacy of our teaching methods, and they make nonsense of any doctrinaire assertions about staff–student ratios.

Research on learning and teaching is notoriously difficult to interpret, for students are astonishingly resilient and adaptable creatures. Give them less teaching, and they compensate by working harder on their own. Introduce some novelty, such as teaching by closed-circuit television, and they may respond favourably not because it is a better method of teaching but because it is a novelty. However, when allowance is made for all these sources of error, the results of research indicate beyond doubt certain simple conclusions about university teaching. The first is that, for formal lectures, large classes are better than small ones, if only for the simple reason that the lecturer takes more trouble to prepare them. As

everyone who has to give lectures knows perfectly well, if he has to address 500 people, and especially if his address is to be televised or filmed, he is, as it were, raised to a temperature of excitement which makes for a better performance.

Secondly, as one would expect, the experiments confirm the value of teaching by discussion in small groups. But what size of groups ? The amount of teaching to be done is increased four-fold if tutorials are taken in groups of three instead of in groups of twelve. Is there any evidence that teaching is always more effective if the groups are very small ? There is not. Indeed, such little evidence as we have indicates that for some subjects a seminar of twelve is more effective than a tutorial of three. Thirdly, there is convincing evidence that students do better in examinations if they are left to work on their own and are not over-taught. This is a truism; three centuries ago Comenius wrote that his object was "to seek and find a method by which the teachers teach less and learners learn more". The novelty is that the methods known to Comenius can now be improved. If students working on their own have access to audio-visual aids they can cover, at their own choice of time and at their own pace of working, the more didactic parts of a course with very little formal teaching.

Fourthly, there is a growing body of evidence—enough now to justify very serious attention—that modern techniques of instruction such as language laboratories, films, taped discussions and (for certain parts of certain subjects) programmed instruction, are useful for some teaching even at university level. These technical devices save the teacher time which he can use more effectively in the intimate encounter of discussion which no modern technique can replace. Far from dehumanizing and mechanizing instruction (a charge frequently brought against them) these modern devices allow the individual to work on his own, at his own pace: they actually encourage individuality. Programmed instruction, for example, which some people ridicule as an instrument for university teaching, is merely a return to the Socratic method (even though the content is not Socratic): the student cannot remain passive; he takes part in a dialogue which makes continuous demands on the learner and evokes a continuous response from the teacher[12].

The introduction of technology into teaching and learning at university level evokes such emotional reactions that it is difficult to persuade some people to contemplate it objectively. Of course, gadgets cannot replace the teacher when he is really engaged in education and not just instruction, for in teaching at the highest level there has to be a feed-back from students to teacher, to which the teaching is constantly adjusting itself. But a good deal even of university teaching is at a lower level than this, and the aim of technology should be to relieve university teachers of this more didactic instruction. Professors once had to reconcile themselves to the invention of printing, which enabled the solitary student, alone in his room with a book, to confront the great teacher through the printed word. It is known that some of them protested, just as, centuries earlier, teachers had protested against writing as a substitute for oral tradition. Now, after nearly 500 years without much change in the technology of higher education, professors will undoubtedly have to reconcile themselves to new inventions, which will enable the student to confront the great teacher in a new and vivid way. Of course, we witness only the rudimentary stages of this transition. We are at that awkward phase when the techniques follow the old vestigial patterns, just as the first written instruction still retained the form of dialogue and the first printing resembled manuscript written with a quill pen. Under the impact of research into the techniques of teaching and learning the whole pattern of education is likely to change. Just as the good teacher of to-day is complementary to the book and the laboratory, so the good teacher of to-morrow will be complementary to the lecture by television, to the voice recorded on tape, and to the scientifically planned programmes for didactic instruction.

The technology of teaching and learning is only one point at which there is need to examine our equipment for investment in man. Another point is the efficiency with which we use the capital spent on universities. It has been estimated that the capital which a university invests, on buildings and equipment alone, for a science student is £2,400, and for a student in technology is £3,500. The bulk of this expenditure is on laboratories.

What assumptions are made in designing these laboratories ? First, that the undergraduate population in the university will not be using them for more than about 26 weeks in the year; secondly, that the programme of practical classes will be so arranged that all, or most, of the students in a course will be doing practical work at the same time; an elementary laboratory, therefore, may be empty for all but two or three afternoons a week in term time. Could the capital which is locked up in this way in the scientific laboratories of British universities be put to better use ? And should we continue to build scientific laboratories to the pattern traditional in British universities ? We simply do not know. What we do know is that research into the utilization of laboratories would be worth doing, for it has been done elsewhere and the results are instructive. A recent analysis in the United States showed that among eighteen different institutions of higher education there was a difference of 400 per cent between the lowest and highest use made of classrooms and laboratories and that in some institutions a rearrangement of the time-table to spread use evenly through the week could have led to very great savings in the capital investment per student educated[13]. A report, published in January 1963 [14], describes how forty institutions of higher education in the United States, ranging from liberal arts colleges to universities with enrolments of more than 15,000, have rearranged their academic years to increase the use they make of their buildings and equipment. Their main purpose in doing this has been to increase the rate of flow of students through higher education.

8. Curriculum

We have so far been discussing the efficiency of the university as an instrument for investment in human resources, and we have dealt with matters which lend themselves to simple statistical and experimental techniques. But to consider only problems susceptible of measurement would be to shirk the really difficult questions about universities, which concern matters which cannot be measured. The assumptions we make about the teleology of a university—its function—are just as much in need of scrutiny as the assumptions we make about its operation. It is at this point we confront the most difficult question of all when we consider universities as instruments for investment in man: What do we want our system of higher education to do for the people we put through it ?

The chief assumption we make about this question is evident from the pattern of courses in English civic universities. In most of them the great majority of students in arts and science are reading for special or honours degrees. This pattern presupposes one of two assumptions: either that these students are going to become professional scientists and scholars, or it presupposes that the training appropriate for an academic is the best training to give students who are not going to become professional scientists and scholars. The second assumption may well be correct, but at present we hold it only as an article of faith; and so it will remain until we know more about the sociology of professional life. The first assumption is easily disproved. In 1959 and 1960, for example, no fewer than 8,441 students graduated in one or other of the single honours subjects: history,

classics, English and modern languages. Of these only 739—about 8 per cent—are reported as having gone on to research and advanced study. Was this intensive training, largely in a single arts subject, the best way to invest in the other 92 per cent of these graduates? In science and technology a larger proportion—about 20 per cent—are reported as having gone on to research and advanced study, and many of the others went into posts where they were required to do scientific work. But again one asks whether the intensive training, especially the long period spent in the laboratory learning practical techniques, was the best way to invest in the 80 per cent or so of graduates in science and technology who did not go on to high-level academic research. It is a system we copied from Germany in the 1860's. Whether it is still the best way to invest the time of a British student in the 1960's we do not know.

We make untested assumptions not only about the pattern of courses, but also about their content. Here is one example. All but the most deeply entrenched pedants agree that education should be relevant to society. It is for that reason that medieval students read theology and renaissance students read Greek. If we begin to catalogue the characteristics of our own age, we soon find that among the outstanding features of modern life is what Peter Drucker has called "application of systematic knowledge to work". This activity is now part even of our cultural atmosphere, invisible because of its very familiarity. People engaged in mass communication, from the advertising agent who writes a toothpaste advertisement or who boosts a new car model, to the scientist broadcasting on the causes of lung cancer or the technology of rockets, assume that their words will be more convincing if supported by scientific evidence, graphs, statistics, data. This—much more than household gadgets and transistor radios—is technology diffusing into the common culture. But education has not yet accepted technology as a form of humanism. The idea that there is no point in learning history unless you are going to become a historian would be treated (and rightly so) with contempt. But the idea that there is no point in learning technology unless you are going to become a technologist still seems perfectly natural. In fact this idea is just as deserving of contempt, for already no man can regard himself as adequately educated who does not understand something of the principles of technology: by which I mean the art and science of the application of systematic knowledge to work.

9. Obsolescence

Finally, there is one more assumption built into our present policy for investment in man which is of critical importance. Education (as Charles Carter said recently) "is not an act of salvation, after which one is safe for eternity". Yet we assume it is: in particular, that a three-year undergraduate course is sufficient to set a man up for a lifetime. Doubtless in the nineteenth century a three-year course in classics did set a man up for a lifetime; but that was in the days when a graduate could assume that he would grow old in a world familiar to him as a youth. We are living in the first era for which this assumption is false, and we have not yet faced the consequences of this fact. The present generation of students will still be employed in the year 2000; but long before then their degrees and diplomas—in any event in science, technology, and the social sciences—will have become obsolete. The only students who can be sure of escaping obsolescence are those very few who will themselves become innovators. Our investment in the rest—the great majority of our human resources—will inevitably be devalued by technological and social change. Measures to combat obsolescence, therefore, become of prime importance.

What measures have already been taken? There is a plentiful supply of scientific and professional journals. But this is not enough: re-education, like education, requires challenge and response, an encounter between teacher and student. Refresher courses offer this, but the extent to which they are used, and the scope and duration of most of them, are pitifully inadequate to meet the need. Let me give you some examples. The Ministry of Education, and many other bodies, arrange short courses for teachers, covering such topics as mathematics, science and audio-visual aids for the teaching of modern languages. These courses are very valuable, but most of them last only for 4–7 days. There are some one-year full-time courses of advanced study, but during 1961–62 they were attended by fewer than 400 teachers[15]. There is no evidence that any educational institution is planning to offer sustained courses of re-education on a scale massive enough to prevent widespread obsolescence among teachers.

In technology the story is similar. Some universities and colleges put on short courses of re-education for technologists, but by no stretch of imagination could these courses be regarded as a comprehensive system to prevent obsolescence[16]. In the training of physicists and chemists there is no sign that any provision is to be made. For medicine the University of London has set up the British Postgraduate Medical Federation, which last year offered courses for general practitioners attended by more than 2,000 doctors; but most of these courses were only for week-ends or single weeks; and again they cannot meet the need for sustained and systematic re-education to prevent obsolescence in the medical profession[17].

So this is how we stand. We already spent large sums, even if they are inadequate, on higher education in Britain. It is probable that we shall be asked to spend a great deal more, in a massive programme for investment in man. A large proportion of this expenditure will be devoted to science and technology. But this investment in man will inevitably depreciate as the volume of effort in science and technology increases throughout the world. There is need for drastic steps to prevent obsolescence. Here is an opportunity for the extra-mural departments of our universities (which are, in any event, seeking for a new inspiration).

Of course universities and colleges cannot alone sponsor a programme to combat obsolescence. There will have to be co-operation from all forms of employers, releasing men on full pay for sustained full-time courses every few years. Perhaps the time may come when a degree or a professional qualification, like a passport, is valid only for a limited number of years, and is renewed only after attendance at a systematic course of re-education. The task of higher education is (in the words of the President of the Carnegie Corporation) to provide a "framework within which continuous innovation, renewal and rebirth can occur". In the disturbing, storm-swept, feverish thirty-seven years remaining to this century, nothing less than this will suffice for investment in man.

I thank Dr. Alvin C. Eurich and Dr. A. H. Halsey who read this address in draft and made many valuable comments on it.

[1] Compiled from information kindly provided by the Secretariat of the Committee on Higher Education and the Canadian Universities Foundation; also from *University Statistics*, 1960 (Commonwealth Bureau of Census and Statistics, Canberra); and de Witt, N., *Education and Professional Employment in U.S.S.R.* (National Science Foundation, 1961).

[2] Compiled from information kindly provided by the Canadian Universities Foundation, and calculated from statistics published by the University Grants Committee and the Australian Commonwealth Bureau of Census and Statistics. The American figure is quoted from Halsey, A. H., Floud, J., and Anderson, C. A., *Education, Economy and Society*, 131, Glencoe, 1961), supplemented by information supplied by A. H. Halsey.

[3] *Education, Economy, and Society*, 222.

[4] Floud, Jean, in *Ability and Educational Opportunity* (edit by Halsey, A. H., 91 (Organization for Economic Co-operation and Development, 1961); also *Early Leaving* (H.M.S.O., 1954); Floud, Jean, and Halsey, A. H., *Brit. J. Sociology*, 8, 33 (1957).

[5] Furneaux, W. D., *The Chosen Few* (Oxford, 1961). Kelsall, R. K., *Sociol. Rev. Mono.*, 7 (in the press).

[6] *Scientific and Engineering Manpower in Great Britain*, 1959, Cmnd. 902 (H.M.S.O., 1959).

[7] Sheer increase in amount of education is not necessarily worthwhile. Thus, Fritz Machlup (*The Production and Distribution of Knowledge in the United States*,, Princeton, 1962) demonstrates how, by increasing the efficiency of American schooling, the amount a child learns between the ages of 6 and 18 could be learnt between the ages of 5 and 15, and that an accelerated programme which brought the school-leaving age down from 18 to 15 might save the United States 10,000 million dollars a year.

[8] Polanyi, M., "The Republic of Science", *Minerva*, 1, 59 (1962).

[9] This assertion is open to challenge but it is supported by several pieces of evidence, for example: (*a*) in 1960, when an increase in student-numbers to 170,000 was contemplated, the President of the London Mathematical Association at the time calculated that 286 new entrants to the academic profession in mathematics would be required, and that the estimated number available was 14 a year; it might therefore take 20 years to accomplish this recruitment. (*b*) It used to be assumed that by and large a recruit to the academic profession should have a first-class honours degree. About 7 per cent of honours graduates are awarded first-class honours. This is at present an annual output of a little more than 1,000. The need for recruits into the academic profession, excluding replacements, is of the order of 8,000 if student numbers are to rise to 150,0000.

[10] This work is summarized in two publications by the Fund for the Advancement of Education, namely, *Decade of Experiment*, 1951–61 (1961) and *Better Utilisation of College Resources* (1959). See also *Ann. Conf. Higher Educ.* (University of Michigan Official Publ. 61, No. 80, 1959).

[11] Cottrell, T. L., *Univ. Edinburgh Gazette*, No. 33, 20 (1962). Joyce, C. R. B., and Weatherall, M., *Lancet*, ii, 402 (1957); i, 568 (1959).

[12] For the results of experiments on some of these techniques: see Green, E. J., *The Learning Process and Programmed Instruction* (1963). Kay, H., *Research and Experiment in Education*, edit. by Tuck, J. P. (Durham, 1961). Lumsdaine, A. A., *Student Response in Programmed Instruction* (1961). Lumsdaine, A. A., and Glaser, R., *Teaching Machines and Programmed Learning* (1960). Schramm, W., *Programmed Instruction Today and Tomorrow* (1962). Stolurow, L. M., *Teaching by Machine* (1961). Fund for the Advancement of Education, *Teaching by Television* (1961). For a general discussion, see Ong, W. J., *College English*, 245 (1960). There are encouraging signs of a change of heart in some British universities over research into university education. In 1963 the University of Lancaster appointed a Research Fellow in Higher Education, and the University of Essex decided to establish a Research Unit for Higher Education.

[13] *California and Western Conference Cost and Statistical Study* (Univ. Calif., 1956).

[14] Tickton, S. G., *The Year-round Campus Catches on* (Fund for Advancement of Education, 1963).

[15] Ministry of Education, *Statistics of Education*, part 2, 72 (1961).

[16] Sir Peter Venables has been kind enough to give me a breakdown of the refresher courses offered by 67 colleges, including colleges of advanced technology and regional colleges. It is as follows:

Duration	*Courses*
2–7 days	169
2–3 weeks	34
4–6 weeks	18
8–10 weeks	8
3 months	2
6 months	1
9 months	3

Under existing regulations of the Ministry of Education a teacher may be seconded to industry full time for 6 months on full salary, but very little advantage has been taken of this, probably owing to the severe shortage of teachers.

[17] British Postgraduate Medical Federation. *Handbook for the Session* 1962–63 and *Report for* 1961–62 (1962).

SUMMARIES OF ADDRESSES OF PRESIDENTS OF SECTIONS

HIGH-VOLTAGE INSULATION

DR. J. S. FORREST chooses for the subject of his presidential address to Section A (Mathematics and Physics) "High-voltage Insulation".

Insulating materials for high voltages may take the form of a gas, a solid, or a liquid, or a combination of these.

The gas commonly used is air at atmospheric pressure, and by spacing the conductors far enough apart, as in power transmission lines, very high voltages can be insulated. In a uniform electric field the breakdown strength of air is 30 kV/cm, but in practice, especially at large spacings between conductors, the field is usually far from uniform and the breakdown voltage gradient is reduced, for example, to 6 kV/cm for gaps of several metres. For very long gaps, as in lightning, the average gradient is only 1–3 kV/cm. It is interesting to note that the whole range of breakdown voltage of air, from short sparks in the laboratory to lightning flashes of several kilometres in length can be broadly represented by a simple power law:

$$V = 0{\cdot}7\, d^{0{\cdot}85}$$

where V is the peak breakdown voltage in megavolts and d the sparking distance in metres.

If the gas pressure is increased the breakdown voltage increases, reaching a value of 300 kV/cm for nitrogen at a pressure of 315 lb./in.^{2}. With certain electronegative gases, still higher values can be attained; for example, 800 kV/cm for sulphur hexafluoride at a pressure of 215 lb./in.^{2}.

Solid insulating materials will withstand even higher voltages, and, under carefully controlled laboratory conditions, figures of about 8,000 kV/cm have been recorded for the puncture strength of mica. Breakdown in solids may, however, occur at very much lower gradients due to relatively slow processes such as thermal instability and discharge erosion. The breakdown strength of solids is very much less over the surface than through the solid itself, and the surface failure of insulation is much the most frequent cause of trouble in practice. If the surface of the solid is contaminated by moisture and conducting deposits, the breakdown strength is drastically reduced. With porcelain or glass insulation such surface deposits give rise to a very irregular voltage distribution over the insulator, and breakdown, due to surface discharges, may occur at average gradients of only 0·2 kV/cm. With organic insulation, these surface discharges may decompose the material and cause permanent damage. Sometimes the damage takes the form of a conducting path of carbon progressively laid down by the discharges—a phenomenon known as 'tracking'. With other materials carbon is not deposited, but erosion of the material occurs and ultimately causes complete breakdown. These phenomena frequently cause failure in practice at average voltage gradients of less than 1 kV/cm.

Hydrocarbon oils are the commonest insulating liquids; they are used either alone or as an impregnant for solid insulation. Transformer oil has a breakdown strength of about 300 kV/cm, but oil-impregnated paper for high-voltage cable insulation has a breakdown strength of 1,000 kV/cm and represents the highest standard achieved in practical insulation technology. The chlorinated diphenyls also have good insulation properties and, as these liquids are non-inflammable, they are used where there is a serious fire risk.

The practical working stress on high-voltage insulators of all types is only a very small fraction of the electric strength of the insulating material itself under good conditions. Some margin is necessary, but attempts should continue to be made to reduce the gap. Progress can be made by achieving more uniform voltage distribution on large insulators, and by inhibiting cumulative and progressive processes which lead to breakdown.

A PHYSICAL APPROACH TO CHEMICAL STRUCTURE

IN his presidential address to Section B (Chemistry), Prof. J. Monteath Robertson reminds us that the past fifteen years have witnessed extraordinary advances in the power of the physical methods that may be used to investigate chemical problems. During that time the

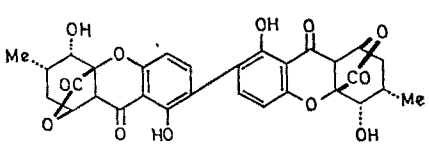
method of nuclear magnetic resonance has been developed and the scope of the other methods of absorption spectroscopy enormously increased by better instrumentation. The old science of X-ray crystal analysis has been transformed by the invention of the electronic digital computer. In other directions equally dramatic advances have been made, as in mass spectrometry, electron microscopy, the use and application of isotopes, the development of the various powerful methods of chromatography, and so on.

The determination of molecular structure is by no means the sole or even perhaps the most important aim of chemical investigation, but it is an important part. The purely chemical method based on degradative investigations can now be powerfully supplemented by an imposing array of physical tools. Many of these new methods resemble the chemical one in that they can be used to inspect certain portions of the molecular structure, identify certain groups, and gradually help to build up a complete picture of the molecule.

The method of X-ray analysis is entirely different. It cannot be used to look at a particular part of the molecule, or diagnose the existence of specific groups. It can only look at the complete structural unit and every atom it contains in a single operation. The waves scattered by the different atoms combine together by the principles of optical interference, and only the resultant can be observed. But if the structure can be solved, the answer obtained is far more complete than that given by any other method. It provides the exact position of every atom in space and so solves the chemical structure and stereochemistry completely in every detail.

There are two main obstacles in X-ray analysis. The first is the fundamental problem of determining the relative phase relationships of the scattered rays. This can usually be overcome by the 'heavy atom' or 'isomorphous substitution' methods[1], where use is made of an added extra atom which alters the resultant amplitude in a way that depends on the unknown phase. The second obstacle is the very formidable amount of computation involved in solving a complex structure. But this problem is now largely overcome by the invention of the electronic computer.

A good recent example of the power of the X-ray method is the determination of the structure and stereochemistry of ergoflavin[2], which can now be written as:



This is one of the colouring matters of ergot, the product of a fungus which grows on rye, and which has long been known to contain a complex group of medically important substances. Prof. W. B. Whalley was able to prepare a suitable heavy atom derivative of ergoflavin which he sent to Prof. Robertson for X-ray analysis at the end of November, 1962. The structure was solved completely by the middle of March this year, less than four months after receiving the crystals.

Work of this kind is greatly increasing our detailed knowledge of the structure of various natural products. A great effort is now being made to utilize these powerful methods to obtain information about the many vastly more complex biological molecules that are so vitally important in living matter. Substantial progress has recently been made, and solutions for some of the simpler protein and enzyme molecules are now in sight.

[1] Robertson, J. M., *J. Chem. Soc.*, 615 (1935); 1195 (1936). Robertson, J. M., and Woodward, I., *ibid.*, 219 (1937).

[2] Eglinton, G., King, F. E., Lloyd, G., Loder, J. W., Marshall, J. R., Robertson, A., and Whalley, W. B., *J. Chem. Soc.*, 1833 (1958). Apsimon, J. W., Corran, J. A., Creasey, N. G., Sim, K. Y., and Whalley, W. B., *Proc. Chem. Soc.*, 209 (1963). Asher, J. D. M., McPhail, A. T., Robertson, J. M., Silverton, J. V., and Sim, G. A., *ibid.*, 210 (1963).

SOME ASPECTS OF DIAGENESIS

THE period between deposition and metamorphism of a sediment, for long the most obscure stage in its history, forms the theme of Prof. J. H. Taylor's presidential address to Section C (Geology). Recognition of its importance to oil geologists in determining the capacity of a rock to hold and transmit fluids and to mining geologists in giving rise to oxidate and reduzate ore deposits has done much to stimulate interest in the sequence of events that takes place.

Recognition of the fact that diagenetic processes represent attempts to attain equilibrium under changing conditions has given rise to the concept of diagenetic grade. Rocks which have been subjected to similar post-depositional history may be said to be of the same diagenetic facies. Mapping of the deposits of the Verkhoyansk geosyncline in Siberia has revealed a series of zones of regional diagenesis passing finally into albite-epidote-rich rocks of the greenschist facies.

Diagenetic changes can conveniently be considered in three main stages: (1) those taking place immediately after deposition, before burial beneath the next layer of sediment; (2) those occurring during early burial and leading to lithification; (3) those operating at considerable depth but not directly due to crustal movement.

Changes prior to burial may result either from transfer of material from the fresh waters of a river to a more saline environment or from changing conditions within the basin of deposition—changes controlled in large part by biological activity. On the sea-bottom *p*H commonly ranges from 7·8 down to 7·0 (somewhat less in restricted environments) and *Eh* from 0·3 to −0·3. Among reactions which take place, perhaps the most widespread are the cation substitutions which modify clay minerals and the transformation of a variety of layered silicates to glauconite.

During early burial *p*H and *Eh* remain major controls of diagenesis. Evidence from sea-bottom cores shows that a downward decrease in *Eh* may be accompanied by either a rise or fall in *p*H. Clay minerals undergo structural modification, carbonate and siliceous concretions develop, primary cementation includes the formation of overgrowths on detrital grains and the partial infilling of pore spaces with a variety of materials of which silica and carbonate are most widespread. Unstable forms of calcite, such as high magnesian calcite and to a less extent aragonite, give place to the more stable form. In sedimentary ironstones some replacement of earlier constituents by chamosite and much replacement by siderite occurs. Pyritization is widespread.

After deep burial, temperature and load pressure replace *p*H and *Eh* as the significant controls. Some indication of the diagenetic environment and of the chemical reactions that have taken place can be gained from a study of deep formation waters, the brines which must presumably largely represent concentrated connate waters. Data regarding these brines are unfortunately distinguished by quantity rather than quality. They indicate a decrease in the ratio of magnesium, sodium, potassium and sulphate to chlorine as compared with normal seawater and an increase in calcium, lithium, strontium, barium and boron. Sodium and potassium have presumably been used in the development of secondary micas, feldspars and zeolites; magnesium has replaced calcium in dolomitization and has also gone into the formation of chlorites and micas. Sulphate has been reduced to sulphide. Complicated sequences involving solution, redeposition and recrystallization of cements can be recognized under the microscope. Interpenetration of grains as a result of pressure cementation is widespread in sandstones, but less common in carbonate rocks. A variety of diagenetic sulphides has been recorded in small amounts in sediments. How far they can be developed in economic

quantities is problematical. In certain deposits they appear to be replacements of diagenetic pyrite but not themselves indisputably diagenetic.

It is pertinent to ask how the great volumes of hot connate brines expressed upwards from compacting sediments differ from the hydrothermal solutions of classical ore genesis. The limited available data regarding composition of mineralizing solutions, from fluid inclusions in ore and gangue minerals and from a recent borehole for geothermal power in southern California, show some remarkable resemblances to deep connate waters. Increased concentrations, relative to chlorine, of potassium and boron, however, suggest additions from some other source.

THE UNITY OF ECOLOGY

DR. F. FRASER DARLING discusses the "Unity of Ecology" in his presidential address to Section D (Zoology).

Both words, 'ecology' and 'economics', stem from the same root, to do with home: both deal with income and expenditure, economics with anthropocentrically considered wealth symbolized as money, ecology in terms of energy and conversion cycles. In communities of plants and animals, ecology is also an observational study of communities which covers the more general definition of ecology as the science of organisms in relation to their environment, and the interrelations of organisms inter- and intra-specifically. Psycho-ecological factors are now being identified and social behaviour can be of critical significance ecologically. Hunting and food-gathering man may be considered a member of the indigenous fauna; agricultural, pastoral, industrial, natural-resource exporting man is like an introduced species, the outstanding difference between him and the rest of creation being his political nature, which is of great ecological significance. The present wave of nationalism in a contracting world could have damaging results on the resource base and the future state of survival of the human species. Man was able to civilize, that is, take advantage of his innate capacity for communicative gregariousness, by learning how to tap the organically derived climax wealth of the planet, thereby giving himself leisure for social and artistic evolution. Originally, the concept of community in ecology was considered in its competitive characteristics; but the fuller present-day thought of plants and animals, including man, being the ecological community embraces co-operative qualities as well, so that the community is greater than the sum of its parts.

There should be no plant, animal and human ecology as separate entities. There is but one ecology, with man being considered actively and historically. History, so commonly recorded politically, might be re-written ecologically, with benefit for the future. The rise and fall of the English wool trade from Saxon times to the end of the medieval period is given as an example. A wider study in space and deeper in time is made of the development of nomadism in the Old World and the place of the horse as a riding animal in triggering the eastward Indo-European migrations and later the westward Mongol movement. Archæological evidences supplement ecological observations and inference.

Comparison is made of old-world nomadism of domesticated animals moved slowly over wild lands at man's wish, with the North American nomadism with the wild bison which developed when the Indians gained the horse from the Spaniard. The tempo of movement was set by the bison and there was consequently no overgrazing or impoverishment of terrain. This nomadism of wild animals on wild lands could have continued indefinitely as a biological continuum, but it specialized the Plains Indian and made him more vulnerable in a changing world from which his resource base was removed. Some reflexion is made on the projected cropping of wild animals on the wild lands of Africa. Ecological management is now possible, but historical consideration and comparison are inadequate. Ecology is always concerned with process, and conservation, which is ecology in action, the social application of ecology, must reach deeper into the studies of history, anthropology and archæology.

BRITISH NATIONAL PARKS

THE national parks of Britain form the subject of Prof. H. C. Darby's presidential address to Section E (Geography). The ten national parks of England and Wales have been created under the National Parks Act of 1949, and they cover about 9 per cent of the country. Much of their character can be explained by two general considerations. In the first place, there is the small size of England and Wales, the limited amount of relatively wild land, and the absence of public domain land from which parks could be created and owned by a national authority as in the United States and elsewhere; the territory within a British national park remains in private ownership. In the second place there is the existence not only of a highly developed system of local government but of very extensive planning legislation, consolidated and extended by the Town and Country Planning Act of 1947. The National Parks Act of 1949 in no way replaced the existing machinery of planning. Planning control within the parks, as elsewhere, remained in the hands of local authorities. It is true that a National Parks Commission was established; but this is essentially an advisory body, and its role is to see that the interests of amenity and access are not lost amid the clamour of many competing claims.

It must be said that the machinery created by the Act of 1949 has the merit of ingenuity. It has made the most of a limited area of land, and it has taken advantage of an established system of planning. It has skilfully used the principle of multiple land use, and it has sought to combine local and national interests in the management of the parks themselves. Yet when all is said, the fact remains that something is not well. Successive debates in both Houses of Parliament have directed attention to weaknesses in National Park arrangements. Wherein does the failure lie ? In the Act itself ? In the operation of the Act since 1949 ? Or in both ?

The Act certainly contained within itself the seeds of difficulty. It charged the Commission it created with two divergent tasks: (*a*) the preservation and enhancement of natural beauty in England and Wales; (*b*) the encouragement of facilities for open-air enjoyment in National Parks. The challenge thus presented to the planner was that of reconciling the different purposes of amenity and access. If this was the challenge presented in 1949, How much greater is it to-day? Only too often a crowd transforms what it has come to enjoy and destroys what it most admires. Rising standards of living, holidays with pay, and about three times the number of motor-cars on the road, have greatly increased the pressure on the remaining areas of open country and especially on the coast. Furthermore, the whole *tempo* of national life has changed, and may yet change to a far greater degree. Some of the main intrusions into the countryside come from Government Departments themselves and from the great statutory undertakings and large private developers—from oil refineries, for example, from water authorities, from the Service Ministries and from the Central Electricity Generating Board. It is inevitable that many proposals for development within a park should come into conflict with the aim of maintaining the scenic beauty and rural solitude of this or that area.

Some of the frustration and despondency about national parks may well spring from the attempt to deal with as much as nearly one-tenth of the country. Clearly, not only the standard of control, but also the degree of financial aid, may need to be different in different parts of our parks as at present delimited. There may well be a case for closely examining all the parks, and for establishing within each some kind of classification from a scenic point of view. The varying needs of open-air recreation also need to be assessed. At present British national parks lack the basic information necessary for sound planning decisions. But the planning officers of nine of the ten parks are also responsible for the remainder of their respective counties, and the staff of the Commission (including administrative, technical and clerical officers) numbers only 30. The simple fact is that there is not enough manpower on the job. How can three field officers cope with the variety of questions that arise ? Very little money has been spent on the parks—taking the country as a whole, less than a penny a person a year. The future of the parks could be transformed with the aid of a relatively modest increase in expenditure, in manpower and in technical expertise. Finally, in the light of 14 years experience and of the changing circumstances of the country, the Act of 1949 now stands in need of amendment.

FUTURE OF THE AEROPLANE IN THE CIVIL AND MILITARY FIELDS

IN his presidential address to Section G (Engineering), Dr. S. G. Hooker describes the "Future of the Aeroplane in the Civil and Military Fields". The cost of developing aircraft and engines is now so high that Government financial support is essential.

In the military field the industry is maintaining the full strength of the Royal Air Force and developing important new aircraft such as the *TSR* 2, *P* 1154 and novel types of transport.

In the civil field it is engaged on the *VC* 10, *BAC* 111 and the Anglo-French supersonic airliner *Concord*.

The exports of the industry are worth between £100 and £150 millions annually, and it is to be hoped that the *Concord* will gain a large share of the market which will arise when the 500 big jets at present in service become obsolete.

If we can produce a transatlantic airliner with a cruising speed of Mach 2·2 the current journey time to New York will be approximately halved, occupying about 3 h. A further increase in cruising speed to Mach 3 would save only about 0·5 h because of the increasing proportion of time spent in take-off, climb, descent and landing. Present knowledge of supersonic flight stems from military aircraft, which have been attaining supersonic speed since about 1950. Mach 2·2 was chosen for the *Concord* because higher speeds would prohibit the use of an aluminium structure or of conventional rubbers and plastics.

The overall thermodynamic efficiency of a turbojet is increased at supersonic speeds and should reach 50 per cent at Mach 2·2.

The engine chosen for the *Concord* is the Bristol Siddeley *Olympus* 593, a development of the engine used in the *Vulcan V*-bomber and *TSR* 2 supersonic strike aircraft. Such an engine will produce horse-power of the order of 100,000 at Mach 2·2 at 36,000 ft., which indicates the order of difficulty of its development.

Great efforts are being made to ensure that the *Concord* is at least no noisier than present jets. The high rate of climb will help to attenuate the noise heard on the ground near airports and if the transition to supersonic speed occurs at something over 40,000 ft. the sonic boom should not be an appreciable nuisance. Sonic boom, however, may limit the size and range of future supersonic airliners.

The *Concord* carries more fuel and less pay-load than a subsonic airliner, but has greater productivity because of its high speed. Compared with a *Boeing* 707, the potential revenue per hour of the supersonic transport is 50 per cent greater, while its operating costs are greater by only 20 per cent.

There is an urgent demand for vertical take-off, single-seater strike aircraft capable of high subsonic speed at ground-level and supersonic speed at altitude. Such an aircraft could well be of the established single-engine type provided that the engine can both lift and propel it. The Bristol Siddeley *Pegasus* has been designed for this purpose and has been flying successfully in the *P* 1127 *VTOL* strike aircraft.

The *Pegasus* has a large front fan which supercharges the high-pressure compressor and supplies by-pass air which is expelled through two nozzles on either side of the engine near the front. The exhaust from the turbines emerges through a similar pair of nozzles at the rear. All four nozzles can be directed downward or in any angular position up to the horizontal to give lift, thrust or a combination of the two.

Such an engine has the following advantages: (1) conventional installation; (2) choice of conventional, short or vertical take-off; (3) freedom from erosion and re-ingestion problems; (4) simplest possible installation and maintenance.

The *P* 1127 was never intended for supersonic speeds and the more advanced Hawker Siddeley *P* 1154, capable of both supersonic speed and vertical take-off, is now being developed.

In the lift/thrust engine for the *P* 1154 the thrust at the front nozzles can be doubled by burning additional fuel in them. The extra power is available for vertical take-off as well as supersonic flight. This type of engine has obvious application to both civil and military transports.

The ramjet is a simple engine depending on high forward speed for efficient operation. At speeds above Mach 3 it is becoming the most efficient powerplant known to man.

Flight at hypersonic speeds—above about Mach 5—is possible provided that suitable combinations of speed and altitude are observed. At such speeds the ramjet is the only possible air-breathing engine.

Studies show the possibility of an aircraft weighing 300,000 lb. and carrying a payload of 20,000 lb. over a range of 4,000 miles at 4,000 m.p.h. at altitudes between 100,000 ft. and 200,000 ft. and may be the reality of to-morrow.

THE TENTACLES OF TRADITION

IN his presidential address to Section H (Anthropology) Mr. Peter Opie considers folklore and "The Tentacles of Tradition". In the nineteenth century men of culture awoke to the possibilities of a new source of knowledge, and urged each other to garner the "fast perishing relics of the common people". Since then the investigation of folklore has grown in significance as it has become evident that the relics are not fast perishing, do not belong only to the common people, and are not necessarily relics.

It is becoming clear that folklore, while retaining ancient characteristics, is a living force which readily adapts to changes in environment. While some customs wither away and their memory becomes romantic to us, others prosper to such an extent that they become ordinary, even tawdry, and possibly commercialized.

In A.D. 960 it was ordered "That every Priest industriously advance Christianity, and extinguish Heathenism, and forbid the Worship of Fountains". To-day it scarcely matters which spring, well, pool, or fountain we visit,

we find the nation to be obsessed by the idea that it should lighten its pocket whenever it sees contained water. At Bridlington more than £35 was found, placed for no valid reason, in the pool of the Illuminated Gardens. At Kingswear in Devonshire the Parish Council collects almost £100 a year from a local wishing well. In Hull, when an ornamental fountain was erected at the corner of Jameson Street and King Edward Street in May, 1956, £67 was found in the fountain before the end of June, and in 1959 it was reported that coins were being thrown in at the rate of £8 a week. To-day churches and charities foster the cult by making pools where no pools were before, to their own pecuniary advantage. It might be argued that people to-day do not throw coins into water out of superstitious belief. But why is it that they are more willing to throw their money into water than to place it in an offertory plate? Is not the answer that if they throw coins into a pool of water they feel they are getting something in return for their money—they are purchasing 'luck'?

One of the causes of malnutrition in Bantu homes is that women of child-bearing age are usually forbidden to drink milk, because it is believed to cause sterility in females. Are we ourselves less haunted by fear of sterility? Belief that sterility will follow certain courses of action has been found associated with the women's services, factory workers, parachutists, and air hostesses. Many of the secret 'facts' which pass by word of mouth behind closed doors about, for instance, conception, contraception, menstruation, and sex-determination turn out to be traditional, and sometimes to have antecedents that go back to the time of Pliny. The fall of man occurs, day after day, when he gives way to his desire to believe in the mysterious and the far-fetched.

In 1962, in a debate in the House of Lords on pest poisons, a peer asked why vermin needed to be poisoned, when his mother-in-law had cleared her chicken-run of rats simply by asking them to leave. Far from being new, this method of disinfestation was practised in Scotland a century ago. It was well known to the Elizabethans, including Shakespeare and Ben Jonson, and was described in classical antiquity. But whereas the writer eighteen hundred years ago had been sceptical, the Under-Secretary of State replying to the debate in the House of Lords had declared that he firmly believed that there was "something in these extraordinary powers" which some people possessed.

It is possible that the extent of man's supernatural and superstitious credulities remains constant, and that the beliefs merely take on more sophisticated forms. There does not seem to have been any slackening in man's desire to believe the unbelievable. Individually we cradle ourselves in comfortable thoughts of sudden fortune, the inevitableness of destiny, the possibilities of re-incarnation, or the compensations of an after-life. The human mind has been equipped with a wonderful capacity for accepting evidence which agrees with its preconceptions. We are transfixed not so much by what we do not know, as by what we think we already know.

THE CONTRIBUTION OF CLINICAL MEDICINE TO PHYSIOLOGY

THE "Contribution of Clinical Medicine to Physiology" is described by Prof. J. McMichael in his presidential address to Section I (Physiology and Biochemistry).

Physicians and physiologists work increasingly together in the analysis of reactions to disease. Sometimes the physiologist points the way to new discoveries of value in medicine, but equally frequently the clinician's observations give an answer to problems before the physiologist is able fully to comprehend the situation. The value of orange and lemon juice in scurvy was recognized by Lind 180 years before vitamin C was isolated. Muddled thinking lost this discovery in the nineteenth century with many disastrous consequences, including the loss of Scott's polar expedition. Endocrine and brain disorders are best studied in man, and the co-operation of an intelligent patient with an observant doctor has repeatedly clarified physiological functions and pointed the way to further investigations.

Clinical observation can proceed a certain distance but completion of the analysis may depend on animal experiment. A good example was the observation of fibrillation of the heart by McWilliam in Aberdeen in 1888. The recognition of fibrillary contraction of the auricles in man was completed by James Mackenzie in 1906.

The use of man for planned physiological experiments was started by J. S. Haldane, who found that the regulation of respiration could be best studied in co-operative human beings. These techniques for respiratory investigations have now been enormously elaborated and constitute the basis of measurement of function of the heart and lungs in human disease. One of the commonest disorders affecting the heart is narrowing (stenosis) of the mitral valve. This obstructs the flow between the left auricle and the left ventricle. Because of this narrow passage the pressure rises in the blood vessels of the lungs. The use of a short-life isotope of oxygen enables measurements to be made of the blood-flow through different parts of the lungs. In mitral stenosis most of the obstruction to flow results from thickening of the arteries in the lower parts of the lungs where blood-flow is retarded. This contrasts with the state of affairs in normal subjects where, owing to the influence of gravity, there is a higher blood-flow in the lower parts of the lungs and a diminished blood-flow in the upper part. These findings give some explanation of why tuberculosis (now a disappearing disease) develops at the apices of the lungs where blood-flow is poor. This disease was known to be rare in mitral stenosis where the blood-flow at the upper part of the lungs is now known to be high. The reason why the arteries of the lungs become thickened and narrowed at the bases may throw light on the mode of development of thickening of the arteries elsewhere in the body. Disease of the arteries is perhaps the major cause of death and studies which began for the purpose of illuminating the lung circulation may in fact have a much wider significance in relation to arterial disease in man.

The clinical observer is exposed all the time to an enormous experience of a multitude of disorders which exercise his complete range of biological knowledge. He must compare continuously his experience and knowledge of disease with the reactions observed in physiological experiment. Modern medicine is becoming increasingly scientific in its methods of study and the mutual efforts of clinicians and physiologists will illuminate innumerable problems on which future individual human welfare depends.

CEREBRAL LOCALIZATION OF PSYCHOLOGICAL FUNCTION

THE idea of cerebral localization of psychological function is discussed by Prof. O. L. Zangwill, president of Section J (Psychology). This idea goes back at least to Gall, whose reputation has suffered unduly on account of his allegiance to phrenology. Although Gall must take his share of blame for the absurdities of phrenology, his ideas have had a good deal of influence on the development of modern neurology, especially through Hughlings Jackson, who derived much of his evolutionary doctrine from Herbert Spencer, in early life a disciple of Gall. It is also noteworthy that Bouillaud, the first to localize aphazia to the frontal lobes, was a strong admirer of Gall.

The development of physiological work on the brain from Flourens to the present day is outlined. It is pointed out that early work on the motor cortex gave rise to the

belief that this part of the brain is in some sense the 'organ' of voluntary movement. This inference cannot, however, be sustained. Nor can the cortical sensory projection areas be accepted as the 'seat' of sensory experience. We appreciate to-day that neither elementary sensations nor elementary movements are appropriate units for the analysis of behaviour and that we should concern ourselves rather with higher-order products of cerebral organization.

The attempt to investigate integrated behaviour patterns by physiological techniques is then considered. It is pointed out that as a number of primitive behaviour patterns concerned with the control of appetite, sleep and the simpler aspects of emotional expression appear to be organized at the diencephalic level, it would seem appropriate to speak of these behaviour patterns as having a measure of localization. Mention is made of the value of techniques of stimulation through implanted electrodes in the unrestrained, conscious animal in extending our knowledge of the functions of these deeper regions of the brain.

The effects of ablation of the brain cortex on the learning and retention of habits in mammals is then discussed. Following the work of Lashley, it is widely believed that habits have no very precise localization and are affected by the extent rather than the locus of a cerebral lesion. But it is important to bear in mind that Lashley himself did not reject the concept of localization. Further, more recent work, especially with primates, has given evidence of the differential localization of a considerable number of acquired behaviour patterns.

The problem of inter-hemispheric relations in learning and performance is briefly considered, with special reference to the work of Prof. R. S. Sperry. This leads on to some discussion of asymmetry of cerebral hemisphere function in man and the problem of cerebral dominance.

In conclusion, it is pointed out that cerebral localization is not so much a matter of delimiting 'centres', each with its own intrinsic 'function', as of specifying integrative foci in systems which may well comprise large areas of the brain and their inter-connexions. In spite of some recent assertions to the contrary, it is contended that examination of the defects resulting from injury to the brain, whether in animals or man, does in fact throw light on the cerebral bases of normal behaviour. It may even turn out that the ideas of Gall, for all their *naïveté*, will, in the long run, prove more relevant to our understanding of brain function than cybernetic models of the kind now in vogue.

THE TROPICAL BOTANIST

BOTANY is a vast subject, growing unwieldy. It stretches from the physical and chemical workings of plant-protoplasm through the grosser structure of cells and tissues to the vegetation which they make. It is concerned with the most important and one of the most abstruse problems of life, namely, photosynthesis. It involves the classification of one of the most complicated sets of entities, namely, the half-million or more living plants which make up the vegetation of sea and land. It comprises the problems of their distribution, their origin and evolutionary connexions, their economic and cultural value to man, and the interpretation of fossil remains. It began as a science about the middle of its range when Linnaeus introduced the system of biological names. It spread with the excitement of the microscope and the voyages of discovery. It spreads with the ultra-microscope, the techniques of modern physics and chemistry, the progress of genetics and ecology, and the accumulation of collections of the world flora, both past and present. It leans on the other sciences, borrows from them, contributes to them and, yet, lacks its own mastery. Its extremes are now so far apart that they cannot be satisfactorily housed, financed, or understood in the same department. The older aspects of classification, plant-structure, life-histories, distribution, ecology, and natural history are referred to, sometimes with contumely, as classical botany. Its younger aspect, which links the minute workings of the plant with physics and chemistry, is being woven into the new science of cell or molecular or microbiology. The study of vegetation takes classical botany into the country. The study of micro-structure builds the new laboratories in the city.

Mr. E. J. H. Corner, in his presidential address to Section K (Botany), is concerned with the future of classical botany. It has ramified, and prospers now in many small fields. It is full of specialists. It has lost most of its applications to the sciences of agriculture, horticulture, forestry, fisheries and medicine. It is impoverished and avoids its main issues. It lacks the integration of a spontaneous science. It seems ripe for overthrow by the rising molecular biology.

This would be disastrous. It would destroy that which should unify our understanding and appreciation of all plants. It would destroy it before it has found its proper footing. Classical botany has still to familiarize itself with the majority of living plants, and this majority, which it has neglected in the tropics, is now in danger of extermination. Classical botany is needed by the applied and lucrative sciences, and the alternative of leaving them to foster the fragments which they require would be as unsatisfactory a surrender.

In order to survive, classical botany must be re-organized. First, it must face and accept the challenge to its stereotyped thought given by A. H. Church in Oxford in 1919. For a generation this outstanding issue has been evaded, but "the beginnings of botany" are in the sea, whence it must be re-developed. Secondly, classical botany must concern itself with the plants, the processes and the results of plant-industries. There is more money in bananas than in buttercups, in mushrooms than in mosses, in seaweeds than in pond-scums, in timber than in taxonomy. Thirdly, it must re-cast its morphology, ecology and classification from the tropical point of view. The neglect of this exuberance has caused the stagnation of the study of the land-flora.

The progress of tropical botany cannot be relegated to the slow rise of pure biology in the tropics themselves at the hands of their own biologists. The tropical countries are being industrialized, their leading students are being made industry-minded, and their floras, which are the richest heritage of plant-life, are being wiped out. Tropical botany must be sponsored from the seats of classical botany in the temperate countries, particularly by the interchange of university personnel. Probably no other natural science needs to develop so much the counter-stream of students to the tropics.

The tragedy of universal deforestation, so often short-sighted and purely for exploitation, brings home to the botanical traveller the fact that his science has no control over its natural resources. Botanists have for too long been content with the crumbs dropped by others from the lavishness of Nature's table. The feast is coming to an end, and botanists must take their stand in the conservation of what remains. They must assert themselves in this administration of world-affairs. Their classical science has the ingredients of leadership for human benefit.

THE BENEFICENT FOREST

THE aim of Prof. H. M. Steven's presidential address to Section K* (Forestry) is to consider what benefits woodlands can bestow, both in general and with special reference to the Highlands of Scotland and similar upland areas in Britain.

The natural climax vegetation on the Earth is some kind of woodland wherever the climate, and in some instances the soil, permits. At one time the whole of Britain was covered with woodland except on the higher hills, the bogs and the marshes. To-day, only 7·1 per cent is woodland. This contrasts with about 25 per cent, which is the percentage of the land area of the world covered with some kind of woodland. If, therefore, the forest is beneficent, we are claiming very little of its benefits.

We are apt to forget that the most fundamental thing in the world is the ability of green plants to utilize part of the energy of the Sun to manufacture products useful, directly and indirectly, to man and all other creatures. Recent work has shown that trees compare favourably with other useful plants in the efficient utilization of solar energy. Soil fertility can be built up by the forest, because most of the nitrogen and minerals in the trees are to be found in the growing tissues, such as leaves and twigs, and also in bark, and not in the timber of the stem; these are not normally removed from the forest and when broken down by innumerable organisms become available again for the growth of the tree. Except on the poorest soils, the nutrients removed from the soil are replaced by the slow weathering of soil minerals and the deposition of nutrients in the rainfall. The forest can be a self-perpetuating natural resource; this is the aim of systematic forestry. The principal alternative use of our uplands is grazing, mainly by sheep. Ecologists and indeed agriculturists agree that this is merely an extractive form of land use in most instances. The animal products so produced are rich in nitrogen and minerals and these are removed from the land; there is no beneficial cycle of returning nutrients as in the forest.

The progressive deforestation of Britain in the past has led not only to declining fertility of the soil but also to an impoverishment of the natural flora and fauna. Even the modest afforestation programme that has been carried out has led to some increase in the rarer woodland birds and mammals. There is already a widespread interest in Britain's native flora and fauna and this is likely to increase in the future.

It is important to know whether forest cover affects surface and sub-surface run-off of water compared with, say, grassland, because water is also an important natural resource. Investigations on this subject are proceeding in many parts of the world, including more recently in Britain, and the Department of Scientific and Industrial Research in 1961 set up a Committee on Hydrological Research. This is a difficult subject, and much more research on it is needed. Even although it is found that there is a lower yield of water under forest, there is general recognition of the higher permeability and greater storage capacity of soils under forest which lead to reduced surface run-off, hence less erosion, a more prolonged yield of water during drought, and lower stream-flood peaks. There is no reason, therefore, why the conservation of water resources should restrict the expansion of the forest area, particularly when much of the potential land for afforestation is in regions of high rainfall.

It is not necessary to argue the role of forestry in helping to stem rural depopulation because this has been emphasized by one committee after another in recent years. The principal contribution of forests to employment is not in the growing of timber but in its processing and utilization, the ratio between these two parts being about one to five. We are now seeing the first fruits of this in the setting up of the Fort William pulp mill, and its consequential effects; this, however, need only be the beginning.

Although these aspects of the beneficence of forests are important, the final role to be mentioned, that of a source of timber and other products, is still more important. Few fully realize that these are among the world's most important primary products with an estimated annual yield of 1,350 million tons. Thus, in terms of timber production also, Britain is making little use of this potential natural resource with only 7 per cent of the land surface devoted to forestry, and an annual import bill of more than £450 million for timber and products derived from it.

The forest policy adopted after the Second World War was to plan for a fully productive forest estate in Britain of 5 million acres, equivalent to about 9 per cent of the land surface—still one of the lowest in Europe. It was estimated that this would require about 3 million acres of additional land for forestry, and the new afforestation would require to be undertaken mainly by the State for economic reasons. The Forestry Commission have, however, experienced continuous difficulties in getting the necessary land, due mainly to the heavy subsidization of agriculture. A revised plan, begun in 1958, fixed planting programmes on a five-year basis but on a falling scale. It is true that, in this nuclear age, it is not necessary to have strategic reserves of timber for a long war, but the Zuckerman Committee, an independent body which did not include any members with forestry interests, stressed the continuing necessity for a vigorous forestry programme in these words: "We may therefore conclude that (regardless of the part which, as we shall argue later, forestry might play in slowing down depopulation and abandonment of vast areas of Great Britain) there is probably a stronger economic reason now than there has been hitherto for investment in planting trees". The policy at present advocated to secure this end is the integration of pastoral agriculture and forestry. Provided this was on a sufficiently large scale, included an adequate proportion of large as well as smaller areas for forestry, and a due proportion of the better as well as the poorer land, it could ensure that Britain had a greater share of the beneficence of the forest. It still poses unsolved problems, and Prof. Stevens concludes by advocating that substantial areas be acquired by the State in a number of upland areas in Britain to investigate and demonstrate the best ways of substantially increasing the productive capacity of such lands by steadily developing and enriching them instead of impoverishing them. He is certain that if the known techniques of improving hill pastures were fully applied it would be possible to release much greater areas of land for an extension of the forest and thus a fuller reaping of its beneficence, while at the same time maintaining hill farming at a reasonable level to provide the foundation stocks for the lowland sheep industry, and also to provide for the use of cattle to develop and maintain the potentiality of the improved grazings.

PERSONAL VALUES IN EDUCATION

WE are reminded by Sir Charles Morris in his presidential address to Section L (Education) that education in the United Kingdom is more generally credited with outstanding success in exploiting the values of the institutional factor than those of the personal factor. Britain's most famous schools and colleges are very famous indeed; and the greatest work of her most celebrated individual teachers has always been seen against the background of particular institutions, to whose service they usually devoted their lives. But for all that, personal relationships have clearly played a fundamental part; and the insistence on the central importance of the development of character, and on the education of the whole man, has kept the individual teacher, making a full use of his personality, in the centre of the stage. But in more recent times in this as in other fields of human endeavour, the immense potentialities of well-designed mechanisms and of imaginative blue-printing have made a powerful impression both on

educational theorists and on public opinion. First, the imaginative administrator and, more recently, the teaching machine, have come very much to the fore. It may be time to ask, Where is Britain going in all this ? Is she being compulsively attracted towards a system of automation, exploiting to the full the possibilities of self-education based on machines and pre-planning ?

In these days, when there are large numbers of students and small numbers of teachers, there are considerable practical difficulties in ensuring that the sparks are able to pass to and fro in this lively relationship and it will undoubtedly be necessary to rely more and more on well-designed systems of instruction, and it will be necessary to invent even more ingenious machines. It will be necessary for teachers to make the fullest possible use of the machine tools of the technology of education, so as to make the human efforts of the individual man or woman more extensive in their impact and more intensively productive. But the key-stone of education lies in an interpersonal relationship. Britain has sometimes been in danger of forgetting this both in the grammar schools and in the universities. She has gone ahead as if good syllabuses and a well-arranged timetable can of themselves alone do the whole job. Certainly they cannot do so; if what is being looked for is true education, though they are quite necessary as conditions, they cannot carry Britain as much as half the way. There is no substitute for the real teacher; and the good teacher has to be able to establish a real relationship, pupil by pupil, with those whom it is his concern to educate. It is certainly going to be difficult enough in our time to ensure that Britain has a sufficient number of teachers. But the most difficult problem of all in the coming years is going to be to see that, in spite of the pressures of numbers, and in spite of the demands of specialism, the good teacher gets a chance to do his true job.

PROVIDING FOOD FOR MAN AND BEAST

BEFORE Section M (Agriculture), Prof. Martin Jones poses the question: Where has the human food come from in the past, and where will it need to come from in the future ?

The present increase in population needs a corresponding increase in the production of food, and the popular idea is that the limit of production will soon be reached as nearly all the land suitable for crop production is already in use.

Such a view ignores what the scientist can do to modify the methods of food production. The problem of food is how to collect the energy from light and convert that light energy into food energy either in the form of fruit and vegetables or as meat and milk. Only about a tenth of the light energy per acre is now utilized by our farm crops, even our most popular crops.

Improvements due to manuring and plant-breeding are playing their part, but the limiting factor over a vast area of the world's surface is the supply of moisture. Man needs only to harness the water and irrigate the large areas of desert in the tropics to bring about a ten-fold increase in the quantity of food available, particularly when combined with the use of modern manures. Experiments have shown that this method is practical and, furthermore, irrigation is spreading so fast that soon it will be regarded as a waste of manures if the requisite amount of water is not supplied with them, especially at the critical stage in the life of the crop.

This increase in the production of food in the tropical and semi-tropical deserts is already being done by enthusiasts who have the confidence to look ten years ahead and are prepared to spend the capital necessary for storing and utilizing the vast supplies of water available, even if it means conducting it through a pipe-line over a thousand miles.

Changes on such a vast scale may alter the distribution of the human race and encourage the people not only to grow their food in these drier areas but also to live there for choice of climate.

Such a development would no doubt guarantee the quantity of food for all; but we have now to guard very carefully that the food is of the right kind for the proper nutrition of the people, particularly young people. Here, with modern crops such as cereals which are easy to mechanize, there is a great danger of the diet lacking sufficient protein and the proper balance of the right kind of proteins, minerals and vitamins.

This balance could be helped very considerably through the use of more meat and milk—animal products based on grass, which requires not only suitable soils and manures but also modern methods of conservation and utilization.

This all adds up to limitless opportunities for an increase in our food production so as to keep pace with the increase in population for at least the next two hundred years, by which time even our proposed methods may again be out of date, if the human being should be able to get his supply of energy through some other means than a green plant. For the time being, however, the green plant is an essential link in collecting the energy of life for us, and it is our duty to study the needs and behaviour of such plants.

PROBLEMS OF PENOLOGY

A SCIENTIFIC penology, the subject of Prof. W. J. H. Sprott's presidential address to Section N (Sociology), is impossible to achieve until the principle of retribution is abandoned. Arguments are given against this principle. If the 'tariff' system of punishment is given up, penology becomes entirely forward-looking. It has three objectives: preventing the offender from offending again, either by deterring him or reforming him, deterring potential offenders, and the protection of the public. Taking the treatment of offenders alone, the first aim is to keep them out of prison, partly on economic grounds but mainly because of the deleterious effects of imprisonment.

To this end research is required into a typology of offenders and also a study of differential treatment. Reference is made to the work at present being carried out in California. Such treatment is particularly directed towards first offenders, but recent research has shown that many habitual criminals are in need of supervisory care on account of their social inadequacy. For them permanent hostels are recommended. The problem of general deterrence demands more research than has been devoted to it. The problem here is: Precisely at what classes of person is the deterrence of potential offenders directed ? Some classes of potential offenders are not likely to be deterred at all. Others may be, and here the question is: What threat will deter them? If deterrence of potential offenders is accepted as a justifiable aim, then some offenders will be sent to prison with this end in view, as will also be the case with those for whom non-custodial treatment has proved ineffective. The issue here is: What reformative influences can be brought to bear on them ?

Reference is made to three experiments now being tried out in Great Britain. The first is the psychiatric prison recently opened at Grendon Underwood, where prisoners referred by prison medical officers are treated and where the problem of treating the psychopath is being explored. The second is the attempt to bridge the gap between prisoner and custodial staff by means of 'group counselling'. The third is the open prison at Ashwell for recidivists where the régime consists of a minimum of supervision by the prison staff and maximum

responsibility given to the prisoners. The third aim of sentencing, the protection of the public, is a matter of weighing values and not a matter for scientific research. The question here is: What offences are considered so serious that society must be protected against them for long periods ? It is suggested that in the case of the persistent petty thief, society must be prepared to take risks. In the case of certain sex offenders and professional criminals society has the right to protect itself.

The final issues discussed are: the indeterminate sentence and the question of a sentencing tribunal. Arguments are brought against both of these. The indeterminate sentence is disturbing to prisoners who are not granted parole. If there is a sentencing tribunal then appeals against sentence will go before the courts and we are back where we started, and if sentencing is to have a general deterrent effect, then sentencing must be done as soon as possible after the trial. At the same time, it is suggested that special sentences such as corrective training and preventive detention should be abolished and the allocation of offenders sentenced to custodial care should be left to the prison authorities.

PRESIDENT FOR 1964 OF THE BRITISH ASSOCIATION

LORD BRAIN

THE RIGHT HON. LORD BRAIN, the well-known neurologist, has been elected president for 1964 of the British Association for the Advancement of Science. Lord Brain will deliver his presidential address at the next annual meeting (1964) in Southampton. The British Association has made what will prove to be an excellent choice, because not only is Lord Brain perhaps the first clinician to be awarded this important honour since Lord Lister, but also, like Lister, he is a medical man who has done much to set medicine on a scientific footing.

Lord Brain was born in 1895 and received his preliminary education at Mill Hill School and New College, Oxford.

At Oxford he was taught zoology by Sir Julian Huxley and for a time had J. B. S. Haldane as a tutor. He also came under the influence of Sherrington and Bazett. Proceeding to the London Hospital for his clinical work, he had as a teacher George Riddoch, fresh from his collaboration with Henry Head on the effects of war injuries on the spinal cord. Riddoch started him on his career in neurology by introducing him to Maida Vale Hospital, to the staff of which he was later appointed, and soon after that he became neurologist to the London Hospital.

There has always been a close link between neurology and ophthalmology, so Brain later joined the staff of the Royal London Ophthalmic Hospital (Moorfields), which led to his interest in the ocular symptoms of thyroid disorders, particularly exophthalmos, and his description of the syndrome exophthalmic ophthalmoplegia helped to clarify the endocrine factors responsible for exophthalmos.

Shortly after the War, his attention was directed to disorders of the joints of the neck as a cause of neurological symptoms, and this helped to open up a new field of clinical neurology dealing with spinal cord syndromes, and more recently with the effects of disease of the cervical intervertebral disks on the circulation through the brain. About this time, in collaboration with others, Lord Brain was one of the first to describe degenerative changes in the nervous system associated with cancer, and this has led to the recognition of a wide range of disorders complicating cancer, but not directly due to the spread of the growth, and he is now directing a team carrying out research in this field, with the support of the British Empire Cancer Campaign.

An early interest in psychology and psychiatry, stimulated by developments in psychological medicine during the First World War, led Brain to investigate the correlations between brain and mind illustrated by the effects of brain damage, and during the Second World War he wrote on some visual perceptual disorders and on the significance of handedness in relation to cerebral function, and recently he has performed work on disorders of language, thereby continuing the London Hospital neurological tradition springing from Hughlings Jackson and Henry Head. The physiology and pathology of perception and consciousness and their philosophical implications remain a continuing interest.

Lord Brain married Stella, daughter of the late Dr. Reginald Langdon-Down, whose father, Dr. John Langdon-Down, described mongolism, which has recently attracted much interest on account of the discovery of its basis in a chromosomal abnormality. Lady Brain has many medical relatives, and a cousin of hers is the wife of Prof. A. V. Hill, a former president of the British Association.

Lord Brain has many honours to his credit; a most intriguing point is that (1) his name is Brain (2) he is a world-authority on the brain; (3) he is the editor of the excellent specialist journal *Brain*.

THE ABERDEEN REGION

The North-East of Scotland

A Survey prepared for the Aberdeen Meeting of the British Association for the Advancement of Science, 1963. Pp. viii+256. (Aberdeen: The Central Press for the Local Committee, 1963.) n.p.

ALTHOUGH it is not usual to start reviewing a book from its end, in the case of this book, *The North-East of Scotland*, it seems the most obvious place. Between the last page and the cover is provided the best introduction to any area—a detailed map. The present one, a 1/5 in. to the mile coloured map, was specially prepared by Messrs. John Bartholomew and Son, Ltd., Edinburgh, for the occasion of the visit of the British Association for the Advancement of Science to Aberdeen—as was the *Survey* itself.

Twenty-four contributors, mainly from the University of Aberdeen, have produced a *Survey* full of information for the members of the Association. It consists of twenty-six essay reviews dealing with various aspects of the area, which for the most vary from two to twenty pages. It opens with a succinct account of North-East Scotland which, like a clenched knuckle, projects from the Grampians to the North Sea. Essentially it is concerned with the counties of Aberdeenshire, Banffshire and Kincardineshire, but in part Moray and Nairnshire are included.

Having thus defined the theatre for the British Association 1963 meeting, the platform is next laid out by essays on the geology and structure (13 pp.), geomorphology (17 pp.), climate (5 pp.), soils (8 pp.), flora (8 pp.), and fauna (13 pp.). North of the Highland Boundary Fault

are rocks of Precambrian age—the Highland Schists, metamorphosed arenaceous and calcareous sediments. Large sills of igneous rocks of Old Red Sandstone age have been intruded into these schists, and vary from granite to basic and ultra-basic igneous rocks. South of the Highland Boundary Fault, rocks of the Old Red Sandstone underlie the whole area. Between the Old Red Sandstone lowlands of Moray and Strathmore the knuckle projects into the North Sea as a complex of lowland and upland plateaux drained by numerous rivers, such as the Dee and the Don. Elements of highland and lowland scenery are interdigitated as straths and glens cut back into the hill masses producing a quilted effect, diversified by the contrast of glacial erosion. For example, the difference in the character of Deeside and Donside is the result of the abundance of fluvio-glacial material in the former:

> "The River Dee for fish and tree
> The River Don for horn and corn".

With increasing distance from the sea, the climate becomes more extreme and is heightened by the basin-like nature of the inland relief. As this pattern alters so also do the soil types, the flora and the fauna. Soils with free drainage occupy the flanks of the hills, imperfectly drained soils, the gentler contours on the lower slopes, poorly drained soils, the footslopes, and very poorly drained soils, the depression and the bottom lands. The flora of North-East Scotland is considered in the *Survey* according to four geographical elements, namely, the Continental Northern, the Northern Montane, the Arctic-subarctic and Arctic Alpine, each containing many interesting and rare species, for example, orchid, saxifrage, mountain avens, sedge, etc. On the other hand, the fauna is treated under six biological communities: mountains (including Arctic species, such as ptarmigan and mountain hare which turn white in winter), moorlands, forests, agricultural land, rivers and lochs, coast and sea. Each has its characteristic fauna, rarely purely natural, but usually a blend of naturally occurring and introduced species. The fauna includes rabbit, deer, wild-cat, stoat, salmon, pike, adder, golden eagle, grouse and seal, etc. The sections dealing with the flora and fauna are supplied with admirable lists of scientific and common names of species mentioned.

Essays follow dealing with the region before 1700, regional settlement and fishing villages 1750–1880. The first mentioned outlines the Mesolithic period with its associated pigmy flints, the area's Neolithic sequence with its important art of pottery making and chambered burial cairns. Then follow the incursive 'beaker folk' from Holland and later the Bronze Age and Iron Age settlements. The picture develops with the Roman penetration, and the Picts and Celtic Christianity. Later came the Viking raids, the feudal castles and the Normanization of Scotland and the attacks from Anglo-Norman England, culminating in the Civil Wars and the dethronement of Mary, Queen of Scots.

Each of the settlement types can be related to a phase in the historical development of the region; the crofts, the product of colonization during the agricultural revolution, representing the successors to the cottar's dwellings of the farming townships. Later come the mixture of agricultural and industrial influences. In the eighteenth century, fishing and farming are predominant in the economy, bringing more intricate contact with the diverse and open society of the mercantile centres.

Three eleven-page sections have been prepared on agriculture, forestry and fisheries. Farming and related industry and trade make up a large part of the economic life of the North of Scotland. Although there has been a movement of labour in the past century towards the townships, improvements have been achieved through automation and the many changes in crop and animal husbandry, which have raised yields per acre and per head of stock. The section on forestry deals with the historical background, State forests, private forests (10·6 per cent of the area), education, research and forest products. The area is one of the most important and most interesting regions for the forester in the whole of the British Isles. It is an area in which trees and woods have played a large part in the social history and where the art and profession of forestry are well understood. The University of Aberdeen is one of the four British universities which has a department of forestry and sets a high standard in its degrees in forestry. The review on fisheries indicates well the major changes which have occurred over the past century, and describes the more than 60 per cent of total Scottish fishing that is centred on the area: Aberdeen itself is the largest fishing port in Scotland and the third largest in the United Kingdom. The North-East collectively has become the chief centre in Scotland for the processing of fish by smoking, filleting and freezing, and for ancillary industries, such as shipbuilding, the manufacture of nets and of fish-meal, ice-factories, ship-repairs and supplies of all kinds. It is interesting to learn that the annual Scottish catch of salmon and sea-trout by angling and by commercial methods amounts to 1,500–2,000 tons—small compared with some species of marine fish, but in value representing an income to fishermen of £1·25–1·5 million.

By far the longest essay in the *Survey* (53 pp.) is that dealing with industries and commerce. The principal industries are fishing, distilling, granite, textiles, shipbuilding, engineering, food preservation and paper manufacture. A characteristic is the high degree of specialism which exists both in the processes of manufacture and in the finished products. Each of the industries is described geographically, historically and from the economic point of view of the annual turnover. For many centuries the manufacture of textiles has held a substantial place in the economy of Aberdeen and other towns, including linen, flax, canvas and, of course, woollen milling. The last-mentioned includes luxury cloth, scarves, plaids, tweeds, twist cheviot and worsted suitings. Whisky is one of Scotland's greatest exports and has had a long and eventful history. Most malt distilleries are to be found to the north of the Tay, and within this area the counties of Banff and Moray are by far the most important—the greatest concentration being in the Spey Valley region. Measured by employment figures, the distilling industry is relatively small, but in terms of value of output and value of exports it ranks very high. The paper industry is no exception, and a great variety of high-quality papers produced at the mills around Aberdeen are consigned to many overseas markets. The area, because of the large deposits of granite of high durability and beauty, holds an important place in the granite industry. As Aberdeen is the focal point of communication in North-East Scotland, the essay is rounded off with a pertinent account of these communication routes, giving details of inland transport facilities, trade, steamship lines, livestock forwardings, etc.

The essays which follow deal with population changes, local dialects, local government in Scotland, schools, the University of Aberdeen, research institutes (including details of the great three—the Rowett Research Institute, the Macaulay Institute for Soil Research and the Torry Research Station), technical education, Aberdeen College of Education, local scientific societies, the museums, the Highland Folk Museum at Kingussie, and scientists of the region.

Most of the essays are supplied with a list of selected references and a general index is provided which makes cross-referencing easy between the essays. All in all, the Local Committee of the Association is to be congratulated on providing a concise, readable account of the North-East of Scotland. Those members of the Association who were able to peruse it before the Meeting will have gained a useful insight of the North-East of Scotland; certainly it will help afterwards to gather together and supplement personal experiences.

(*Continued from page 876*)

In addition to finding the value x_2 (0, 5), the nineteen quantities x_2 (5, 10), x_2 (10, 15) . . . x_2 (95, 100) were recorded and again totalled to give X_2 (5, 10), X_2 (10, 15) . . . X_2 (95, 100). These totals refer to the number of times cell 2 spikes occur in successive 5-msec intervals after the firing of cell 1, up to 100 msec.

We were also interested in whether or not the firing of cell 2 influenced the subsequent firing of cell 1; so the same quantities were recorded with the roles of the two cells interchanged. These totals are X_1 (0, 5), X_1 (5, 10) . . . X_1 (95, 100).

We now assume the cells are firing independently of each other and calculate the expected values of the X_1 $(a, a + 5)$ and X_2 $(a, a + 5)$. Let there be n_1 and n_2 spikes of cell 1 and 2 respectively and the duration of the record be T seconds. Further, let m_1 and m_2 be the mean rate of firing per second of each of the two cells. Then n_1/T and n_2/T are the sample estimates of m_1 and m_2. The probabilities of a cell firing in a randomly selected short time δt are $m_1\delta t$ and $m_2\delta t$ for cell 1 and 2 respectively. For a longer period Δt, the expectations of the number of spikes are $m_1\Delta t$ and $m_2\Delta t$. Taking $\Delta t = 5$ msec, the estimate for the expected value of any x_2 $(a, a + 5)$ is $n_2/200T$ and for any x_1 $(a, a + 5)$ is $n_1/200T$.

Let us now consider, for clarity of exposition, a specific one of our totals, say, X_2 (0, 5), which has in fact been analysed in detail and is described here. This value is the total number of spikes in n_1 separate 5 msec intervals, in each of which the expected number of spikes is $m_2/200$. The numbers in the various intervals cannot be regarded as independent random variables because the distribution of the intervals depends on the statistical properties of the sequence of spikes of cell 1. However, the expectation of the sum is equal to the sum of the expectations even when the numbers are not independent (see ref. 2, p. 73) and hence the expectation of X_2 (0, 5) is $n_1m_2/200$. All the X_2 $(a, a + 5)$ have the same expectation for which the sample estimate is $n_1m_2/200 = n_1n_2/200T$. The corresponding quantities for X_1 $(a, a + 5)$ are $n_2m_1/200 = n_1n_2/200T$. The expected value is therefore the same for both X_1 and X_2.

Without a very detailed statistical analysis, which we hope to make later, it is not possible to give a completely rigorous test for whether an observed value for an X_1 or X_2 differs significantly from an expected one. However, it is essential to have some idea of what deviations may be regarded as significant and fortunately it is easy to derive an approximate test for a cell which fires fairly slowly relative to the interval considered and which occurs in a pair of cells, both of which do not have an entirely regular firing pattern.

We now write p_1 or p_2 for the probability that, respectively, cell 1 or cell 2 fires in a 5-msec interval. Clearly $p_1 \approx n_1/200T$ and $p_2 \approx n_2/200T$. We suppose p_1 and p_2 to be small so that $1-p_1 \approx 1$ and $1-p_2 \approx 1$, and we also assume that the probability of two or more firings of either cell in one interval may be neglected. Consider now X_2 (0, 5). Its distribution will be given approximately by a binomial distribution with parameters p_2 and n_1 (not strictly because the different x_2 (0, 5) are not entirely independent). This distribution in turn is approximated by a normal distribution with mean n_1p_2 and standard deviation $\sqrt{n_1p_2(1-p_2)}$ (see ref. 2, section 6.4). Using our sample estimate for p_2 we test the significance of the observed value of X_2 by assuming it to be a member of a normal distribution with mean $m = n_1n_2/200T$ and standard deviation $\sigma = \sqrt{m}$. Owing to the symmetry of the result, the same test applies to X_1. This test should be quite reliable for our results because, with one exception, all values of p_1 and p_2 are very small.

The other parameter of cell discharge which we considered was a function of the number of spikes from one cell of a pair counted in the 100-msec interval following the discharge of a spike in the other cell. This function was the mean, M_1, of all twenty X_1, that is, $M_1 = \Sigma X_1/20$ or the mean, M_2, of all twenty X_2, that is, $M_2 = \Sigma X_2/20$. Thus M_1 is the number of spikes from cell 1 present in the mean of 20 successive 5-msec intervals following a spike in cell 2 and vice versa for M_2. This 5-msec period will be referred to as the averaged 5-msec interval. In view of the analysis outlined in the preceding paragraphs it seems reasonable to compare the distribution of each of the two quantities M_1 and M_2 with a normal distribution having a mean $m = n_1n_2/200T$ and standard deviation $\sigma_1 = \sqrt{(m/20)}$.

To summarize, we have examined the question of whether the firing of one of a pair of cells (say, cell 1) influences the probability of discharge of the other (cell 2), by considering the number of spikes of cell 2 present in (i) the first 5-msec interval and (ii) the average of the twenty successive 5-msec intervals after the discharge of a spike from cell 1. We have analysed these results to see whether the values so observed deviated from the expected values which were estimated.

In order to assess the statistical significance of any deviation, a parameter was calculated which was a numerical statement of the magnitude of the deviation. In the case of the data from the first 5-msec interval, the parameter was $\lambda = (X-m)/\sigma$ and in the case of the averaged 5-msec interval $\Lambda = (M-m)/\sigma_1$. If the value of $|\lambda|$ or $|\Lambda|$ exceeded 3, it was considered that the deviation of observed from expected value was likely to occur by chance only rarely ($P < 0{\cdot}01$) and hence reasonable to conclude that the two values came from different populations.

Experimental results. Our experimental records comprise a total of 16,770 spikes corresponding to eight recording points with two cells and one recording point with three cells. In the case of three cells, the analysis was performed separately for each of the three possible pairs.

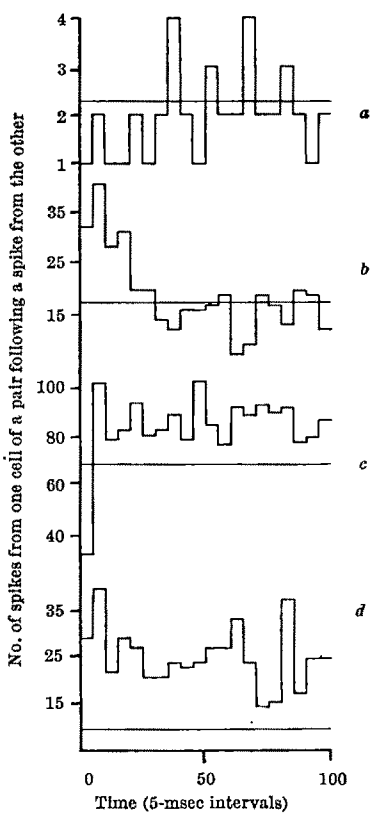


Fig. 2. Histograms showing, for selected pairs of cells, the number of spikes from one cell in successive 5-msec intervals after the firing of the other. The expected number is drawn as a horizontal line. The cells plotted are: (*a*) pair 8, cell 2 following 1; (*b*) pair 6, cell 1 following 2; (*c*) pair 2, cell 1 following 2; (*d*) pair 7, cell 2 following 1

Table 1. Properties of the Cells

n_1 is the total number of spikes due to cell 1 which were counted. m_1 is the mean rate of discharge of cell 1 in spikes/sec and p_1 the probability that cell 1 will fire in any randomly selected 5-msec interval. n_2, m_2 and p_2 are corresponding values for cell 2. T is the number of seconds over which the discharge of the corresponding pair of cells was observed

Cell pair	n_1	m_1	p_1	n_2	m_2	p_2	T	Cat
1	1,149	2·09	0·0104	46	0·084	$4·2 \times 10^{-4}$	551·0	35
2	355	2·31	0·0115	5,811	37·81	0·189	153·7	35
3	385	1·17	$5·87 \times 10^{-3}$	123	0·38	$1·87 \times 10^{-3}$	328·1	35
4	605	4·91	0·0246	168	1·36	$6·82 \times 10^{-3}$	123·2	36
5	641	11·50	0·0575	318	5·70	0·0285	55·8	36
6	554	3·92	0·0196	853	6·03	0·0302	141·4	36
7	1,792	3·79	0·0190	498	1·05	$5·27 \times 10^{-3}$	472·4	36
8*	2,707	6·35	0·0678	35	0·08	$8·7 \times 10^{-4}$	426·5	35
9 (12)	158	1·46	$7·31 \times 10^{-3}$	133	1·23	$6·16 \times 10^{-3}$	108·0	36
9 (23)	133	1·23	$6·16 \times 10^{-3}$	439	4·06	0·0203	108·0	36
9 (31)	439	4·06	0·0203	158	1·46	$7·31 \times 10^{-3}$	108·0	36

* Refers to 10·7-msec. intervals

Table 2. Correlation of Activities of Cells

X_1 and X_2 are the numbers of discharges of cells 1 and 2, respectively, in the first 5-msec interval following discharge of the other cell. M_1 and M_2 are the means for the first twenty 5 msec (except for cell pair 8, for which the interval was always 10·7 msec). λ and Λ measure the deviations from the expected values, significant deviations being underlined

Cell pair	Expected No.	X_1	λ_1	X_2	λ_2	M_1	Λ_1	M_2	Λ_2
1	0·48	0	−0·69	1	0·75	0·55	0·45	0·65	1·10
2	67·10	30	−4·53	50	−2·09	82·4	8·35	84·7	9·61
3	0·72	0	−0·85	3	2·68	2·3	8·31	2·6	9·88
4	4·13	3	−0·55	0	−2·03	4·7	1·27	0·4	−8·20
5	18·28	21	0·64	21	0·64	25·3	7·35	24·8	6·82
6	16·71	32	3·74	30	3·25	18·3	1·74	9·95	−7·40
7	9·45	23	4·41	28	6·04	28·4	27·58	24·3	21·62
8	2·27	2	−0·18	1	−0·85	3·15	2·60	2	−0·81
9 (12)	0·97	0	−0·99	0	−0·99	1·8	3·75	2·05	4·89
9 (23)	2·70	9	3·83	6	2·01	4·4	4·62	4·85	5·84
9 (31)	3·21	2	−0·68	0	−1·79	1·85	−3·40	2·5	−1·77

For one of the recording points with two cells the interval of time discussed in the last section was 10·7 msec, not 5 msec, owing to a different rate of recording on film. This is indicated in Table 1.

The mean rate of discharge (m_1 and m_2, Table 1) of the nineteen cells ranged from 0·082 to 37·8 spikes per sec with only one cell firing at a rate exceeding 11·5/sec. Accordingly, the probability that a cell would fire in any randomly selected 5 msec interval (p_1 and p_2, Table 1) was, with this one exception, low.

First 5-msec interval (Table 2). In four pairs of cells the number of spikes observed from one cell in the first 5 msec following a spike from the other, was significantly ($|\lambda| > 3$) different from the expected number. In cell pair 2, λ_1 was a negative quantity, which means that the discharge of cell 1 was reduced to below the expected level following a spike from cell 2. The value of λ_1, in pair 9 (23), was positive; so the number of cell 1 spikes following a cell 2 spike was significantly greater than the expected value. In cell pair 7, λ_1 and λ_2 were significant and positive. That is, the number of spikes of cell 1 following a discharge from cell 2, and the number of spikes of cell 2 following a spike from cell 1, were significantly greater than the expected value. A similar relationship existed between both cells of pair 6.

Averaged 5-msec interval (Table 2). In two cell pairs (1 and 8) the observed number of spikes did not differ from the expected number ($|\Lambda| < 3$). That is, the discharge of a spike from one cell did not influence the probability of discharge of a spike from the other. In three cell pairs (4, 6, 9 (31)) the deviation of the observed value from the expected value was significant for one of the cells of each pair. In each case, Λ had a negative value. This parameter was both positive and significant for the two cells in each of the remaining pairs.

The magnitude and sign of Λ for a given cell pair bore no obvious relation to the magnitude and sign of λ for that cell pair.

The way in which the values X (0, 5), X (5, 10) . . . X (95, 100) for one cell of a pair fluctuated about the expected value was not constant from one cell to the next. We have illustrated four fairly typical histograms of these fluctuations in Fig. 2. Each block of the histogram represents the mean number of spikes of one cell in a 5-msec interval following the discharge of a spike in the other cell. The twenty intervals from this event to 100-msec later are plotted. The horizontal line in each histogram represents the expected number of spikes. The histogram of Fig. 2*a* is that of cell 2 following cell 1 of pair 8. It may be seen that the observed number fluctuates irregularly about the expected value. In this case (Table 2) λ_2 and Λ_2 were both well below 3. Fig. 2*b* is the histogram for cell 1 following a spike in cell 2 in pair 6. After the first 20 msec, during which the number of cell spikes was increased well above the expected number, the histogram falls precipitously and fluctuates close to the expected level. In this case λ_1, > 3, but $|\Lambda_1| < 3$. The histogram of cell 1 following cell 2 of pair 2 is illustrated in Fig. 2*c*. For the first 5 msec the number of spikes is well below the expected level ($\lambda_1 = -4·53$), and thereafter remains above it, not returning to the expected level throughout the 100 msec analysed ($\Lambda_1 = +8·35$). Cell pair 7 also shows a rather curious pattern of behaviour. The data for cell 2 following a spike in cell 1 are plotted in Fig. 2*d*. Following the cell 1 spikes the number of spikes of cell 2 is increased for each of the twenty 5-msec periods, at no time returning to the expected value. We anticipate that if we had continued our analysis for a long enough period (for example, 10 sec) following the spike in cell 1 the observed number would fall to the expected number, for it must do so ultimately.

Discussion. With the exception of two cell pairs (1 and 8) the firing of one cell of a pair was influenced by the presence of a spike in the other. Sometimes (6 cell pairs) the influence operated both ways. These effects were demonstrated by at least one of the two analyses undertaken. The influence lasted a variable length of time. In some instances (Figs. 2*c* and *d*) a discharge from one cell altered the probability of discharge in the other for 100 msec. This effect was seen in a pair of cells which fired relatively slowly (pair 7, Fig. 2*d*) and in a pair (pair 2, Fig. 2*c*), one cell of which fired relatively rapidly (38

spikes/sec). The effect thus seems to be independent of the rate of discharge of the interacting units.

When the observed number of spikes differed significantly from the expected number in both cells of a pair the deviation was positive. When the observed number differed significantly from the expected number for only one cell of a pair, the deviation usually (4 out of 5 occasions) was negative. The physiological interpretation of these findings is almost entirely a matter of speculation. Where both cells influence each other they may do so by mutual facilitatory interaction. It is possible, however, that the discharge of the two cells is governed by a source common to both. If the activity of this source periodically waxed and waned, it could certainly produce the coupling effects which have been described. There is no suggestion whatever that the supposed source is common to all pairs of cells showing mutual interaction.

This hypothesis is less plausible for those pairs in which the firing of only one of the cells was influenced by the other. In such pairs it is possible that one cell tends (usually) to inhibit the other, though we might have expected the effect to have been reciprocal (see, for example, ref. 3).

Finally, it is of considerable interest that each of the two cell pairs (1 and 8) which showed no evidence of interaction contained a regularly firing cell[4] which discharged one spike every 12 sec approximately.

We thank the Radio Astronomy Group at the Cavendish Laboratory for their advice and assistance, the staff of the Mathematics Laboratory, especially Dr. David Wheeler, for their help and advice with programming, and the Medical Research Council for financial assistance.

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A RAPID METHOD FOR EXAMINING STEAM-CORROSION AND STEAM-CORROSION INHIBITION

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CORROSION investigations have been carried out in the past both as practical tests and laboratory investigations. In the former so-called 'field tests', long corrosion periods were used and the attack was usually measured by registering the change in weight of the test sample and also by comparison with standard samples the corrosion of which had been graduated according to certain scales of measurement. The corrosion specimens varied in size and shape depending on the test involved. In field tests, it is desirable to use flat specimens with large surface areas. The laboratory investigations, on the other hand, were performed using small test samples of different shape. When the corrosion in these latter tests was not measured electrochemically, the test periods were often long, the length depending on the use to which the laboratory-type corrosion tests were to be put[1–3]. Other workers[4–6] have investigated a number of volatile amines in steam-heating systems and obtained evidence of considerable reductions in corrosion. Analogous investigations have, however, not been performed with regard to instrument sterilization in autoclaves.

The aim of the present investigation has been to investigate the corrosion of instruments during their sterilization in autoclaves and the possibilities of inhibiting this effect. For practical reasons, it has been necessary to carry out the investigations on small specimen rods. Moreover, during instrument sterilization, the corrosion times were relatively short. In order to examine more thoroughly the degree of corrosion and the effect of the corrosion inhibitor, model experiments were performed in glass apparatus in which the test samples were subjected to the influence of the same corrosion medium as in the autoclave (that is, steam and steam condensate) or to this medium together with a certain amount of inhibitor, that is, volatile amines. The temperature of the steam and steam condensate was 98°–99° C and the pressure atmospheric, and in this respect the experimental conditions differed from those in autoclave sterilization where both the pressure and temperature are higher.

De-ionized water or de-ionized water containing 0·1 per cent *cyclo*hexylamine was poured into a number of 'Pyrex' vessels equipped with ground-glass countercurrent coolers (Fig. 1). In each apparatus, a test sample was placed above the liquid surface on a 'Pyrex' glass support. The apparatus was then immersed in a constant-temperature water bath set at 99° C. Losses of liquid from the vessels were prevented by the counter-current coolers. Immersion lasted 120 min.

In this way the test sample was subjected to the influence of both steam and condensate. The specimen rods, which were cylindrical (length 45 mm and diameter 5 mm), were made from the basic materials used for different dental instruments. In this report, results are given for one type of material (SANDVIK 21*T*10*P*. Analysis (per cent): C = 1·25; Si = 0·15; Mn = 0·3; Cu = 0·25; W = 1·7; V = 0·1; Pb = 0·2) used in dental burrs.

The specimen rods, which had been stored by rubbing with oil, were selected at random, washed with ether and rinsed in de-ionized water as cleansing liquid. Then they were 'set to zero' by means of ultrasonic cleaning immediately before the start of the tests (Narda ultrasonic apparatus: Ser. 600).

Five test samples were cleaned at a time. Each sample was cleaned by itself in a separate 'Pyrex' test-tube using 5 ml. de-ionized water. The five test-tubes were fixed in special positions in a support in the ultrasonic apparatus. The ultrasonic cleaning has been carefully standardized (time, water temperature, depth below water surface,

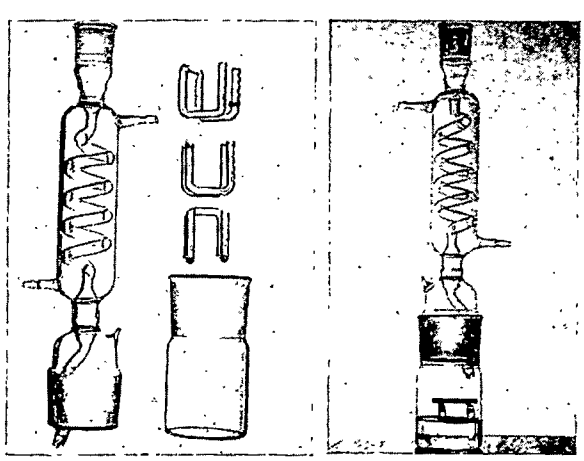


Fig. 1. 'Pyrex' corrosion apparatus. Individual parts on the left and assembled on the right

frequency, height of test-tube above bottom of ultrasonic tank, volume of liquid in tube, etc.) and each specimen rod has had, during the whole of the experiment, a definite position in the ultrasonic tank. Each cleaning period for each separate specimen lasted 2 min, and each sample was each time cleaned 10 times consecutively (cleaning period 1, 2, 3, etc.) in fresh test-tubes using de-ionized water (Fig. 2). Experiments have shown that the specimen rods, after this treatment, are satisfactorily free from oxidation impurities and from any damaged grains and grain boundaries (Fig. 2) resulting from the surface treatment. The different specimen rods were, in addition, more 'similar' to each other at the beginning of the corrosion experiment than they were before the ultrasonic cleaning (Fig. 2)[7]. After the corrosion test, the specimen rods were once again purified in an analogous manner.

The amount of 'corrosion products', expressed as the amount of dissolved iron/cm² surface area, was determined by analysing for the amount of iron in each cleaning solution from each separate cleaning period using a modified orthophenantroline method[7].

A comparison of a series of experiments on one type of test specimen shows that, if the steam contains *cyclo*-

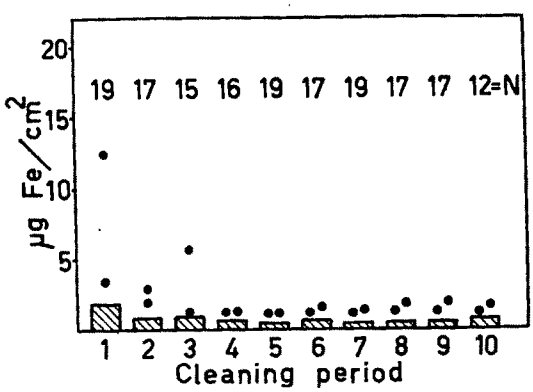


Fig. 2. Amount of iron dissolved out per ultrasonic cleaning period during standardization of specimen before corrosion experiment. N = number of determinations. Shaded areas indicate the mean values for each cleaning period

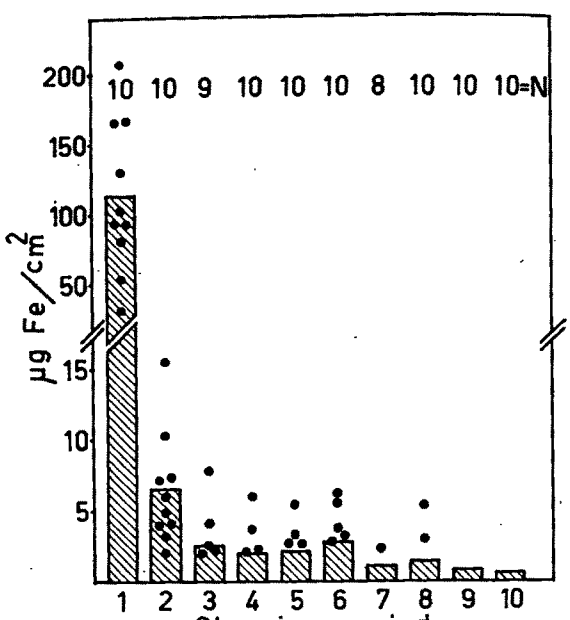


Fig. 3. Amount of iron dissolved out per cleaning period for the ten samples in Fig. 2 which were subjected to corrosion by steam treatment alone. The specimen rods were chosen at random. Shaded areas show the mean values and the points the individual values which exceeded 2 µg/cm². N = number of determinations. Amount of iron in the cleaning solution for cleaning period 1 includes also the corrosion products in the feed water

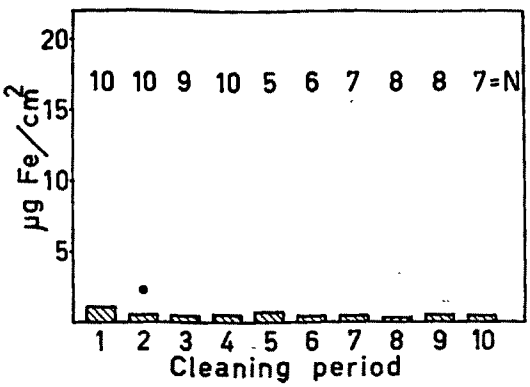


Fig. 4. Amount of iron dissolved out per cleaning period for the ten samples in Fig. 2 which were subjected to corrosion by steam together with 0·1 per cent *cyclo*hexylamine. The specimen rods were chosen at random. Shaded areas show the mean values and the points the individual values which exceeded 2 µg/cm². N = number of determinations

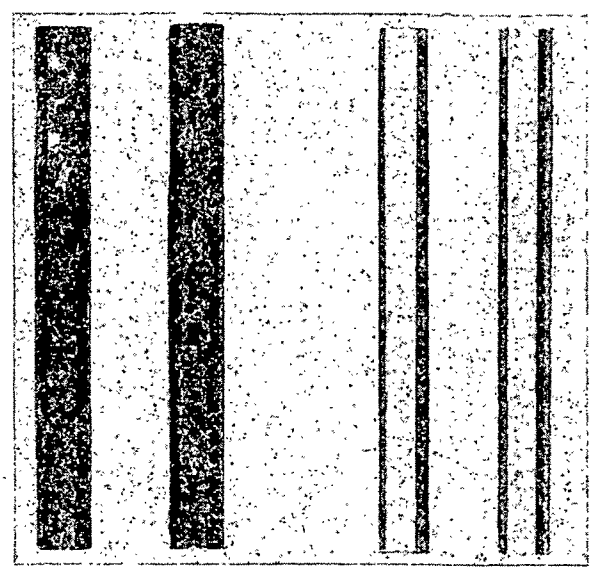


Fig. 5. Left, specimen rods subjected to steam and steam condensate; right, to the same corrosive medium but with *cyclo*hexylamine present as inhibitor. The specimen rods were cleaned ultrasonically in the manner described

hexylamine, practically no corrosion is obtained at atmospheric pressure even after applying steam and steam condensate at 98°–99° C for 120 min (Figs. 4 and 5) while steam and steam condensate by themselves give rise, under the same conditions, to a high degree of corrosion (Figs. 3 and 5). The experiments thus show that small, well-standardized specimen rods are suitable for corrosion studies of this type and that *cyclo*hexylamine is a volatile inhibitor possessing a considerable corrosion inhibiting effect in an atmosphere of steam. Moreover, they show that corrosion investigations can be carried out using short test periods provided that well-standardized experimental conditions and a sensitive method of measurement are used.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Distinguishing between the Solar, the Interplanetary and Geomagnetic Fields

In a paper[1] to be published I derive the number density (n) distribution of particles trapped in a magnetic tube of force specified only in so much as the speed and the magnetic moment of each such particles are invariants. The derivation extends and amends that of Dowden[2]. The density distribution is made to relate to that at a point (denoted by a prime) where the number density per steradian is isotropic or nearly so. Collisions and external forces are neglected. It is found that at a part of the tube where $B > B'$, $n = n'$ and where $B \ll B'$:

$$n \approx KB \qquad (1)$$

where K is constant.

It follows that different tubes of force are distinguishable by different K values.

Smith[3] and others used the empirical relation:

$$f_c^2 = af_H \qquad (2)$$

(where $a \approx 10^6$ c/s) to describe the whistler medium of the Earth, where f_c is the plasma frequency and f_H the gyrofrequency of electrons. This is equivalent to equation (1) so that $K = a/4\pi ce$. Thus for the Earth $K \approx 10^4$ cm^{-3} gauss^{-1}.

Assuming a dipole coronal magnetic field of 1 gauss at the Sun, Ingham's[4] distribution of electron density in the outer solar corona implies a value of $K \approx 10^7$, or $a \approx 10^9$ for the corona. My assumed value of magnetic field, however, may be inappropriate for the corona.

Using a value of $B = 10^{-4}$ gauss for the interplanetary field near the Earth and $n = 1$, $K \approx 10^5$ and $a \approx 10^7$.

It is suggested then that geomagnetic field may be distinguished from interplanetary field by measurement of either the pair (n, B) or the pair (f_c, f_H). Likewise interplanetary field may be distinguished from solar field. The distinction may be difficult if irregularities exist in the fields, for it may be expected that the K value for any particular tube of force could differ from the mean for its parent magnetic field. It would be interesting to know whether the magnetic field at 1 A.U. from the Sun is part of the solar field.

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PHYSICS

Pressure Temperature Projection of the Three-phase Line of the Gallium Antimony System

Previous work has been carried out on the phase diagram of the gallium antimony system by several workers[1-3]. However, their work was limited to the composition temperature projection of the phase diagram.

The interest shown in the semiconducting properties of gallium antimonide[4] makes it desirable to grow stoichiometric single crystals. Crystals so far produced have, however, contained large numbers of electrically active impurities[4]. Since most workers grow their crystals with conditions such that antimony can freely evaporate, it is possible that this may be due to antimony deficiency[5].

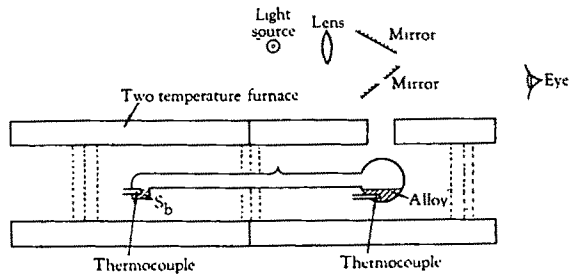


Fig. 1. Experimental apparatus

This communication describes an investigation undertaken to see whether the equilibrium pressure at the melting point was sufficiently large to cause this effect.

The apparatus used was very similar to that used for the Ga–Te system[6] and is shown in Fig. 1. The starting materials were zone refined GaSb and Sb. Before loading the materials were etched. (GaSb was etched with HF / HNO_3, 1 : 1 and the Sb with HCl / H_2O_2 / H_2O, 5 : 1 : 4.) The tube was evacuated and sealed off at a pressure of about 10^{-6} torr.

It was found that the alloys could be supercooled by up to 20° C so that only melting points were taken. The mode of operation was that the system was allowed to reach equilibrium for times of about 70 h just below the three-phase line. The melt temperature was then raised in about 3° C steps at intervals up to 24 h and the temperature at which the solid liquid transition occurred was noted.

Platinum–platinum 13 per cent rhodium thermocouples were used to measure the temperatures, which were computed from the thermocouple outputs using standard tables[7].

The results obtained are shown in Fig. 2, where they are plotted in terms of melt and reservoir temperatures. The pressures shown are taken from Honig's collection[8]. No attempt has been made to correct the reservoir temperatures for the effect of the pressure gradient in the tube. At the worst this would give a pressure correction of the form[9]:

$$\frac{P_A}{P_R} = \sqrt{\frac{T_A}{T_R}}$$

where P and T are pressure and temperature and the suffices refer to the alloy (A) and the reservoir (R). With

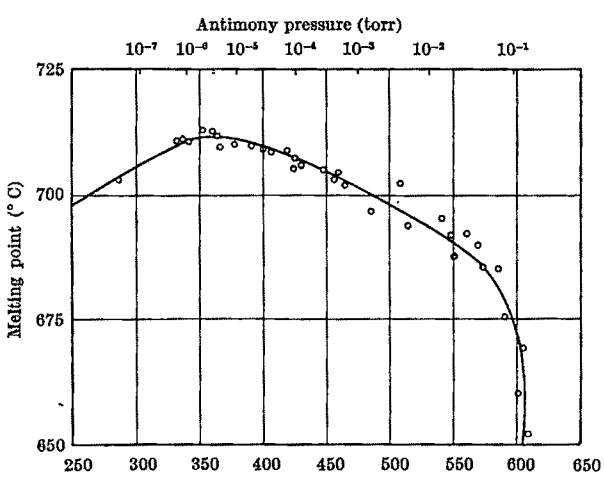


Fig. 2. Experimental results

T_A = 1,000° K and T_R = 600° K, P_R would be lower than P_A by about 30 per cent, which is equivalent to a 5° C correction to T_R.

Fig. 2 shows a maximum melting point of 712 ± 5° C, which is in reasonable agreement with the published values. At the maximum melting point the antimony partial pressure is (3 ± 1) × 10^{-6} torr (T_R = 360 ± 10° C). Depending on the diffusion coefficient, with this partial pressure up to about 2 × 10^{15} atoms per sec could be lost from each square centimetre of solid surface. This could lead to serious antimony deficiency.

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British Standard of Radioactive Iodine-131

In 1952 it was agreed[1] to define the above standard in terms of the γ-ray dose-rate in air at 1 cm from a point source of 1 mc. of iodine-131. Such a procedure, utilizing a graphite ionization chamber, resulted at that time in greater precision and reproducibility than could be obtained by particle counting. It was estimated that the derived disintegration rate would not differ from the absolute value by more than ± 5 per cent.

Recently, the National Physical Laboratory, which is responsible for such standards in the United Kingdom, has issued certificates, in the case of iodine-131, giving disintegration rate values obtained both from ionization chamber measurements and from 4πβγ coincidence counting.

In future, standards will be based on the 4πβγ coincidence method since the inherent greater accuracy is now attainable. Activities quoted may be related to previous standards using a value for the ratio

$$\frac{4\pi\beta\gamma \text{ activity}}{\text{Ionization chamber activity}} \text{ of } 1{\cdot}024 \pm 0{\cdot}002.$$

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Variation of Bed Height with Bubbling in an Air/Catalyst Fluidized Bed

During a preliminary investigation of mass transfer from bubbles in a fluidized bed composed of cracker catalyst (mean particle size 60μ), it was noticeable that the presence of bubbles caused a reduction of the height of the bed which was obtained when either a bubble flowstream or individual bubbles were injected into the bed. It appears that gas flows preferentially from the continuous phase into the bubble, and that the hold-up of gas in the continuous phase is reduced because the rising velocity of the bubbles is about 100 times that of the gas in the continuous phase.

The apparatus used for the investigation consisted of a 'Perspex' tube 9-in. diameter with a 'Vyon' distributor and beds of up to 60 in. deep could be accommodated. Preliminary results were obtained using either a single orifice ⅛-in. diameter at the centre of the bed, or three ⅛-in. diameter orifices placed symmetrically in the bed.

By injecting a single bubble, the bed was caused first to expand by an amount equal to the volume of the bubble. Immediately the bubble had left at the top, the bed collapsed to a point well below the initial height. Further, when the bed flow-rate was less than about 1·5 times the minimum fluidizing velocity, bubbles injected at flow-rates of up to 400 ml./min failed to reach the surface. Gas from the bubble was transferred to the continuous phase, and the height of the bed was slightly increased.

From the measurements of the reduction in height of bed it was possible to calculate the effective bed flow-rate and hence the amount of air transferred from the bed to the bubbles. Using either a single or a triple orifice for a given total bubble flow-rate, the rate of air removal from the bed was constant. Thus, within limits, the method of bubble formation had no apparent effect.

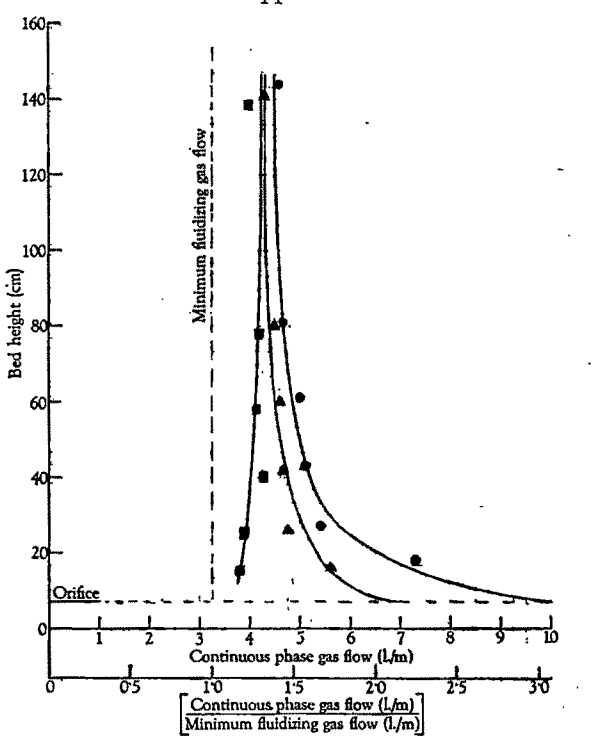


Fig. 1. Injected bubble flow-rate 240 l./m. ●, Initial bed flow-rate 10·0 l./m; ▲, initial bed flow-rate 7·0 l./m; ■, initial bed flow-rate 5·0 l./m

The results of experiments carried out at one particular rate of injection of bubbles into the bed are shown in Fig. 1. The rate of flow of gas in the continuous phase at equilibrium is plotted against height of bed for a range of initial bed flow-rates. It will be noted that, in all cases, the curves tend at large bed-heights to a constant value. The transfer is most rapid immediately above the orifice and the limiting value can be approached from either direction.

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Microwave Inversion Efficiency of Orange Ruby

Recently some ruby, grown by a vapour-phase process, has been found to be orange coloured rather than the normal pink. Some of this has been investigated as a potential maser material by taking a small sample (~10 mg), containing 0·05 per cent Cr^{3+}, double pumping

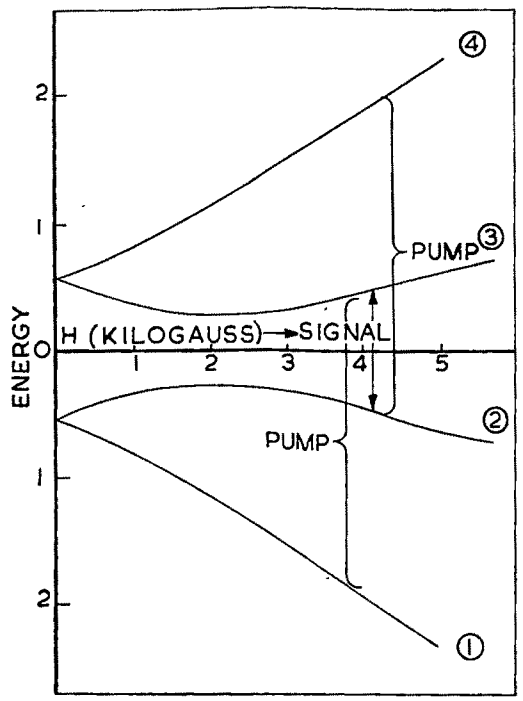


Fig. 1. Energy-levels of Cr^{+++} in ruby. Magnetic field at 54° 44′ to C-axis (energy expressed in kMc/s)

at 23 kMc/s and observing the resulting inversion of the 2–3 transition at 9 kMc/s (Fig. 1). This was then compared with the inversion obtained from a sample of pink ruby of similar size and concentration, placed in the microwave cavity, alongside the orange ruby. The c axes of the two crystals were arranged to be at 90° to each other and at 45° to the radiofrequency magnetic field in order to achieve similar transition probabilities. The inversion ratio I, defined as:

$$I = \frac{\text{peak inversion}}{\text{peak absorption}}$$

when measured at 1·4° K, was found to be 1·5 for the pink ruby, while that of the orange was much bigger, being of the order of 5·0 for most samples.

The paramagnetic spectrum of the orange ruby shows the normal Cr^{3+} lines and also a broad line ~200 gauss wide with $g \doteqdot 2$, which is almost isotropic with respect to magnetic field direction. Measurement of the Cr^{3+} in alumina energy-level diagram[1] shows that the broad line is coincident with the idler transitions, 4–3 and 2–1, when the crystal is being operated in the double pump position. It is thought that this produces a rapid relaxation path between levels 4–3 and 2–1, thus producing an enhanced inversion between levels 3 and 2, a process very similar to that used in the first successful maser[2].

Chemical analysis of this orange ruby has not as yet revealed any appreciable quantities of chemical impurities. It is thought that this broad line originates either from a valence state of chromium other than Cr^{3+}, possible Cr^{+} which has a $^6S_{5/2}$ ground-state, or from some colour centre formed in the vapour phase process due to unusual flame conditions. Some undoped corundum, grown by this method, is now being examined for colour centres.

It would appear that this orange ruby is worth further investigation as it may prove to be superior to the normal pink ruby as maser material. We hope to carry out some inversion measurements at liquid nitrogen temperature to see whether relaxation times remain favourable for an enhanced inversion at higher temperatures.

We thank Thermal Syndicate, Ltd., Wallsend, for supplying the ruby, and the Chemical Inspectorate, War Office, Woolwich, for the chemical analyses of the specimens.

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METEOROLOGY

Effect of Carbon Dioxide on Evaporation of Water

As meteorologists probe more deeply into the physical processes involved in the growth of cloud droplets, they are rapidly led to a conclusion which physical chemists have appreciated for quite some time: the varied and unique properties of ordinary water suggest a highly complex structure much of which still remains obscure. On this point there is little disagreement. There is considerably less agreement, however, on the best way in which to re-examine some of the classical concepts on which our facile and inadequate models of liquids are based. Evidence mounts that a fresh start must be made before we can obtain a realistic and fruitful working model. In this connexion, we have chosen to scrutinize the classical concept of evaporation.

Recently, Weyl (personal communication) suggested that the interaction of the overlying gas with the surface may tend either to facilitate or impair evaporation of the liquid, depending on the nature of the liquid or gas, respectively. Consequently, the nature of the overlying gas should influence the evaporation rate of water. According to Weyl, evaporation depends on the rate at which a polymerized liquid is changed into single molecules. Since such a rate is subject to catalysis, an overlying gas may serve as a catalyst in this reaction. Weyl predicted that carbon dioxide would cause an increase in evaporation rate of water, other conditions being equal. In view of this, an experiment was designed to test Weyl's hypothesis.

In order to test the effect of overlying gases on the evaporation rate of water, two identical 'Lucite' chambers were constructed to house shallow brass evaporation dishes 2 cm in diameter. Under uniform ambient conditions this would ensure equal evaporation from both dishes. The diminishing height of the water meniscus over the dishes filled with distilled water provided a measure of evaporation rate; the menisci were optically projected on to a grid on which the lowering of the meniscus apex was recorded every 15 or 30 min, depending on the evaporation rate, for periods of 3–4 h.

Initially, one chamber was ventilated with carbon dioxide, the other with air. The water sample in the chamber containing carbon dioxide was observed to evaporate most rapidly. Efforts were made to regulate the flow of the gas by use of flow meters but it was felt that it would be extremely difficult to provide equal ventilation due to differences in temperature and density of the gases involved. Also, significant amounts of carbon dioxide were observed to become dissolved in the water of one chamber. No bubbles were observed to migrate to the surface. The stable bubbles of carbon dioxide that formed on the bottom of the dish suggested that the effect of the gas on the evaporation rate could be examined by bubbling carbon dioxide through the distilled water before placing it in the chamber and omitting ventilation of the chamber. Thus, the effect of dissolved carbon dioxide on the evaporation rate of water could be compared with the evaporation rate of water free of carbon dioxide. A variation of this experiment consisted of

saturating distilled water samples with hydrogen, helium, oxygen, nitrogen, and air, respectively.

As a result of fifteen sets of observations, the water containing the dissolved carbon dioxide consistently evaporated more rapidly than the control water sample. The increase in evaporation varied between 15 and 50 per cent. This was undoubtedly due to the fact that the amount of carbon dioxide dissolved in the water was not kept constant. The gas was bubbled through the water at various temperatures; since solubility decreases with increasing temperature one could expect a smaller effect at higher temperatures. This, in fact, was observed: when the temperatures were higher and the evaporation rates were also higher, the increase in evaporation rate due to carbon dioxide in solution was less.

On the average the measurements revealed a 30 per cent increase in evaporation of the water containing the carbon dioxide over the control samples. No significant difference in evaporation rates was detected when other gases were substituted for carbon dioxide. The results of these experiments suggest the desirability of continuing the investigation with more elaborate experimental design using various gaseous environments.

All possible experimental errors were scrutinized. First, it was suggested that the carbon dioxide escaping from the water surface would sink in the lighter environment of air and set up convection currents which would enhance evaporation of the water. To prevent this, carbon dioxide was introduced into the chamber so that buoyancy effects would be avoided. This did not alter the results reported here. Second, since carbon dioxide strongly absorbs infra-red radiation, we examined the possibility that the sample containing carbon dioxide would absorb more heat from the projector beam than the plain water sample and thereby evaporate faster. To test this, the projector lamp was turned on only for the very short periods of time required for recording the height of the meniscus.

The more rapid evaporation of the sample containing carbon dioxide continued. Third, to ensure that the control and test chambers were identical and that they were subject to essentially the same ambient conditions, many runs were made in which both chambers contained plain water samples. In each case, the evaporation rates were then the same.

Using the most pessimistic estimates of these and various other possible errors, I found that their influence on the evaporation rate is negligible when compared with the observed effect reported here.

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Alignment of Cirrus Clouds along the Magnetic Meridian

Cirrus clouds appear very often in a regular array of parallel bands. Alexander von Humboldt[1] discovered in the course of his meteorological observations in South America and Siberia that these arrays follow frequently the line of the geomagnetic meridian of the locality; he invented the term "bandes polaires" for this phenomenon. Even very casual observations confirm the truth of his statement. He suspected some connexion between the appearance of these 'polar bands' and that of the aurora borealis. In the light of present-day knowledge of the composition of cirrus clouds, it is possible to put forward an explanation of this alignment. Since the constituent ice particles are known to form around dust particles of partly meteoric origin, they will align along the lines of force of the geomagnetic field in those cases in which the dust core consists of a magnetic material. It follows incidentally that the concentration of magnetic meteoric dust in the upper atmosphere should increase from the equator towards the poles, a hypothesis which is confirmed by Sobermann's[2] recent work on the composition of noctilucent clouds above northern Sweden.

Humboldt[3] observed, furthermore, that the alignment of cirrus clouds is more often seen and is of longer duration near the equator than in northern latitudes where their direction gradually changes from that of the magnetic meridian to an east–west line. This movement of the cirrus bands may be due to the action of the jet streams which occur at approximately the same height as cirrus clouds. It seems therefore that observations of the occurrence and movement of aligned cirrus bands may be of use in the investigation of the force and direction of jet streams.

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GEOLOGY

F_2 Quartz Veining in the Manx Slate Series

The geological foundation of the Isle of Man is an aged, complex sequence of slates, flags and greywacke, termed the Manx Slate Series. This thick geosynclinal pile, apparently of Cambrian age, outcrops over the greater part of the Island.

Previous research by me[1] has demonstrated the polyphase structural history of the Manx Slate Series during the Caledonian orogeny. Three distinct episodes of tectonic movement (F_1, F_2, F_3) each formed large-scale folds, a suite of congruous minor flexures and an axial-plane cleavage (S_1, S_2, S_3).

The F_1 fold-system is characterized by steep, acute flexures and the accompanying S_1 is a flow cleavage in pelite. The subsequent F_2 and F_3 folds possess a more open, right-angled style, but are easily distinguished by their difference in orientation. The axial planes of the F_2 flexures are flat-lying or gently inclined while those of the F_3 generation dip steeply and consistently to the north-eastern quadrant. The related S_2 and S_3 are strain-slip cleavages. A syn-tectonic F_1 metamorphism produced the mild, chlorite-zone alteration which characterizes these strata[2].

Throughout the Isle of Man the plicated Manx Slate Series is traversed by conspicuous quartz-veining. Individual veins usually vary from a fraction of an inch up to several inches thick though instances measurable in feet are not uncommon. This veining only rarely accompanies the more severe F_1 deformation. The widespread injection of most of the white vein-quartz occurred during the second (F_2) movement-phase and is characteristically associated in the field with mesoscopic F_2 structures. The veining becomes particularly intense and abundant at loci where minor F_2 folding is severe, emphasizing its syn-kinematic nature. The most favourable host rocks are the uniform pelite of the slate formations and the thinly striped strata of the various banded groups. The veining is less abundant in flaggy units. In the Barrule Slates, the contrast between the dark pelite and the white quartz is particularly striking.

The F_2 quartz-veining adopts three main modes of occurrence controlled to a large extent by the time of emplacement during the F_2 movement. These are: (1) concordant veining; (2) discordant veining; (3) irregular veining.

Concordant veining was emplaced along either bedding or S_1, whichever was the dominant S-plane, during F_2 flexural-slip. After injection, such veining was later implicated in the crumpling and effectively outlines the F_2 contortions. In places the veining is cut by

the undeformed S_2 developed in the host rock during the later growth of the F_2 system by planar-slip.

Discordant veining was emplaced at a later stage in the F_2 movement along the already formed S_2 and therefore transects bedding and S_1. Small veins of this type have the form of thin, planar lenses with a lenticular cross-section. On a larger scale the quartz adopts the form of thick pods or augen which thin out rapidly towards their margins. A favourite site for these larger bodies, up to several feet thick, is along the axial planes of mesoscopic F_2 flexures.

Much *irregular veining* of F_2 age also traverses the Manx Slate Series with no regard for the pre-existing S-planes. The injection of such veining evidently took place throughout the F_2 movement. The earlier influxes are affected by F_2 crumpling and cut by S_2 while the later material is only disrupted by the cleavage. A proportion of the irregular veining, injected at the close of the second movement, anastamoses discordantly through all the F_2 minor structures.

The bulk of the quartz-veining in the Manx Slate Series is of F_2 age. Only small amounts were occasionally injected during the last (F_3) phase of folding. Such veining is restricted to sporadic localities where F_3 minor crumpling is more strongly developed than usual. In the south-western tip of the Island it has been observed along minor F_3 slides and may occupy tension gashes opened in competent quartzite by F_3 bedding-slip.

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Source of the Glencoe Ignimbrites

Ignimbrites[1,2] are a characteristic component of the typically post-orogenic basalt–andesite–rhyolite volcanic association. These pyroclastic deposits are now generally accepted[2] as having been produced by eruptions of *nuées ardentes* in which solid particles, both rock and crystal fragments, glass shards and pumice fragments are suspended by turbulent flow in gas, probably derived in part from the glass shard and pumice material itself[3]. Such a fluidized system[4] forms the basal avalanche of a *nuée ardente*. While associated with several types of volcanic structure, most of those ignimbrite deposits with a volume greater than 10 km^3, and the source of which is known, have been related either to calderas or, for the larger volume (> 1,000 km^3) deposits, to volcano-tectonic depressions[3]. No intrusive igneous body has yet been recognized which, on clear-cut evidence, served as a feeder for extensive ignimbrite flows[3].

At Glencoe, Scotland, an approximately cylindrical mass of metamorphic rocks overlain by a thick pile of Lower Devonian volcanics subsided in two stages within ring-faults. During the cauldron subsidence the early and main Glencoe Fault Intrusions were emplaced as discontinuous ring-complexes up the ring faults[5]. Within the lava sequence, consisting largely of andesites and rhyolites, recent work by me has lead to the recognition of two thick ignimbrite sheets within Groups 2 and 5 (ref. 5). Both ignimbrites are rhyodacites, the lower being a lithic tuff rich in fragments of the underlying rhyolites and andesites, the upper a crystal tuff containing abundant felspar crystals. At the present level of erosion the lower horizon thickens towards the centre of the cauldron suggesting a caldera form at the time of eruption. The presence of Group 2 (ref. 5) rhyolite lavas and of Group 3 (ref. 5) volcanic conglomerates towards the margins confirms this view. The vulcanicity, rather than being "the quietly welling, effusive type", as originally thought[5], was explosive in nature.

Calderas are often the surface expression of the emplacement of ring dykes and complexes in depth by cauldron subsidence[6,7], accepted by most workers[7] as the product of magmatic stoping. Reynolds[4,6], however, has suggested that such complexes may be emplaced as fluidized systems of gas, solid fragments and, if the temperature is high enough, liquid droplets. On reaching the surface, this forms ignimbrite. Part of Reynolds's evidence[6] for fluidization comes from the published description[5] of the type locality of the main Glencoe Fault Intrusion at Stob Mhic Mhartuim, recently studied in detail by me. Here the porphyritoid facies of the fault intrusion is in contact with the downfaulted metamorphic rocks within the cauldron subsidence. The contact is marked by a narrow zone of "flinty crush-rock", originally interpreted as a product of frictional heat generated on the fault-surface during subsidence[5]. As Reynolds[6] suggests, in actuality it is an extremely fine-grained tuffisite formed by gas attrition of the conduit walls during the emplacement of the fault intrusion as a fluidized system. A similar contact is found against the country rocks outside the cauldron subsidence.

On this evidence it is suggested that the two ignimbrite horizons within the volcanic pile were derived respectively from the early and main Glencoe Fault intrusions during two periods of cauldron subsidence. The porphyritoid facies of the fault intrusions contains phenocrysts of plagioclase varying in composition from labradorite to oligoclase, hornblende, biotite and occasional pyroxene in a fine-grained felspathic matrix[5]. The ignimbrites show a more uniform composition. The crystal fragments present are predominantly oligoclase-andesine. Some quartz, not obviously of xenocrystal origin, is found in the upper horizon.

Ferromagnesian minerals, apart from occasional biotite flakes and rare pseudomorphs after hornblende, are generally absent. The ignimbrites thus appear to have been derived from the more acid varieties of the fault intrusion porphyrites, the hornblende and much of the biotite having been destroyed during their emplacement. Hornblendes and some biotites in the porphyrites are often rimmed by corrosion borders of magnetite dust and alteration products, formed by oxidation under near-surface conditions[8]. On eruption this process would be accentuated, producing the abundant magnetite dust now found in the ground-mass of the ignimbrites.

It is generally accepted that ignimbrites form by the explosive vesiculation of a glassy magma with the subsequent transport of the glass shards thus formed in a fluidized system, the pumice fragments representing the initial stages in this process[2]. Glass shard and pumice textures are not seen in the groundmass of the fault intrusion porphyrites but may have been destroyed by recrystallization. As the porphyrites are 'frozen' fluidized systems, pumice fragments may have been 'flushed' from the system during the earlier stage of flow. However, the lack of glass shard textures, which are generally retained despite recrystallization, suggests that liquid droplets, rather than glass shards as such, were originally present. On reaching the surface these would vesiculate by exsolution of gas to give glass shards. It may be that, towards the surface, the liquid droplets coalesce on the conduit walls to form a glassy layer afterwards disrupted to form the angular pumice fragments characteristic of ignimbrites[2].

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CHEMISTRY

A Secondary Process in the Liquid-induced Crystallization of Polyethylene Terephthalate

It is known that amorphous polyethylene terephthalate may be crystallized by treatment with certain organic liquids[1]. With the polymer in the form of a film the process is accompanied by the appearance of opalescence, generally attributed to sub-microscopic spherulites, and is a function of time, temperature and film thickness[2]. In these investigations the thickness was at least equal to 2×10^{-2} cm. On extension of the work to include thinner films, it was observed that not only was there an increase of opalescence on treatment with a typical liquid such as acetone but also that after a certain time, afterwards referred to as an induction period, an almost opaque front was seen to move across the sample.

Use of reflectance and transmission microscopy indicates that the phenomenon is basically an internal one and involves a particulate structure, the size of the particles being of the order of a few microns. X-ray diffraction reveals no sharp distinction between samples which have undergone this change and those removed from acetone just before the process would have occurred. Figs. 1 and 2 show that the induction period is both temperature and thickness dependent, the latter suggesting that the reason why the phenomenon has not been previously noted, if it had taken place, was due in part to a high degree of opalescence which may have masked the process. Although it is premature to conclude a mechanism at this stage, it is suggested that it may be due to an internal fracture effect which occurs when the two main diffusion and associated crystallization fronts approach each other, abstracting amorphous material in two directions and resulting in a void in the centre of the sample. In this context it may be significant to note that it is found that the phenomenon does not apparently happen when diffusion is restricted to occur from one direction only.

We wish to thank Imperial Chemical Industries Ltd. for the polyethylene terephthalate film, and also to thank Mr. P. Blakey for assistance in the X-ray diffraction aspects.

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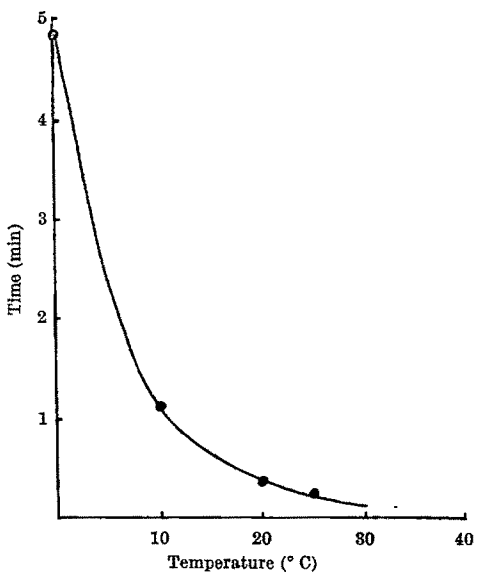


Fig. 1. Induction period versus temperature of acetone

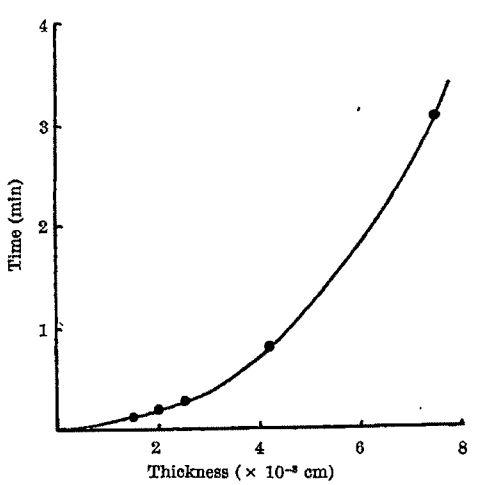


Fig. 2. Induction period versus film thickness

Solvated Electrons in Alkali Glasses

Evidence has recently been obtained which strongly suggests that 'solvated' electrons in water at room temperature have an absorption band in the 15,000 cm^{-1} region[1]. Jortner and Sharf[2] found a similar absorption in frozen aqueous solutions of alkali metal hydroxides at 17,000 cm^{-1} after exposure to 200 kV X-rays, which they attributed to trapped electrons, although they favoured an 'expanded' orbital on the cation as the trapping centre[2].

It has been suggested[3] that 'solvated' electrons, if such species exist, will not normally be produced by radiolysis of molecular solids since there is no opportunity for bulk reorientation of solvent molecules. However, if a frozen ionic solution were irradiated and anion vacancies could be generated by some means, then electrons could well be trapped as in *F*-centres to give the required 'solvated' electron centre.

Table 1. Details of that Feature of the Electron Spin Resonance Absorption Spectrum of γ-Irradiated Aqueous Sodium Hydroxide and Potassium Hydroxide Glasses at 77° K attributed to Trapped Electrons

System	*g* factor (± 0·0005)	Line width ± 1·0 gauss
10 molar NaOH in H_2O	2·0006	16·1
20 molar NaOH in H_2O	1·9995	18·5
10 molar KOH in H_2O	2·0005	11·8
20 molar KOH in H_2O	1·9997	11·6
10 molar NaOH in D_2O	2·0007	6·1
10 molar NaOH in 1 : 1 (by vol.) mixture of H_2O and D_2O	2·0009	11·8

We postulate that this is in fact the reaction observed by Jortner and Sharf, the hydroxyl ion being especially effective as an anion since it is the conjugate base of the solvent and can readily move, on demand, by proton migration. A systematic investigation of such systems by visible and electron spin resonance spectroscopy has been undertaken, and the results seem to support the model. Those features which are particularly relevant are summarized in Table 1 and will be briefly discussed.

There was no marked variation in the visible absorption spectrum with change in concentration of water or in the nature of the cation. On exposure to visible light the band in the 17,500 cm^{-1} region was lost, and a broad absorption in the near infra-red appeared. At the same time, one feature in the complex electron resonance spectrum of the γ-irradiated material was lost.

By subtracting the spin resonance spectra obtained before and after bleaching, an accurate plot of the absorption of the blue entity could be derived (Table 1). In most instances the absorption was symmetrical, and we direct attention especially to the following features:

(1) The line-width is greatly reduced on going from water to deuterium oxide.

(2) There is only a small cation dependence of the line-width, and there is no sign of any hyperfine structure from the cations.

(3) The g-values are almost independent of cation and are close to that found for solutions of alkali metals in ammonia and amines (about 2·0005).

These and other aspects of our results are in good accord with expectation for an 'F-centre' having water molecules rather than cations as the nearest neighbours, but they do not seem to be in accord with Jortner and Sharf's model[2].

The other paramagnetic species formed on γ-radiolysis has properties which suggest an O^- centre with strong environmental interaction. These results will be reported in detail elsewhere.

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Mercuration of Diazomethane

As a possible route to *bis*-oxymethylmercury acetate, $Hg(CH_2CO_2CH_3)_2$, we have examined the reaction of diazomethane with mercuric acetate. Although a reaction has been stated to occur[1] no details of the products were reported. The reaction in ether was slow and heterogeneous. There was no evidence of methylenation of the mercury–oxygen bond, as apparently occurs with aryl-mercury benzoates[2], nor of reduction to mercurous acetate. Instead, when diazomethane (0·03 M) was slurried in ether with dry mercuric acetate (0·015 M), methyl acetate was formed (50 per cent after one day, 90 per cent after five days), together with an insoluble and dangerously explosive pale yellow solid (90 per cent), and small amounts of a soluble yellow oil. This oil, obtained by evaporation of the filtrate and separation from polymethylene on a short silica-gel column, is apparently *bis*diazomethylmercury, $Hg(CHN_2)$, analogous to the long known *bis*(carboxyethyldiazomethyl)mercury[3], $Hg(CN_2CO_2Et)_2$, although the yield (5 per cent) is much lower. It showed a strong localized absorption at 2,105 cm^{-1}, indicating a diazoalkane grouping, and evolved nitrogen with acids. Bromine decolorized it, giving mercuric bromide, nitrogen and some bromoform.

The solid (obtained by filtering and drying) was insoluble in all normal solvents except strong acids which detonated it. The infra-red absorption spectrum (mulls in medicinal white oil and 'Fluorolube') showed no evidence of alkyldiazo groupings, or of hydrogen attached to imide nitrogen or acetylenic carbon, and very little carboxylate. It is suggested that this material is formed by condensation of mercuric acetate with isodiazomethane to form a polymer $(-HgCNN-)_n$. Diazomethane can isomerize in the presence of base[4] and both imide nitrogen and acetylene groups mercurate readily. The infra-red spectrum showed strong bands at 1,960 and 1,710 cm^{-1}, suggesting a group containing cumulative multiple bonds, such as occur in allenes, azides and carbodiimides[5]. After reaction for 20 h, the infra-red spectrum of the precipitate indicated that carboxylate and imide groups were present, suggesting that the initial attack is at the acetylenic hydrogen rather than at the imide group, as with methyl lithium[4].

$$2CH_2N_2 \rightleftharpoons HCNNH$$

$$\downarrow HgAc_2 \qquad \downarrow HgAc_2 + CH_2N_2$$

$$AcHgCHN_2 + CH_3COOCH_3 \qquad AcHgCNNH + CH_3COOCH_3$$

$$\downarrow CH_2N_2$$

$$(-HgCNN-) + CH_3COOCH_3$$

This isodiazo structure is supported by the absence of this type of product when diazoethane and diazoacetic ester are treated with mercuric acetate, since they are not known to isomerize. With excess diazoacetic ester, *bis*(carboxyethyl-diazomethyl)mercury (m.p. 95°–98°, dec., from ether; lit. m.p. 104° dec.) was obtained in high yield. Similarly, diazoethane gave little insoluble material, apart from free mercury.

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BIOCHEMISTRY

Quantitative Spectrophotometric Determination of Indolyl-3-acetic Acid

Indolyl-3-acetic acid (IAA) can be determined either by non-specific biological methods[1] or by chemical methods[2]. Most of the quantitative work with auxins has been based on bioassays. This is probably due to their high sensitivity, and because larger amounts (300 g) of plant tissue are necessary to maintain precision when using chemical methods[3]. This communication describes a spectrophotometric technique which offers a means of precise, sensitive, quantitative estimation of IAA from small amounts of plant material.

'Free' auxins were extracted from the apical regions (including cotyledons) of *Phaseolus vulgaris*, var. Dutch Brown beans, using methanol as a solvent[2]. An aliquot of the crude extract representing 1·5 g fresh material was chromatographed on Whatman No. 1 paper and the identity of IAA confirmed by chromogenic reagents which included *p*-dimethylaminocinnamaldehyde (DMCA)[4]. The solvent was isopropanol/ammonia/water (8 : 1 : 1) and IAA was normally located by spraying a portion of the paper with DMCA. The corresponding R_F region (0·32 to 0·35) from the unsprayed portion of the paper was eluted with 3·5 ml. of methanol and the absorbance of the eluate between 230 and 300 mμ was measured using a Beckman *DK*1 spectrophotometer.

The absorption curve (Fig. 1*B*) obtained from the eluate of the region R_F 0·32–0·35 was identical in its characteristics with that of synthetic IAA (Fig. 1*A*). When the same procedure was followed with a greater quantity (3 g) of fresh material, resolution was unsatisfactory and contamination of the eluate occurred as evidenced by the

second absorption peak at 273 mμ (Fig. 1*D*). This was probably due to interference of an unknown compound obtained from R_F 0·29–0·32 (Fig. 1*C*). The contamination could, however, be eliminated either by the use of less material or by superior resolution, the latter being achieved by allowing the solvent front to flow off the paper.

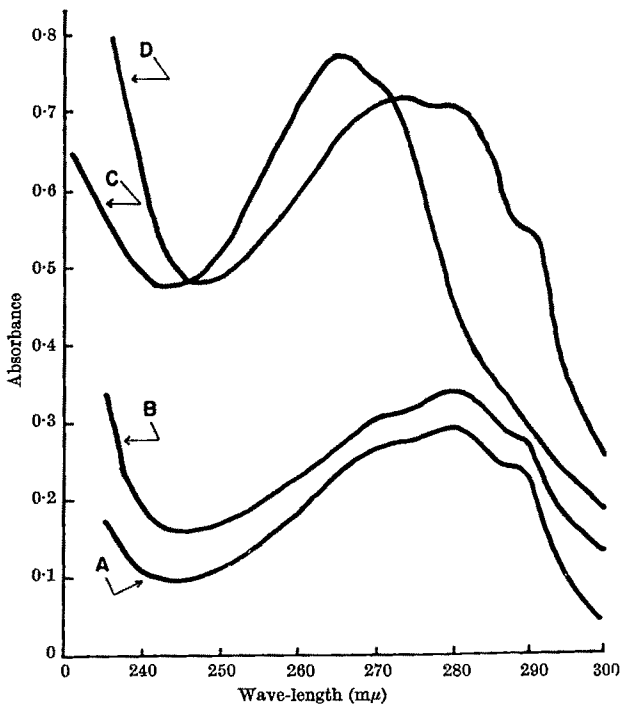


Fig. 1. Ultra-violet absorption curves. *A*, Synthetic IAA (10 μg/ml.); *B*, IAA (R_F 0·32–0·35) from 1·5 g fresh weight; *C*, unknown compound (R_F 0·29–0·32) from 1·5 g fresh weight; *D*, contaminated IAA (R_F 0·32–0·35) from 3 g fresh weight

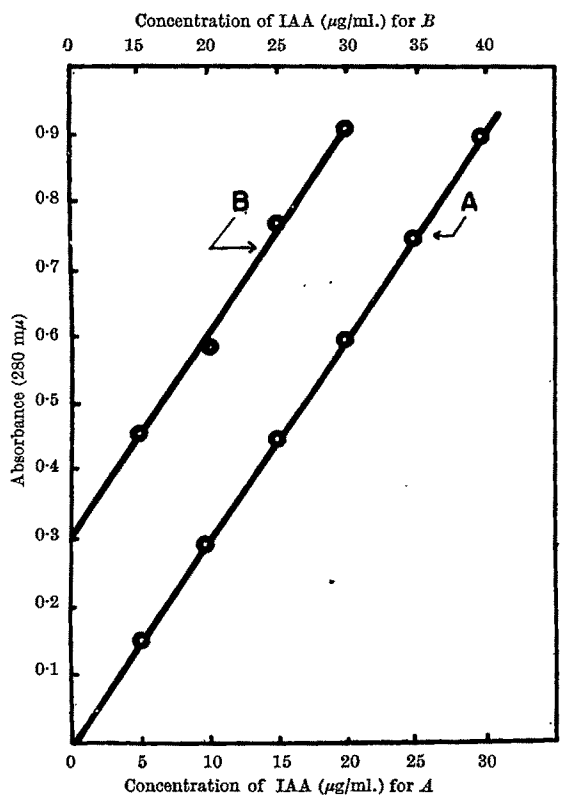


Fig. 2. *A*, Relationship between concentration and absorbance of synthetic IAA in methanol; *B*, IAA extracted from 1·5 g fresh material + 0, 5, 10, 15, 20 μg/ml. synthetic IAA

The absorption at 280 mμ of methanol solutions of synthetic IAA obeys Beer's law over at least the range of 1–30 μg/ml. (Fig. 2*A*). The sensitivity of spectrophotometric measurements was determined to be 0·2 μg/ml. at a concentration of 9–10 μg/ml. When known concentrations of synthetic IAA were added to the crude extract and the same isolation procedure followed, the absorption values of the eluate at 280 mμ were additive (Fig. 2*B*). A mean error of 1·1 per cent (of the mean concentration) was established using 10 replications of 1·5 g of fresh material.

The foregoing procedure represents a simple method for the qualitative and quantitative determination of IAA in extracts from relatively small amounts of plant material. Although this method is somewhat less sensitive than a bioassay it has the advantage of greater simplicity, precision and specificity.

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A New Reticuloendothelial System Stimulating Agent ('Restim') from Shark Livers

We have previously reported a reticuloendothelial system stimulating agent ('restim') derived from cell walls of yeast[1]. The purpose of the present communication is to describe briefly a similarly active material composed of lipid derived from shark livers.

A variety of sharks were caught in the Gulf of Mexico. These included: lemon (*Negaprion brevirostris* (Poey)), nurse (*Ginglymostoma cirratum* (Bonnaterre)), (great) hammerhead (*Sphyrna mokarran* (Ruppell)), (lewin) hammerhead (*Sphyrna lewini*), black nose (*Carcharhinus acronatus* (Poey)), bull (*Carcharhinus leucas* (Muller and Henle)), tiger (*Galeocerdo cuviere* (LaSueur)), brown (*Carcharhinus milberti* (Muller and Henle)), dusky (*Eulamia obscura* (LaSueur)).

Previous experience indicated that species, sex, age, and season of the year may play a critical part with regard to the presence of any specific lipid in elasmobranch liver. In the case of the substance we sought—designated 'restim-*S*'—sex and age made no difference. Lemon, tiger and hammerhead sharks gave high yields. (Season of the year cannot be adequately evaluated, for many species are caught only in certain months in specific areas.)

The lipid which is active begins to deteriorate within 15–20 min after the shark's death. Sharks which have been dead for longer periods are not used. It has been our convention to bring the sharks in alive and kill them at once. The livers are removed. Blood and bile are rapidly drained out of the elongate lobes. The liver is cut into chunks and immersed in cold absolute ethanol for 30 min. The liver is then removed and packed in frozen carbon dioxide and shipped from the Gulf of Mexico to this Institute. Very often these livers, instead of being light yellow-brown, appear much darker and exhibit dark brown or almost black mottling. According to Dr. Ross Nigrelli of the New York Aquarium (personal communication), this is not an infrequent concomitant of stress. Such livers manifest both glycogen and fat depletion, and the discoloration is presumably ceroid and hæmosiderin. Such livers are not used.

One kilogram of fresh shark liver is homogenized in a blender with 2 l. of absolute ethanol. This homogenate is then diluted with 1 l. of ether and 250 ml. of hexane, and then allowed to stand for 2–3 h. It is then filtered. The freshest liver gives the most active lipid; but, because of its freshness, one extraction will not remove all the lipid. Therefore, the extraction procedure is repeated by taking up the wet residue in 1 l. of ethanol, 1 l. of ether and 250 ml. of hexane. It is then filtered once more, the residue discarded, and the solvents are removed *in vacuo*. The concentrate is then taken up in hexane and the hexane layer is filtered through anhydrous sodium sulphate. The hexane is then removed under vacuum, and the remaining lipid is dissolved in anhydrous ether. The ether solution is then treated with five volumes of cold acetone, and the mixture is kept in the freezer at −28° C for 24 h. After centrifuging at −2° C, the yellow acetone-rich supernatant is discarded. The residue is transferred into a flask with anhydrous ether. The ether is removed by distillation, and the lipid is stored at − 28° C under nitrogen. The yield is about 20 per cent by weight of wet shark liver.

This lipid is still only partially purified in this form but quite active in stimulating phagocytosis by the carbon clearance technique. Further purification is obtained by fractional crystallization. The chemically fractionated material obtained is put through a silicic acid column. A colourless, odourless, active fraction is thereby separated from inactive fractions. This is more stimulatory on phagocytosis than the crude lipid by a factor of 3- to 4-fold. The experiments reported herein are with the partially purified 'restim-*S*'.

For intravenous injection, the lipid, 5 mg/ml., is emulsified in 5 per cent glucose containing 0·025 per cent 'Ninol' (Stepan Chemical Co., Chicago, 6, Illinois). The particle size of the lipid emulsion is less than 1–2μ. This is important in the prevention of lipid microemboli.

Standard rabbit pyrogenicity investigations showed no significant temperature rise over a 6-h period with an intravenous dose of 350 mg per rabbit.

Determination of the effect of the partially purified shark lipid restim on non-specific and specific resistance of mice to challenge by *Salmonella typhimurium*[1] was made in the following manner. A pool of 60 mice was divided into six groups of 9–10 mice each. All mice used were males, weighing 20–25 g each at the beginning of the experiment. Group 1 received 'restim' 3 days before vaccination with 0·2 ml. of 1 : 1,000 stock vaccine[1]. 'Restim' was given intravenously, 1 mg suspended in 0·2 ml. of 5 per cent glucose. Fourteen days after immunization, an identical dose of 'restim' was given.

Group 2 mice received identical treatment except that the second dose of 'restim' was omitted. In the case of Group 3, the initial dose of 'restim' was omitted. Group 4 received vaccine only, Group 5 received both doses of 'restim' but no vaccine, and Group 6 received neither 'restim' nor vaccine.

On the twenty-third day following the first 'restim' dose (19 days post-immunization), all mice were challenged simultaneously with 0·2 ml. of a suspension of *S. typhimurium*, determined by plating to contain 3×10^6 viable bacterial cells. Deaths were observed daily for 20 days.

Fifty per cent survival with 95 per cent confidence limits were determined by Litchfield's method[2]. These data, together with percentage mortality, are given in Table 1.

The data indicate that the crude 'restim-*S*' alone neither prolonged life nor increased survival. An increase in 50 per cent survival and in survivorship was shown for all mice receiving the vaccine, as would be expected. When 'restim' was given in addition to vaccine, further increases, particularly in survival time, were demonstrated. Optimum results in increase of survival time were obtained with two doses of 'restim', although the best result for increased survivorship was obtained with one post-vaccinal dose of 'restim'. The statistical significance of this result, however, is doubtful. The data reflect similarity to results obtained with 'restim' derived from yeast[1].

Table 1. Effect of Shark Lipid 'Restim' on Survival Time and Survival in Mice challenged with Two 1,000,000 LD_{50}'s of *S. typhimurium*

Treatment	SD_{50} (95% conf. lim.)	% Survivors (20 days)
'Restim' 1 dose before and 1 dose after vaccine	16·5 (10·9–24·9)	40
'Restim' 1 dose before vaccine	10·5 (7·25–15·2)	33
'Restim' 1 dose after vaccine	8·6 (6·4–11·5)	60
Vaccine only	8·0 (6·0–10·6)	30
'Restim' only, 2 doses	2·4 (2·2–2·7)	0
No treatment	2·2 (1·9–2·5)	0

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A New Metabolic Regulator: its Stimulation of Citric Acid Production and Anti-sulphonamide and Streptomycin Effect

Although at present many data are available concerning the production of citric acid by fungi[1–5], very little is known of the mechanism by which the production of this acid is regulated. Its production in *Aspergillus niger* varies greatly with the strain and the medium[6,7]. The same strain, under various conditions, is capable in one case of converting practically all the sugar available into citric acid; in another case production is decreased by the conditions of the reaction practically to zero.

From the foregoing, it follows that a regulation system exists which controls the utilization of sugar either in the direction of citric acid synthesis or in the direction of biomass production and respiration.

In searching for such a system, we have succeeded in obtaining from the fungus a substance which stimulates citric acid synthesis, and regulates its production (regulator *C*). Regulator *C* may be obtained from various strains of *Aspergillus niger*, and similarly from *A. terreus*. The method of isolation has already been described[8].

Although we have not yet succeeded in isolating regulator *C* in the pure state, we conclude from its properties that it is acid in character, stable at elevated temperatures, capable of dialysis, unstable in alkalis, practically insoluble in lipophilic solvents. Fig. 1 shows the effect of regulator *C* on the production of citric acid for several strains of *Aspergillus niger*, on synthetic and molasses-containing substrates, for a nine-days old surface culture. The *K* 10 strain is a production strain on molasses; in the case of the other strains there are no marked differences between production on synthetic and molasses-containing substrates. The regulator has increased production in all cases, even when production was relatively high as, for example, in the case of the *K*10 strain on molasses.

It has been proved at the same time that regulator *C* does not influence the rate of citric acid decomposition in the case of this fungus.

In further work, the effect of the regulator *C* on the formation of citric acid by ground mycelium has been examined. Mycelium was ground first on a propeller-type mixer, and the operation was finished in an instrument devised by Nossal[9].

The regulator was first separated from the mycelium by means of dialysis. Citric acid formation occurs only in the ground material to which the regulator has been added (Fig. 2). The regulator slows down production of other acids, and synthesis is oriented only in the direction of citric acid formation.

Table 1. Influence of Regulator *C* on the Growth of Micro-organisms

Micro-organism	Inhibitor	Regulator	Turbidity
Streptococcus faecalis	*p*-Toluene sulphonamide 1×10^{-2} moles		
	–	–	1·13
	–	+	2·25
	+	–	0·20
	+	+	1·15
	p-Aminomethyl benzol-sulphonamide 1×10^{-2} moles		
	+	–	0·50
	+	+	1·17
	Streptomycin 10 μg/1 ml.		
	+	–	0·20
	+	+	1·20
Escherichia coli	–	–	1·89
	–	+	2·70
	+	–	0·68
	+	+	2·05

Growth on Bouillon medium has been evaluated turbidimetrically. Measurements were carried out after 24 h of cultivation at 36° C.

It is known from the literature that sulphonamides inhibit very intensively the production of citric acid[10]. According to our experience, this inhibition can be proved only for some strains of *Aspergillus niger*. We have shown that regulator *C* is able to reverse this inhibition, and that even its stimulating effect can be manifested, so that citric acid production is higher than for the control. *p*-Aminobenzoic acid does not have these effects (Fig. 3).

Coenzyme A, which is assumed to be necessary for citric acid synthesis[11,12] and the function of which is, according to Indian authors, inhibited by sulphonamides, has neither been found capable of stimulating production, which agrees with earlier results[13], nor of eliminating the effect of sulphonamides.

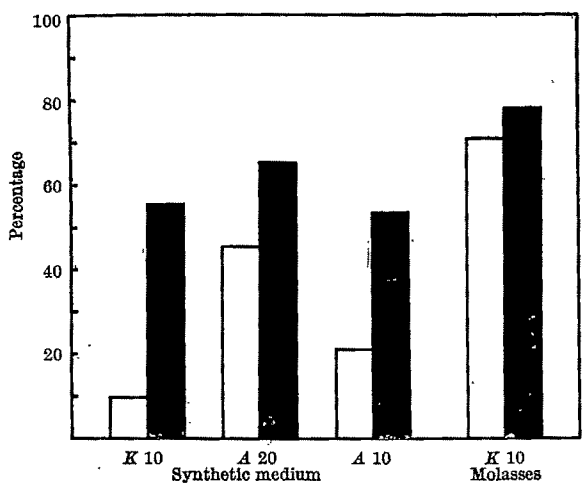


Fig. 1. Influence of regulator *C* on citric acid production, which is plotted as percentage of sugar introduced on the *y* axis. *K* 10, *A* 10, *A* 20 *Aspergillus niger* strains. The first columns express production-levels in control flasks, the second columns express the same value after addition of regulator *C*

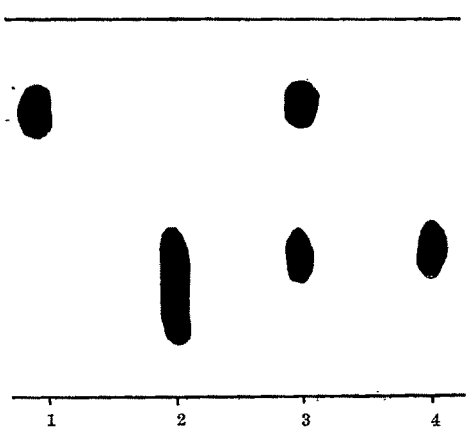


Fig. 2. Production of citric acid by ground mycelium. 1, Citric acid sample; 2, broken mycelium without addition of regulator *C*; 3, citric acid synthesis after addition of regulator *C*; 4, regulator *C*

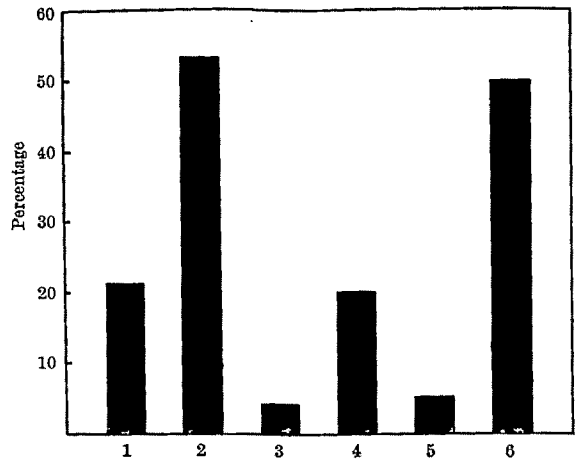


Fig. 3. Elimination of the inhibitive influence of sulphonamides on citric acid production. This production is plotted as a percentage of sugar introduced on the *y*-axis. 1, Citric acid production in the control; 2, citric acid production after addition of regulator *C*; 3, citric acid production after addition of *p*-toluenesulphamide at a concentration of 7×10^{-4} moles; 4, citric acid production after addition of *p*-aminobenzoic acid; 5, citric acid production after addition of *p*-toluenesulphamide and *p*-aminobenzoic acid; 6, citric acid production after addition of *p*-toluenesulphamide and regulator *C*

With regard to the fact that regulator *C* is capable of eliminating the effect of sulphonamides, its effect on the growth of bacteria has been investigated, especially on *Streptococcus faecalis* and *Escherichia coli*. We have found that the regulator substantially stimulates the growth of these micro-organisms, and further that it eliminates the inhibitive effect of sulphonamides and streptomycin on the propagation of these microbes (Table 1).

We have adopted several methods of purifying regulator *C*, and we have tested the preparations obtained with regard to their effect on the production of citric acid, the growth of microbes and the elimination of the effect of streptomycin. We did not, however, succeed in finding any difference in the efficiency of these preparations in the aspects named. This shows that the effects are due probably to the same regulator.

The results of earlier work[6] and of these experiments permit the conclusion that the high production of citric acid by fungi is independent of the formation of a new enzymatic system in the mycelium, as well as of the inhibition of its decomposition, but that it depends on the presence of a regulator, which in the function of a coenzyme controls sugar metabolism in the direction of citric acid production. Its effect on the growth of micro-organisms and the antagonistic influence on sulphonamides and streptomycin, furthermore, show that the substances, or several substances of which the regulator consists, have a more general function in the metabolism of micro-organisms.

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In vivo Degradation of Histamine-adenine Dinucleotide Phosphate to Histamine Ribonucleoside

ENZYME-CATALYSED interactions of histamine with pyridine coenzymes, leading to formation of histamine-substituted dinucleotides, were previously observed in our laboratory[1–3]. These findings were later confirmed by Muraoka *et al.*[4,5] and Devi *et al.*[6]. In more recent experiments, 2^{14}C (ring)-labelled histamine was administered to guinea pigs[7], or it was incubated with mast cell preparations[8] and guinea pig lung mitochondria[9]. In all experimental set-ups, after deproteinization of the homogenized carcass or the incubation mixtures, carrier (non-labelled) histamine dinucleotides were added to the supernatant. After re-isolation and repeated (up to 15 times) purification by alternative ionophoretic and chromatographic procedures, the dinucleotides were examined for radioactivity (autoradiography). By this procedure, traces of *de novo* biosynthesized dinucleotides were invariably detected. In addition, however, unidentified labelled product(s) appeared during the isolation procedures. On the assumption that histamine-adenine dinucleotides are rapidly degraded in the body or in tissues, we recently studied the fate of histamine-adenine dinucleotide phosphate (structurally: histamine-ribose-pyrophosphate(ribose-2′-phosphate)adenine) after intraperitoneal injection of the labelled compound in mice and guinea pigs. This is a preliminary account of our findings in mice.

Young adult male mice (about 25 g) of either the *AKR* or the Swiss strain were used in these experiments. Ten to twenty μmoles of 2^{14}C(ring, histamine)-labelled histamine-adenine dinucleotide phosphate[3], with specific radioactivity of 30,000–50,000 c.p.m. per μmole counted in a windowless flow-counter, were dissolved in 1 ml. of sterile 0·15 M sodium chloride and injected intraperitoneally. Amounts of total radioactivity excreted in the urine of an *AKR* mouse are shown in Table 1. Aliquots from the first day's collection of urine were run ionophoretically[2] at *p*H 7·9 along with appropriate 2^{14}C(ring, histamine)-labelled controls (Fig. 1). The radioactive material from the urine moved towards the negative electrode at the same rate as did histamine ribonucleoside prepared *in vitro* through the consecutive action of nucleotide pyrophosphatase and 5′-nucleotidase[2,10]. The radioactive spot from urine was ninhydrin-positive and, after elution from the paper, part of this material was subjected to acid hydrolysis in a sealed tube (2 N hydrochloric acid, 2 h, 115°). Only after this treatment did the eluate from the paper react in the orcinol test for ribose[11] and coupled with diazotized sulphanilic acid[12], indicating the liberation of ribose and histamine. This is in harmony with previous findings regarding the behaviour of the histamine-ribeos bond to acid hydrolysis[13]. The material in the eluate was free of (esterified) phosphorus[14]. Larger quantities of purified histamine ribonucleoside were obtained from two-dimensional paper chromatograms of the urine. In the first dimension, the chromatogram was developed in 80 parts *n*-butanol, 10 parts 95 per cent ethanol and 30 parts concentrated ammonia[15]. Separation was achieved by development in the second dimension with 0·025 M formic acid/ethanol, 1 : 1. The radioactive material moved as a single band and behaved as did the *in vitro* prepared histamine ribonucleoside. Preliminary evidence indicates that histamine ribonucleoside is also a product of histamine-adenine dinucleotide phosphate metabolism in the guinea pig.

The foregoing results confirm previous *in vitro* observations[5,10], which indicated that histamine ribonucleoside or histamine-adenine dinucleotides were not substrates for oxidative deamination. They are also in agreement with

Table 1. EXCRETION PATTERN OF RADIOACTIVE MATERIAL IN THE URINE OF AN *AKR* MOUSE AFTER INTRAPERITONEAL INJECTION OF 2^{14}C(RING, HISTAMINE)-LABELLED HISTAMINE-ADENINE DINUCLEOTIDE PHOSPHATE

Time (h after injection)	Vol. collected urine (ml.)	Total c.p.m.
24	0·6	477,000
30	0·2	58,000
46	0·7	6,335
54	0·2	470

Male *AKR* mouse of 25 g body-weight; 17·4 μmoles of 2^{14}C(ring, histamine)-labelled histamine-adenine dinucleotide phosphate of specific radioactivity of 34,000 c.p.m. per μmole; other conditions as described in the text. Data from ref. 10.

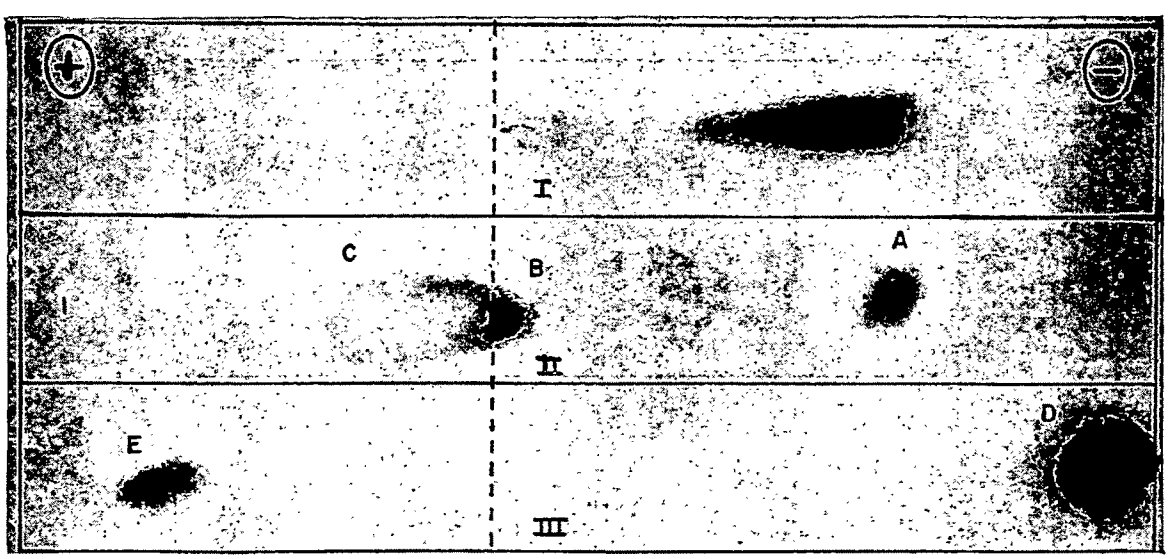


Fig. 1. Relative positions of histamine-adenine dinucleotide phosphate and other 2^{14}C-(ring, histamine)-labelled compounds in a paper ionopherogram. *Tris*-HCl buffer at *p*H 7·9, specific resistance 200 ohms at 4° C, 62 V h per cm. Other conditions as described previously (ref. 2). Spots were applied along the dotted vertical line.

Horizontal line No. I. Aliquot from the first day's collection of mouse urine (see Table 1). Longer exposure to the X-ray film (see below) revealed no additional spots. The longer dimensions of this spot may be due to the salt content of the specimen (urine), although the presence of two closely related compounds cannot be excluded at present.

Horizontal line No. II. Aliquot from an *in vitro* incubation mixture of histamine-adenine dinucleotide phosphate with a partially purified preparation of nucleotide pyrophosphatase from *Crotalus adamanteus* venom (ref. 20) and 5′-nucleotidase (ref. 21). In addition to histamine ribonucleoside (*A*), this mixture contained histamine-adenine dinucleotide (*B*), most probably derived from histamine-adenine dinucleotide phosphate by the action of a 2′-nucleotidase present as a contaminant in the snake venom preparation, and an as yet unidentified product (*C*) (probably histamine ribonucleotide).

Horizontal line No. III. Aliquots from chromatographically purified solutions of histamine (*D*) and histamine-adenine dinucleotide phosphate (*E*) (ref. 3). Spots developed by contact radioautography with Kodak 'No-Screen' medical X-ray film. This photograph is a composite of which horizontal lines I and II of the ionopherogram were exposed to the film for 14 days, while horizontal line No. III was exposed for 3 days. Spots *B* and *E* were also visible under ultra-violet light with a 'Mineralight *SL* 2537' lamp

the view that formation of a histamine-ribose bond represents a catabolic process[16] and it is not involved in the mechanism of histamine release, as proposed by Yamasaki *et al.*[17]. Conclusions similar to ours were also recently reached by Muraoka *et al.*[5] on the basis of *in vitro* studies. According to preliminary evidence which appeared a few years ago[16], histamine ribonucleoside may also be formed by enzymatic interaction of histamine with 5-phosphoribosyl-1-pyrophosphate. However, these findings have not been confirmed as yet in our laboratory. Thus, it is possible that histamine dinucleotides are the sole source of histamine ribonucleoside in tissues of adult animals[19]. In view of this, search for the ribonucleoside among the excretion products of histamine, especially in correlation to the animal species and to the particular state of sensitization of the animal, might prove of considerable interest.

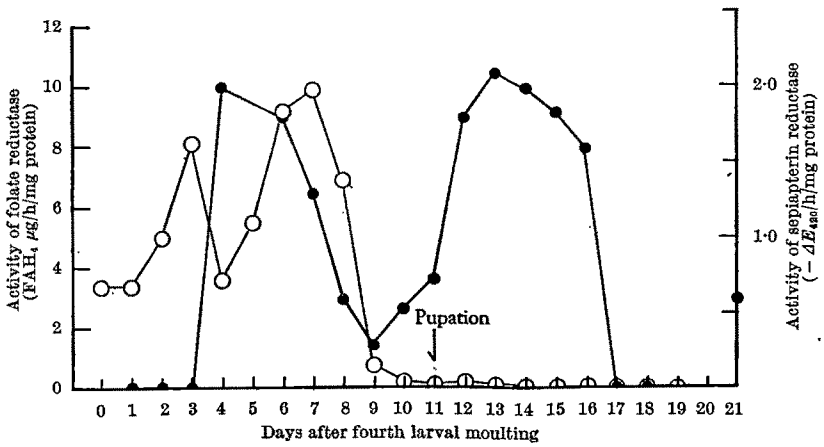


Fig. 1. Activities of reducing sepiapterin (—●—) and folate (—○—) in the developmental stages of the wild-type silkworm

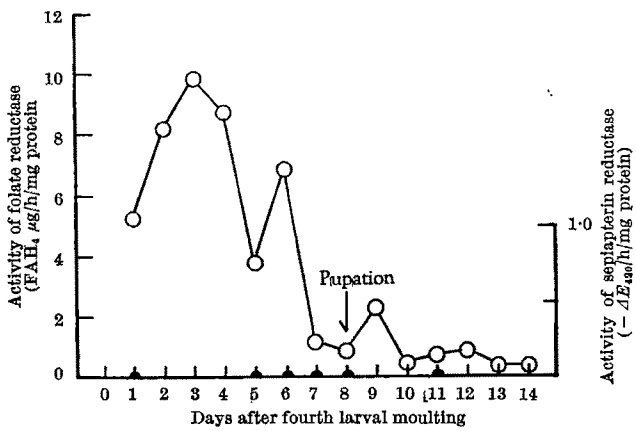


Fig. 2. Activities of reducing sepiapterin (—●—) and folate (—○—) in the developmental stages of the mutant *lemon* of the silkworm

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Occurrence of Two Different Enzymes in the Silkworm, *Bombyx mori*, to reduce Folate and Sepiapterin

The mutant *lemon* of the silkworm is characteristically yellow in colour, accumulating a fluorescent pigment, xanthopterin-B_1, in the skin. Extract of the fat body of the wild-type silkworm converts this pigment into a non-fluorescent product[1]. There is some evidence to support the view that xanthopterin-B_1 is identical with sepiapterin[2], which is known to be reduced, in the presence of reduced nicotinamide dinucleotide phosphate (NADPH), by a folate reductase from chicken liver and *Drosophila*[3,4].

In the acetone-powdered fat body of the wild-type silkworm we noticed that the power to reduce folate was practically lost after six months cold-preservation while the activity to reduce sepiapterin remained unchanged, and we inferred that two different enzymes, folate reductase and sepiapterin reductase, were responsible for attacking the respective substrates. Further experiments with improved preparations also supported this inference.

To obtain sepiapterin reductase solution, the fat body was homogenized with 10 volumes of McIlvaine's buffer (*p*H 5·0) and twice centrifuged (12,000 and 40,000 r.p.m., 30 min each time); clear supernatant was used. Activity of this preparation was determined by measuring the decrease in optical density at 420 mμ ($-\Delta E_{420}$) per h (of incubation at 37° C) per mg protein, of the reaction mixture of sepiapterin[5] (crystallized from the fly mutant *sepia*, 0·02 μmole), NADPH (0·2 μmole), buffer (*p*H 5·0, 30 μmoles), and enzyme (0·1 ml.), brought to a total volume of 1·0 ml.

For the enzyme of folate, the fat body was extracted with 2 volumes of potassium phosphate buffer (0·1 M, *p*H 6·0), centrifuged (12,000 r.p.m., 30 min), filtered and dialysed against distilled water (about 15 h). A mixture of folate (0·136 μmole), NADPH (0·2 μmole), buffer (*p*H 5·0, 100 μmoles), and enzyme (0·5 ml.), brought to a total volume of 2·5 ml., was incubated for 1 h at 37° C and then heated for 3 min over boiling water; the amount of the resultant tetrahydrofolate (FAH_4) indicated the

enzyme activity, expressed in μg/h/mg protein[6]. Protein was determined by the method of Lowry *et al.*[7].

Both preparations from the wild-type independently rise and fall in activity during larval development after the fourth instar moulting (Fig. 1). Rapid and unrecovered loss of the folate-reducing activity can be seen in earlier pupal days when the activity of sepiapterin reductase, once much depressed, starts to rise again. Fig. 2 illustrates the results with similar preparations from the mutant *lemon*, which, as could be expected from the nature of accumulating xanthopterin-B_1, shows no activity of reducing sepiapterin during the whole period. Rise and fall of the folate-reducing activity in the mutant occurs rather like that in the wild-type. Chemical separation of these reductases is in progress.

This work was aided by a research grant from the Ministry of Education.

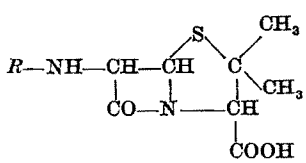
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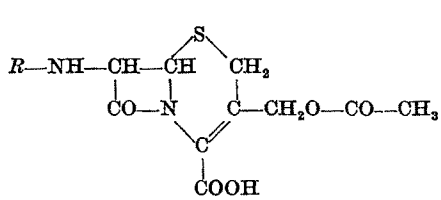
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Microbial Degradation of Cephalosporin C

The antibiotic, cephalosporin *C*, is closely related to the penicillin family and displays the same mode of action[1].



Penicillin *N*



Cephalosporin *C*

R = D-α-aminoadipic acid

Although cephalosporin *C* possesses the β-lactam ring—the substrate site of penicillinase action—it is resistant to this enzyme and, in fact, acts as a competitive inhibitor. It is, however, attacked by a 'cephalosporinase' found in crude preparations of penicillinase from *Bacillus cereus*[2] and by a citrus acetyl esterase[3]. Although little is known about the site of attack by cephalosporinase, its action causes inactivation of the antibiotic with the loss of 260 mμ absorption and liberation of one acidic group. The esterase converts cephalosporin *C* to deacetylcephalosporin *C*, which retains the properties of ultra-violet absorption and resistance to penicillinase but which is a less-active antibiotic. We have now found that these two enzymatic activities are present in many bacteria and actinomycetes but not in the moulds examined.

About 200 freshly isolated marine micro-organisms (we thank T. Demny for providing these isolates and for classifying them into bacterial, actinomycete and fungal groups—they were all isolated from marine environments) were first screened for the ability to grow in the presence of 50 μg/ml. of crystalline cephalosporin *C* (provided by E. Inamine and T. Jacob) and to degrade the antibiotic. The method was similar to that used by Gots[4] to detect penicillinase producers. Each Petri plate, containing cephalosporin *C* and 0·1 per cent yeast extract in nutrient agar and seeded with *Escherichia coli* W, was inoculated with ten different isolates. Sixty-six of the 117 bacterial isolates, 36 of the 43 actinomycete cultures and 37 of the 41 fungi were resistant to the antibiotic and formed colonies at 28° C. The presence of satellite zones of *E. coli* around and under the colonies indicated degradation of cephalosporin *C* since the *E. coli* cells could not grow in the presence of 50 μg/ml. of the antibiotic. Table 1 shows that 70 per cent of the growing actinomycetes and 41 per cent of the bacteria degraded the antibiotics; the fungi lacked this ability.

Table 1. Degradation of Cephalosporin *C* by Marine Micro-organisms

Medium	Parameter	No. of isolates: Bacteria	Actinomycete	Fungi
Agar	Colony formation	66	36	37
	Cephalosporin *C* degradation	27	25	0
Liquid	Cephalosporin *C* degradation*	27	25	—
	Complete cephalosporin *C* destruction*	14	22	—
	Deacetylation of cephalosporin *C**	20	19	—

* Under one or more of the five experimental conditions described in the text.

Further study of the 52 cultures which were capable of degradation on agar was accomplished by exposure of cells to solutions of cephalosporin *C* under five different experimental conditions: (*a*) *Growing cells.* Cells were grown in Bacto Penassay broth containing 1 mg/ml. cephalosporin *C* for 1 day at 28° C on a shaking machine. (*b*) *Resting cells, pH* 7. Cells were incubated without shaking in 0·05 M phosphate buffer containing 4 mg/ml. cephalosporin *C* plus toluene at 28° C for 1 day. (*c*) *Resting cells, pH* 5·5. Same as (*b*) using 0·05 M citrate buffer. (*d*) *'Adapted' resting cells, pH* 7. Cells which were first grown for 1 day in the presence of 1 mg/ml. cephalosporin *C* were centrifuged and exposed to 4 mg/ml. cephalosporin *C* in 0·05 M phosphate buffer plus toluene for 4 h at 37° C with shaking. (*e*) *'Adapted' resting cells, pH* 5·5. Same as (*d*) using 0·05 M citrate buffer.

After the exposures, the incubation mixtures were centrifuged. The supernates (50 μl.) were chromatographed on Whatman No. 1 filter paper for 16 h in the descending direction. The solvent (isopropanol : pyridine : water; 65 : 5 : 30) was allowed to drip off the end of the sheet. Standards of cephalosporin *C* and deacetylcephalosporin *C* were also chromatographed. The antibiotic spots were detected by bioautography on large agar dishes seeded with *Bacillus subtilis*. Deacetylcephalosporin *C* migrated more slowly than cephalosporin *C* under the foregoing conditions.

Judging from the size of the cephalosporin *C* zone and the presence or absence of a deacetylcephalosporin *C* spot, we found that all 52 cultures attacked cephalosporin *C* under one or more of the incubation conditions. Three-quarters of the bacteria and actinomycetes tested showed esterase action (Table 1). Approximately half the bacterial isolates and almost all the actinomycetes were so active under one or more conditions that no cephalosporin *C* spot was detected. Quantitative bioassay of such supernatants by an agar-diffusion technique indicated more than 90 per cent destruction in these cases.

To complement the foregoing, 273 known bacterial species, selected at random from the stock culture collection, including almost all representative genera and/or species, were screened by the resting cell method (*b*). Also included were 25 different genera and/or species of yeasts. These cultures had no known previous contact with cephalosporin *C*. Of the bacterial cultures, 113 or 41 per cent, degraded cephalosporin *C* completely, whereas 16, or 7 per cent, formed the deacetylcephalosporin *C*, without further destruction of the molecule. Only one yeast was found to deacetylate the antibiotic, but none was capable of complete destruction.

Ninety-nine cultures of actinomycetes, freshly isolated from soil samples, were also tested directly in the resting

cell method (*b*) for a short incubation period of only 4 h. Within this period, 9 per cent of the actinomycetes completely destroyed the cephalosporin and another 10 per cent deacetylated the substrate.

Thus, bacteria and actinomycetes are potent producers of enzymes capable of destroying or deacetylating cephalosporin *C*. In no case, however, was a culture found which could remove the aminoadipic acid side chain from the molecule, thereby producing the cephalosporin nucleus—7-aminocephalosporanic acid.

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PHYSIOLOGY

Evidence of the Mechanism of Ouabain Inhibition of Cation Activated Adenosine Triphosphatase

Ouabain has been shown to inhibit electrolyte transport in a number of tissues, including erythrocytes[1,2], brain and kidney[3,4]. Ouabain also inhibits that adenosine triphosphatase obtained from membrane and sub-cellular preparations which is stimulated by sodium plus potassium ions[5–7]. Recent work has shown that this enzyme must play an important part in the mechanism of active ion transport[7–10].

We have been able to demonstrate[11] that a heavy microsome sub-fraction of guinea pig kidney cortex sedimented at 40,000*g* from a tissue homogenate in 0·25 M sucrose and afterwards treated with deoxycholate by a procedure modified from Skou[8] contains in high yield a sodium plus potassium-activated adenosine triphosphatase reaction

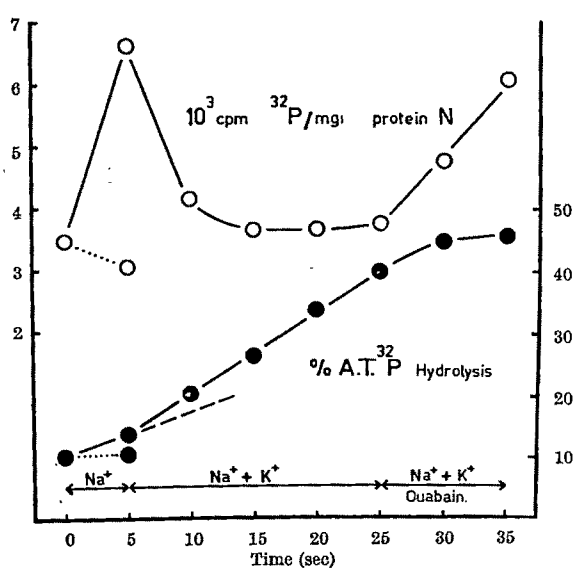


Fig. 1. Incorporation of phosphorus-32 from AT^{32}P into a microsomal adenosinetriphosphatase preparation incubated at 37° in a system to which the sequential addition of 80 mM sodium ions, 20 mM potassium ions and 0·1 mM ouabain was made. O—O, ^{32}P incorporation with Na+, then Na+ and K+, then Na+ and K+ and ouabain; O - - - O, ^{32}P incorporation with K+; ●—●, percentage hydrolysis of AT^{32}P with Na+, then Na+ and K+, then Na+ and K+ and ouabain; ● - - - ●, percentage hydrolysis of AT^{32}P with K+. The results are derived from a typical experiment

Table 1. Incorporation of Radioactive Phosphorus from AT^{32}P into a Na+- + K+-dependent Adenosinetriphosphatase Preparation from Guinea Pig Kidney Cortex

Additions*	c.p.m. ^{32}P per mg protein N	Percentage ^{32}P released from AT^{32}P
None	26,000	8
80 mM Na+	53,000	8
80 mM Na+ + 0·1 mM ouabain	48,000	7
20 mM K+	15,000	7
20 mM K+ + 0·1 mM ouabain	14,000	6
80 mM Na+ + 20 mM K+	18,000	15
80 mM Na+ + 20 mM K+ + 0·1 mM ouabain	31,000	10

* Microsomal enzyme incubated with 0·02 mM AT^{32}P for 10 sec at 37° in 0·05 M imidazole-glycylglycine buffer *p*H 7·6.

Table 2. Binding of ^{22}Na to a Na+- + K+-dependent Adenosine-Triphosphatase Preparation from Guinea Pig Kidney Cortex

Additions*	Specific activity c.p.m. ^{22}Na/mg protein N
None	14,000
ATP	131,000
ATP + 0·1 mM ouabain	150,000
ATP + 2 mM K+	70,000
ATP + 2 mM K+ + 0·1 mM ouabain	87,000

* Incubated for 5 sec at 37° with carrier-free ^{22}Na at *p*H 7·6 in 0·05 M imidazole-glycylglycine buffer.

which is inhibited by ouabain and has other characteristics associated with active ion transport systems[6,7,12].

In experiments using this microsomal ATPase preparation and AT^{32}P as substrate we have been able to demonstrate the incorporation of phosphorus-32 into a phosphorylated compound which appears to be an intermediate in the overall hydrolysis of AT^{32}P to ADP and orthophosphate labelled with phosphorus-32 (ref. 13). Table 1 shows that the level of incorporation of labelled phosphorus into this microsomal material is greatly increased in the presence of sodium but not potassium ions, and is insensitive to ouabain. When both sodium and potassium ions are present together the hydrolysis of ATP is increased, but the level of phosphorus incorporation is reduced to near control values. On the other hand, addition of 0·1 mM ouabain to the sodium and potassium containing system results in inhibition of ATP hydrolysis and increased incorporation of phosphorus into the microsomal enzyme protein.

On the basis of these and other experiments in which sodium and potassium ions and ouabain were added in sequence to the incubation system (Fig. 1) we propose that the overall hydrolysis of ATP by this sodium plus potassium activated ATPase system proceeds by at least two stages. The first step is the formation of an intermediate compound which is dependent on the presence of sodium ions. The second step of the reaction is the subsequent phosphorolysis of this intermediate compound with the release of orthophosphate; this latter reaction is both dependent on potassium ions and is completely inhibited by low concentrations of ouabain.

The suggested mechanism is as follows (cf. ref. 13):

$$\text{Enzyme} + \text{AT}^{32}\text{P} \xrightarrow{x\text{Na}^+} [\text{E} \sim {}^{32}\text{P}]\text{Na}_x + \text{ADP} \qquad (1)$$

$$[\text{E} \sim {}^{32}\text{P}]\text{Na}_x \xrightarrow{y\text{K}^+} \text{E} + {}^{32}\text{P}_i + x\text{Na}^+ + y\text{K}^+ \qquad (2)$$

$$\text{Enzyme} + \text{AT}^{32}\text{P} \xrightarrow{x\text{Na}^+ + y\text{K}^+} \text{Enzyme} + \text{ADP} + {}^{32}\text{P}_i$$

Ouabain inhibits the hydrolysis of ATP by blocking the K+-dependent phosphorolysis and hence turnover of the intermediate complex (reaction 2).

In an examination of the sodium plus potassium activation of an 'intact' membrane ATPase system Whittam has reported an asymmetry of this system which orients the site of sodium activation internal to, and potassium activation external to, the cell surface[14]. Dunham and Glynn[5] have previously shown an antagonism between ouabain and K+ in this reaction, and these findings imply that the site of this antagonism is at a potassium-sensitive activation site, which is probably external to the cell surface, as also suggested by Post and Albright[15].

Preliminary experiments in which sodium-22 was incubated with this enzyme preparation have shown that there is an ATP dependent binding of sodium ions to this microsomal enzyme system, similar to that described by Järnefelt with brain microsomes[16].

The addition of K^+ to the incubation system resulted in a marked reduction in the binding of sodium-22. Ouabain did not reduce this initial sodium binding but at low concentrations could, in these experiments of only 5-sec duration, partially overcome the effect of potassium ions (Table 2). In other experiments of longer duration the effect of ouabain became more pronounced.

These findings also suggest that the action of ouabain is not on the formation of the phosphorylated complex, which is an intermediate in this reaction, or on the binding of sodium ions to it; but rather ouabain specifically inhibits the K^+-dependent phosphorolysis reaction which normally leads to the release of orthophosphate. In the presence of ouabain this intermediate tends to accumulate as its turnover is blocked.

The precise nature of the phosphorylated complex is as yet unknown, but total phospholipid extraction revealed 85 per cent of the total radioactivity (phosphorus-32) in a residual fraction containing all the original protein. This argues strongly against the complex being phospholipid in nature, for example, phosphatidic acid as suggested by Hokin and Hokin[17], and is in accord with the more recent findings of others[3,18–20].

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Polystyrene Membranes for Covering Oxygen Electrodes for Use in Tissue

Bare electrodes for measuring tissue oxygen tension polarographically have several disadvantages. The electrode current depends not only on the oxygen tension at the cathode surface but also on the diffusion constant of oxygen and the blood flow pattern in the tissue in that region. In addition, the electrode current slowly falls when the bare cathode is exposed to tissue or blood. Clark[1] overcame this latter difficulty by using electrodes covered with a membrane for measurements in blood. The remaining difficulties in tissue can be overcome by using a membrane-covered electrode together with a pulsed voltage[2]. In this way the region around the cathode surface which is depleted of oxygen, due to the oxygen reduction process, is limited to the membrane and the thin saline layer behind it and does not extend into the tissue, where there is an unknown diffusion constant and blood flow pattern. The membrane-covered electrode, in contrast with the bare electrode, may be calibrated *in vitro* before use and the calibration will be valid for conditions *in vivo*. A number of membrane materials have been tried, for example collodion, rubber, polyethylene, polytetrafluoroethylene and 'Cellophane'. In the past we have found the most suitable membranes to be latex rubber sheaths. A disadvantage is that the electrodes must be mounted in the sheaths shortly before use, which can be a very lengthy operation. Furthermore, the rubber can move relative to the cathode and cause a change in calibration. Bartels *et al.*[3,4] reported that electrodes for use in blood samples can be covered with polystyrene and used with a continuously applied voltage. We have found that if our electrodes are coated with polystyrene and are used with a pulsed voltage, the more exacting requirements for measurements in tissue are met, and the disadvantages associated with rubber membranes are avoided. Such polystyrene-covered electrodes are independent of stirring effects outside the membrane, have a time response of a few seconds, are not poisoned by tissue and are easy to prepare. Further advantages are that there is no need for a membrane holder in the electrode assembly and the electrode tip can be made small and streamlined. Electrodes with an overall diameter of only 0·3 mm have been made and used satisfactorily in tissue.

The electrode system which we usually use has been described previously[5], and consists of 'Diamel'-coated gold wire 0·2 mm in diameter sealed with cold setting 'Araldite' into silver tubing of 0·3 mm internal diameter and 0·5 mm external diameter. The gold forms the cathode and the silver tube the anode of the electrode assembly. The external surface of the silver tubing is roughened on coarse emery paper to provide a key for the polystyrene. It is wiped clean and dipped into a 7 per cent w/w solution of polystyrene in trichlorethylene. The electrode is inverted and the membrane left to dry for 0·5 h under an electric light bulb. It is then soaked in physiological saline for at least a day before use in tissue. The electrodes are used in conjunction with the polarograph already described[6]. A 0·7-V pulse is applied for 0·5 sec every 10–20 sec. The current is sampled shortly before the end of each pulse.

If the electrode is tested in physiological saline immediately after the membrane has dried, no current flows between anode and cathode. If the end is left dipping in saline the system slowly becomes conducting. This may be accelerated by dipping the electrode for a few minutes in a mixture of 96 per cent ethanol in water saturated with sodium chloride, before it is left in the physiological saline. It appears that a layer of saline forms between the cathode surface and the inner surface of the membrane. Electrodes which have been coated by membranes which are too thin show a large increase in current when the medium outside is stirred. If the membrane is too thick either no current flows at all, or the current which does flow is not appreciably influenced by changes in oxygen tension in the outside medium. When the membrane is of the correct thickness its response to a change of oxygen tension in the outside medium is half complete in 2–5 sec and, when dipped in air-equilibrated saline, vigorous stirring does not increase the current by more than between 2 and 3 per cent. There is a linear relationship between oxygen tension and electrode current up to four atmospheres absolute. There is a background current in the absence of oxygen which is about a quarter of the total current obtained in air-equilibrated saline. This proportion is often greater when the membrane is too thick. The background current has a temperature coefficient of 1·5 per

cent per deg. C, whereas the oxygen reduction current shows no temperature dependence.

These electrodes have been successfully used in tissues over the past year.

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Stereochemical Theory of Olfaction

In his recent article on this subject J. E. Amoore[1] ascribes the odours of substances to the size and shape of their molecules: the olfactory epithelium, he suggests, contains receptor sites of definite shapes and dimensions, and a substance with a molecule of configuration appropriate to fit into one of these sites will have one of the primary odours.

It is well known that some people are unable to detect the odours of certain substances: hydrogen cyanide is a case in point, many being unable to detect it. In fact, a few years ago, R. L. Kirk and N. S. Stenhouse[2] showed that ability to detect it was inherited, a sex-linked recessive gene being possibly involved. There are also other odours (such as vanilla, violets, etc.) which cannot be detected by everybody. In the case of hydrogen cyanide there is a further complication. Those who can detect it describe it as having an almond odour. The same kind of odour, though different in degree, is reported for benzaldehyde and nitrobenzene: however, people who are unable to detect hydrocyanic acid can, I believe, detect both the latter substances.

It would be of interest to hear how these phenomena can be fitted into Amoore's theory. In his article, he refers to the almond odour but not in the present connexion.

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Fullman (preceding communication) has raised the important question of how cases of partial anosmia can be fitted into my stereochemical theory of olfaction[1]. Unfortunately, the question cannot be answered as succinctly as it can be posed, because I have no single comprehensive answer. Each chemical example of partial anosmia will probably need special consideration.

I would anticipate that, by analogy with colour-blindness, certain individuals might be deficient in one type of receptor (or more), and hence lack the ability to perceive a whole class of primary odours. This kind of anosmia has been reported to appear artificially during recovery from vitamin A deficiency[2], and during recovery from accidental damage to the olfactory area by an alcoholic solution of gymnemic acid[3]. The majority of reported anosmias, however, involve not large classes of odours but circumscribed families of chemicals, or even single compounds, as pointed out by Fullman.

Where a family of chemicals cannot be perceived by a subject, it could be suggested that his receptor sites for the corresponding primary odour have an abnormal shape. For example, Guillot[4] found a number of individuals who normally could not perceive the macrocyclic musks, one subject who could not detect the steroid musks, and one who could perceive neither, but this last case was able to smell the nitro musks. Guillot explained this by postulating at least three fundamental odours for the musk sensation. However, Johnston and Sandoval[5] have shown that muskiness is a valid primary category, organoleptically and semantically. I would attempt to explain Guillot's observations by suggesting that his deficient subjects have musk receptor sites which are abnormally short (say 10·5 Å long instead of the normal 11·5 Å), while retaining the usual width of 9 Å. Such a site would exclude the longer steroid and macrocyclic musk molecules while still accepting the shorter, wider nitro musks. More detailed consideration of Guillot's observations will have to await refinement of my postulated dimensions for the normal musk site.

Most of the known subjects with partial anosmia have been tested on far too few compounds for it to be possible to map out the true extent of their anosmia. In the absence of evidence to the contrary, I suggest that such anosmias are to be explained as indicated here, by an abnormal configuration of the receptor site in the deficient individuals.

However, Fullman cites the example of hydrogen cyanide, which some persons are unable to smell, but those who can do so sometimes consider it to have an odour reminiscent of bitter almonds. It should be noted at the outset that Meyer, who made a special study of the odour of liquid HCN and its aqueous solution, reported that the smell of HCN is quite different from that of bitter almonds, with which he considered it was frequently incorrectly associated. He described its odour merely as penetrating and pungent[6]. Actually (assuming that the almond odour is not simply due to an impurity) hydrocyanic acid represents a yet more fundamental difficulty than those mentioned by Fullman, for I am rather at a loss to explain why it has an almond odour at all. Its molecule is very small, far too small to satisfy any of the three receptor sites (camphor, floral and pepperminty) which I postulated[7] go to make up the complex almond odour. In fact I conceded that hydrocyanic acid represents the only odorant example I encountered which really defied all reasonable explanation[8].

I am inclined to treat hydrocyanic acid as a special case for the time being, rather than distort the whole stereochemical theory to include one recalcitrant exception. It must be remembered that hydrocyanic acid possesses a number of special properties, very rarely found in a single compound. It is an exceptionally strongly associated liquid, very chemically reactive, and exceptionally poisonous. Any or all of these three unusual properties might conceivably account for its odour, as follows.

Hydrogen cyanide molecules, even in the vapour state at room temperature, have a remarkable tendency to associate by hydrogen bond polymerization into chains of indefinite length[9]. Lateral association of such chains by dipole attraction might also be anticipated. Perhaps in this way assemblages of hydrocyanic acid molecules build up to sufficient size to satisfy the three receptor sites to produce an almond odour. Alternatively, the chemical reactivity of hydrocyanic acid suggests the possibility that it might combine with some compound present in the nasal mucus or the olfactory epithelium, thereby converting it into a molecule which has an almond odour. Wright[10] noted that hydrocyanic acid does not fit into his (vibrational) odour theory either, and suggested that its odour is produced indirectly, on account of its chemical reactivity. Meyer[6] observed that hydrocyanic acid anæsthetizes the olfactory nerves in a few seconds, so that the odour soon cannot be perceived. Possibly the various primary odour receptors are anæsthetized at different rates. If the other four receptor types were extinguished before the camphor, floral and pepperminty types, then the residual resting discharge in these three would remain

for a while, and might give an impression of an almond odour.

The further complication in the smell of hydrocyanic acid is that those who cannot smell it are nevertheless able to detect the almond odour of benzaldehyde and nitrobenzene (see, for example, Guillot[11]). This means that their neural circuits for recognizing the complex odour of almond are intact, and that one must look elsewhere for an explanation of their deficiency. Small variations in the shapes of the receptor sites will not explain the phenomenon either, because three sites are involved, and multimolecular association has to be postulated in order to fill the receptor site at all. Individual variability in the susceptibility of different subjects' olfactory receptors to the anæsthetizing action of hydrocyanic acid would be a better explanation, in that their receptors might be anæsthetized before they could even detect an odour in the first place. This will explain the very variable assessments of the odour of hydrocyanic acid by different observers. Perhaps the best explanation is afforded by the hypothesis that hydrocyanic acid itself has only a pungent odour, but combines with some substance in the nose to produce an almond-smelling compound. This would be in accord with the evidence that inability to smell hydrocyanic acid is inherited along Mendelian lines[12]. Presumably the precursor of the almond-smelling adduct is absent from the nasal sinuses of the partially anosmic minority[10].

I have dealt in some detail with the paradox of hydrogen cyanide's odour, and some people's anosmia to it, because it represents the sole serious objection to my theory, so far as I am aware. My simple stereochemical theory cannot account for its almond odour without introducing special supplementary assumptions. I consider that the odour of this altogether exceptional molecule is brought about indirectly by one or other of the foregoing mechanisms, and I believe that in due course it will turn out to be 'the exception that proves the rule'.

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A Metabolic Regulating Device based on the Actions of Human Growth Hormone and of Insulin, Singly and Together, on the Human Forearm

There have been several reports from the laboratories of F. G. Young (Ketterer, Randle and Young[1], Korner and Manchester[2], Korner[3]) suggesting that growth hormone directs the action of insulin on muscle 'away from carbohydrate toward protein synthesis'. Although some of the intimate detail of the proposed hypothesis concerning growth hormone action may be erroneous, the basic notion, as advanced by Korner[3], that, in the presence of growth hormone, some actions of insulin are preserved, whereas other effects are blunted, is very attractive. Placed in juxtaposition to observations from this laboratory on the effects of close intra-arterial injection of insulin and of human growth hormone, singly and together, on muscle and adipose tissue metabolism *in situ* in the forearm of man, it provides a clearer picture of the day-to-day role of human growth hormone and of insulin, and thus of their place in the economy of the normal adult.

We propose that, during a day, metabolism is dominated alternately by the action of insulin, or of human growth hormone, or of the combined effects of the two, in a three-phased cycle determined by the intake of food. Exposure to insulin in the 'immediate post-prandial period' encourages storage of carbohydrate and fat (phase I), exposure to human growth hormone plus insulin in the 'delayed post-prandial period' encourages protein synthesis (phase II) and exposure to human growth hormone in the 'remote post-absorptive period' encourages mobilization and peripheral oxidation of fat and retards translocation of glucose into muscle and adipose tissue (phase III).

The hypothesis was suggested by the following previously recorded observations:

(1) Yalow and Berson[4] and Glick, Roth, Yalow and Berson[5] have measured insulin and human growth hormone levels in blood in the basal state and following an oral glucose load. There is a 7- to 10-fold increase in plasma insulin concentration 30–60 min after a glucose meal and plasma human growth hormone falls, usually to zero. Plasma human growth hormone returns to basal values two to three hours after the glucose load, when plasma insulin concentration is still many times higher than its basal value. During the next few hours, plasma human growth hormone continues to rise, while insulin gradually returns to its basal concentration. If no food is taken and fasting persists for several days, insulin usually disappears from plasma[6] and human growth hormone concentration rises to levels reported in patients with acromegaly[5].

We can therefore consider three phases as man cycles between feast and famine (Fig. 1). In phase I, the immediate post-prandial period, peripheral tissues are exposed to insulin mainly. In phase II, the delayed post-prandial period, both insulin and human growth hormone circulate in high concentration. If the food-free interval is prolonged, human growth hormone concentration persists at a high level and insulin almost disappears (phase III, the remote post-absorptive period); that is, human growth hormone acts nearly alone.

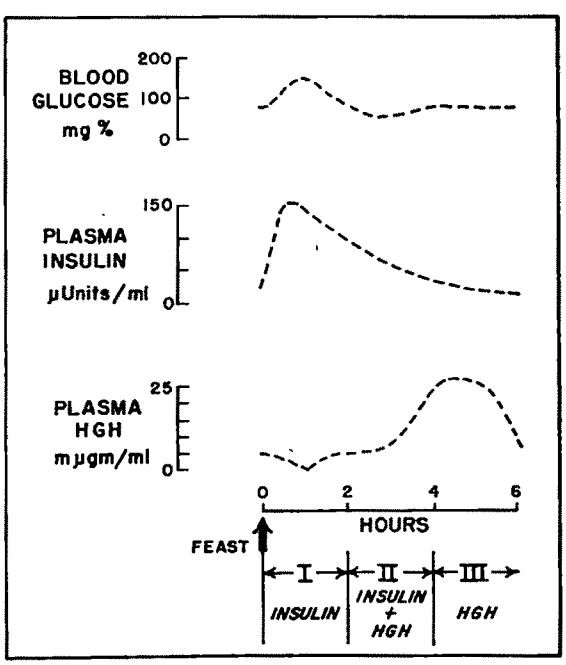


Fig. 1. Suggested variations in plasma insulin and human growth hormone concentrations during one feast–famine cycle. Curves are from Yalow and Berson (ref. 4) and Glick, Roth, Yalow and Berson (ref. 5)

(2) Close intra-arterial injection of insulin alone[7,8] in a concentration similar to that obtaining in the immediate post-prandial period produces the following effects on forearm metabolism in normal man: (*a*) a 10-fold increase in glucose uptake by muscle; (*b*) a 6-fold increase in uptake of glucose by adipose tissue; (*c*) obliteration of free fatty acid release from adipose tissue. The glucose taken up by peripheral tissues is stored rather than oxidized.

(3) Close intra-arterial injection of human growth hormone in normal subjects[9] produces the following: (*a*) enhanced release of free fatty acids from forearm adipose tissue; (*b*) enhanced uptake of free fatty acids by forearm muscle; decreased glucose uptake by forearm tissues.

(4) Present ideas on the interaction of insulin and human growth hormone may be summarized as follows. It has been shown in many species (the cat[10], the dog[11], the rat[12] and man[13]) that growth hormone will induce nitrogen retention only in the presence of 'adequate' amounts of insulin. This has given rise to the notion, reviewed recently by Korner[14], that growth hormone shuts off insulin's action on glucose uptake in order to let insulin exert its protein-anabolic effect. This is not quite our view. An alternative proposal will be made later. Whatever the mechanism, an insulin requirement for the protein-anabolic effects of human growth hormone is recognized. The missing link on which we have required information is: What effect does insulin exert on the fat-mobilizing properties of human growth hormone? To this end the following observations were made:

In six subjects with active acromegaly, the output of free fatty acids from adipose tissue (as reflected by negative arterio-superficial venous free fatty acid differences across the human forearm) was enhanced in the basal state, a finding which is consistent with endogenous over-production of human growth hormone. Close intra-arterial injection of insulin into the brachial artery in doses previously standardized in control subjects met with the anticipated poor response with respect to glucose movement into muscle (Fig. 2) and adipose tissue, but insulin-induced inhibition of free fatty acid release from forearm adipose tissue was at least normal (Fig. 3).

These observations were also true when insulin was administered in similar fashion to three normal men in whom endogenous human growth hormone production was stimulated by a 66-h fast[15], and we have reproduced

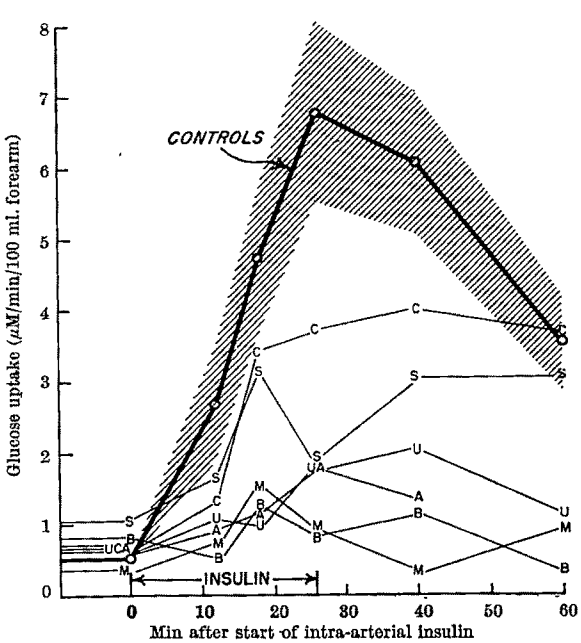


Fig. 2. Effect of insulin on forearm glucose uptake in acromegaly. Shaded area represents mean ± *S.E.* of mean among ten normal subjects (ref. 7). Individual lines indicate glucose uptake in acromegalic subjects, identified by initials

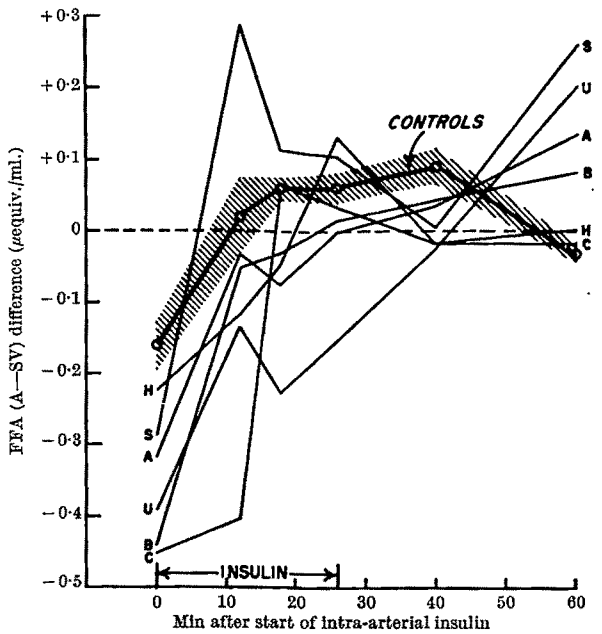


Fig. 3. Effect of insulin on arterio-superficial venous free fatty acid differences in acromegaly. Shaded area represents mean ± *S.E.* of mean among five normal subjects (ref. 8). Individual lines indicate arterio-superficial venous free fatty acid differences before, during and after insulin in acromegalic subjects, identified by initials

the same effect in five normal subjects by intra-arterial infusion of human growth hormone (0·2 μg/kg min) and insulin (100 μU/kg min) simultaneously. In them, there was but little response with respect to glucose uptake by forearm, suggesting that human growth hormone had inhibited insulin action, but there was total inhibition of release of free fatty acid, suggesting that insulin had exerted its full effect. It is therefore incorrect to speak of human growth hormone as inhibiting insulin. It is probably preferable to consider that the two hormones interact to behave like a new hormone that does not stimulate glucose uptake by muscle or adipose tissue but does reduce or obliterate release of free fatty acid from adipose tissue and, we suggest, also stimulates protein synthesis.

We are led to the following synthesis: in the first hour or two of 'feast' (phase I), insulin, acting alone, encourages storage of carbohydrate and fat; after the second hour (phase II), human growth hormone is released but acts solely as an anabolic agent, the levels of endogenous insulin inhibiting its fat-mobilizing action and catalysing its protein-anabolic effects. In the remote post-absorptive period of 'famine' (phase III), human growth hormone acts exclusively as a fat-mobilizing agent since the circulating plasma insulin-level is below that which can either inhibit release of free fatty acid or stimulate the anabolic action of human growth hormone. These notions would resolve the apparent paradox of attributing to human growth hormone a central role in metabolic adaptation to starvation[16], which can be looked on as an exaggeration and perpetuation of the post-absorptive state.

We are therefore suggesting that whether human growth hormone is predominantly protein-anabolic or lipid-catabolic may be a function of the plasma insulin concentration, which, in turn, depends on whether or not the animal has recently eaten. The consequence is that carbohydrate, fat and protein storage is promoted during the post-prandial period, and lipid catabolism and oxidation and glucose preservation is made easy during the post-absorptive period, so that the supply of substrates is smoothed out over the day.

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Photodynamic Effect of Diidroquinidine on Isolated Hearts of Mammals

THE photodynamic action of diidroquinidine chloridrate on *Paramecia*, blood serum and isolated heart of a frog has been observed by Pace *et al.*[1]. Examination of this particular action of diidroquinidine on the isolated heart and the effect of some photodynamic substances (that is, hæmatoporphyrin) on the function of isolated hearts of mammals and amphibians[2,3] suggested the opportunity of investigating the photodynamic effect of diidroquinidine on isolated mammal hearts by electrocardiographic technique.

Hearts from 10 guinea pigs and 10 rabbits were used. The animals were anæsthetized by an intraperitoneal injection of ethylic urethan, 25 per cent, and the hearts isolated by the usual Langendorff technique. Aorta was incannulated and hearts were washed and perfused with a Ringer solution at 37° C and constant oxygen tension. Two platinum electrodes were applied to the right atrium and left ventricle and the ECG was recorded in lead I by an ink electrocardiograph. The mV was 0·5 cm. The diidroquinidine solution was 10^{-4} M in the experiments carried out on the guinea pig hearts and 10^{-3} M in those on the rabbit hearts. Light was supplied by a low-pressure mercury lamp at 6×10^3 ergs/cm²/sec, 50 cm from the heart and filtered through 5 mm of glass so that $\lambda > 320$ mμ.

The diidroquinidine solutions at 37° C were injected into the Ringer perfusion pipe. After 2 min for rhythm stabilization and regularization a first record in the dark and under irradiation showed no modification of the ECG. The diidroquinidine solution was then placed in the dark.

After 2 min the first ECG modifications were recorded. In the guinea pig heart a slowing down of the rhythm could be observed (Fig. 2) followed by a period of tachycardia with elevation of the ST segment. In some cases these modifications shared a monophasic wave pattern. At this stage the records in the dark showed a reduction in the voltage of the ventricular complexes. A further perfusion with diidroquinidine and irradiation determined bradycardia after about a 2 min period; the monophasic wave disappeared and ST returned to the base line with normalized *T* wave. Further perfusions with diidroquinidine in the dark confirmed the bioelectric phenomenon described in the first part of the experiment. Using rabbit hearts (perfusion with diidroquinidine in the dark), a remarkable prolongation of *QRS* with the pattern of a bundle branch block was noted. The same alterations described with the guinea pig hearts could be recorded.

In one case (Fig. 1), we recorded a complete auriculoventricular block 2 : 1, which disappeared after diidroquinidine perfusion and irradiation and reappeared as a complete auricoventricular block after cutting down the irradiation: only the *P* waves could be recorded. At this point the irradiation determined the immediate appearance of abnormal but rhythmical *QRS* complexes that showed a revival of the heart function. After about

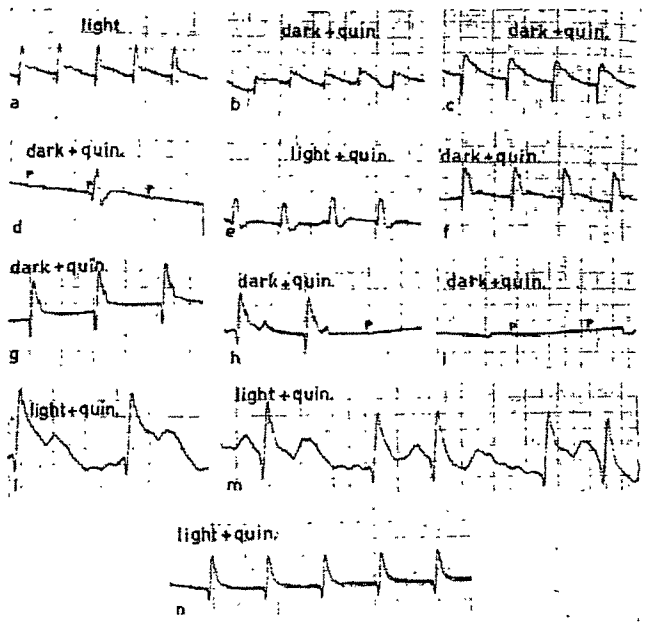


Fig. 1

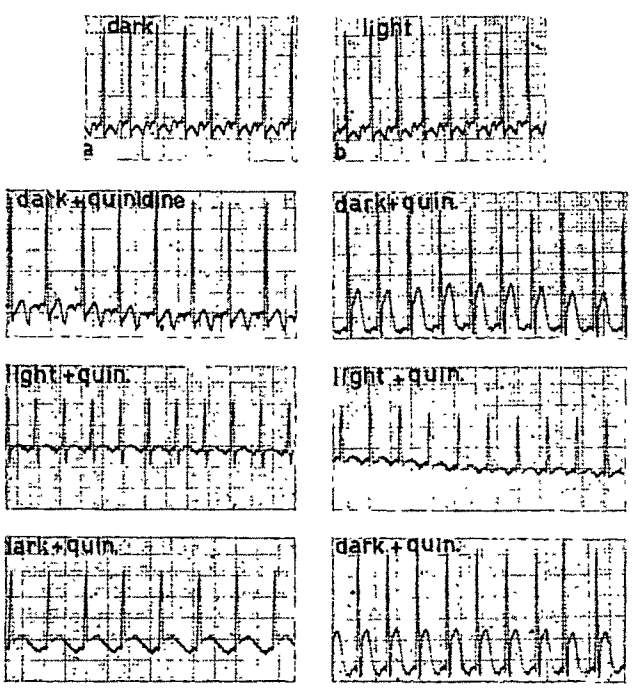


Fig. 2

2 min the rhythm became regular and the records showed the normal (original) pattern.

The modifications induced on the ECG by diidroquinidine chloridrate solutions in the dark can be identified with the well-known effect of this substance on the specific conduction tissue and on the muscles of the heart, that is, a slowing down in the frequency and conduction of the stimulus as far as a partial and complete auricoventricular block, and toxic damage—probably on metabolic grounds. The latter alterations can be observed on the ECG on account of the modifications of ST and of the *T* wave. A monophasic lesion wave can be noted in some cases.

Examination of our records during irradiation and diidroquinidine perfusion shows in all cases the electrical modifications to be interpreted as an improvement of the heart functions. Indeed, the normalization of ST, of the *T* wave and of the ventricular complexes and chiefly the disappearance of the auricoventricular blocks, indicate that the normal action of the diidroquinidine was modified by light so that the substance has, in this case, a photosensitizing function.

This experiment is in accordance with our previous work on diidroquinidine as a photodynamic substance. The importance of the concentration of the substance to the photodynamic action must be pointed out, as while concentrated solutions induce a total and not reversible damage, more diluted solutions improve and regularize the functioning of the heart.

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PHARMACOLOGY

Antitussive Activity of a New Local Anæsthetic Compound : Benzobutamine

Investigations of the relationships between the antitussive activity and other pharmacological properties of many known drugs[1] led us to conclude that local anæsthetic effect was very important in order to predict the antitussive activity of new compounds, especially if this action is accompanied by other pharmacological effects such as the antispasmodic and antihistaminic ones. A systematic investigation was then carried out on new synthetic derivatives possessing local anæsthetic properties in different degrees. The compound *N*(2-benzoyloxymethyl-2-phenylbutyl)-*N*,*N* dimethylamine (benzobutamine)[2] was found to have the most interesting antitussive activity among a series of many other related compounds. Benzobutamine displays local anæsthetic properties and, at the same time, shows antispasmodic, anticonvulsant and antihistaminic effects. In this communication a description of the pharmacological properties of this drug is given.

The local anæsthetic activity of benzobutamine was investigated in rabbits by instilling three drops (0·1 ml.) of an aqueous solution in the conjunctival sac, and observing the disappearance of the corneal reflex elicited by touching the corneal surface with a nylon thread. A comparative examination demonstrated that benzobutamine is as active as lidocaine.

The antispasmodic activity of benzobutamine, studied on the isolated rat gut in Tyrode at 37° C, was found to be the same as that of 'Trasentine®': the concentration effective in reducing 50 per cent of the effect of standard doses of barium chloride is 1·5 μg/ml. for both substances. In a little higher concentration (2 μg/ml.) benzobutamine inhibits also and in the same degree (50 per cent) the effect of histamine on isolated guinea pig ileum. Inhibition of the action of acetylcholine on the isolated rat ileum was produced only by higher concentrations of the product, that is, 5 μg/ml.

Although benzobutamine, given in toxic doses, produces clonic and tonic convulsions, it prevents the tonic phase of maximum electro-shock seizures in 50 per cent of mice when given in doses of 40 mg/kg intraperitoneally. Mice are also completely protected from pentamethylenetetrazol (120 mg/kg intraperitoneally) induced convulsions and death by 80 mg/kg of benzobutamine given 30 min before.

When injected subcutaneously in the rat in doses of 20 mg/kg, benzobutamine produces a slight analgesic effect, as determined according to Randall and Selitto[3].

The antitussive activity (Table 1) was tested in guinea pigs in which the cough was induced by inhalation of acrolein according to the previously described method[1]. The reduction of 50 per cent of the cough was obtained by 3·5 mg/kg subcutaneous injection of benzobutamine. Under the same experimental conditions the isodynamic doses of codeine, benzononatine, dextromethorphan and narcotine were found to be 4 mg/kg, 3 mg/kg, 6 mg/kg and 3·5 mg/kg respectively[1].

Table 1. Antitussive Activity and Acute Toxicity of Benzobutamine and Other Antitussive Agents

Products	Cough inhibition ED_{50} (mg/kg)				LD_{50} (mg/kg s.c.)
	a	*b*	*c*	*d*	
Codeine	4 s.c.	1 s.c. 7 orally	1·5 i.v.	4 s.c.	300 (ref. 7)
Dextro-methorphan	6 s.c.	—	2 i.v.	1–2 s.c. (ref. 5)	275 (ref. 8)
Benzononatine	3 s.c.	> 2 s.c.	1·5 i.v.	> 4 s.c.	230 (ref. 9)
Narcotine	3·5 s.c.	2 s.c.	> 20 i.v.	> 8 s.c.	390 (ref. 10)
Benzobutamine	3·5 s.c.	1 s.c. 5 orally	0·5–1 i.v.	> 8 s.c.	> 1,000

a, Acrolein inhalation in guinea pigs; *b*, aerosol of sulphuric acid in dogs; *c*, electrical stimulation of the superior laryngeal nerve in cats; *d*, electrical stimulation of sub-mucous tracheal tissues in dogs.

In dogs the cough induced by an aerosol of sulphuric acid (0·5 N) according to Winter and Flataker[4] was significantly reduced (56 per cent) by benzobutamine at 1 mg/kg subcutaneously and at 5 mg/kg orally. Codeine, at doses of 1 mg/kg subcutaneously and 7 mg/kg orally, produced the same degree of cough inhibition. Narcotine gave the same results in doses of 2 mg/kg subcutaneously, while benzononatine was found to be ineffective at doses of 2 mg/kg subcutaneously.

Benzobutamine was found to be inactive on the cough induced by electrical stimulation of sub-mucous tracheal tissues by means of chronically implanted electrodes, as described by Stefko and Benson[5], in doses up to 8 mg/kg subcutaneously. In the same test, benzononatine and narcotine were also found to be devoid of any inhibitory effect. Only codeine, morphine and dextromethorphan appeared to be active in reducing this experimental cough.

On the cough response, elicited by electrical stimulation of the superior laringeal nerve in anæsthetized cat according to Domenjoz[6], benzobutamine produced an inhibitory effect in doses of 0·5–1 mg/kg intravenously. The duration of this effect after the latter dose was of about 20 min. The isodynamic doses of other known antitussive compounds were found to be 1·5 mg/kg for codeine, 2 mg/kg for dextromethorphan and 1·5 mg/kg for benzononatine. Narcotine appeared completely ineffective also in doses of 20 mg/kg.

In rabbits and dogs benzononatine moderately depresses respiration only when given intravenously in doses of 2–5 mg/kg. Table 2 compares the effects of benzobutamine and codeine on the rate of respiration of rats (5 per group) previously treated with phenobarbitone (120 mg/kg intraperitoneally).

The results indicate that benzobutamine produces insignificant respiratory depression in doses of 10 mg/kg subcutaneously, while codeine, already at 5 mg/kg subcutaneously, is able to decrease the rate of respiration.

Table 2. Effects of Codeine and Benzobutamine on the Respiratory-Rate (per min) of Rats given Phenobarbitone (120 mg/kg i.p.)

Time (min)	0	10	20	30	40	50	60	80	100	120	140	160	180
Controls	120	126	108	116	116	132	120	168	144	140	124	132	130
Codeine:													
10 mg/kg s.c.	126	112	74	66	45	64	42	42	34	20	62	38	64
5 mg/kg s.c.	120	98	94	101	86	86	94	88	106	98	114	162	166
Benzobutamine:													
10 mg/kg s.c.	122	130	116	108	110	116	138	110	120	116	166	116	133

Each figure is the average of data obtained from five animals. Lines under figures indicate a significant difference ($P \leq 0.05$) from controls.

The acute toxicity of benzobutamine was determined in *CF*-1 mice, and compared with that of other antitussive agents (Table 1). Long-term toxicity examinations were carried out in rats and dogs. No pathological signs were found at the end of a six months' treatment with oral doses of 25 mg/kg and 50 mg/kg in rats, and 30 mg/kg and 60 mg/kg in dogs.

Extensive clinical investigations, carried out according to a double blind schedule, have confirmed the good antitussive activity of benzobutamine in men and the absence of any side-effect[11].

The results obtained with benzobutamine confirm the previously mentioned hypothesis[1] of a relationship between the antitussive activity and other pharmacological properties.

The two most important properties of benzobutamine—local anæsthetic and spasmolytic—may be considered both as peripheral ones, and one could suggest that its mechanism of action resembles that suggested by Bein and Bucher[9] for benzononatine, which is practically the only antitussive agent the local anæsthetic properties of which are particularly marked. According to these AA. benzononatine acts through an anæsthesia of pulmonary receptors which are reached by the drug because of its peculiar tropism. However, other local anæsthetic agents proved to be effective in experimental cough[1], and, moreover, benzobutamine possesses probably a certain degree of activity on the central nervous system as suggested by its anticonvulsant action.

The demonstration of the activity of benzobutamine in man indicates that among the methods of screening for antitussive compounds the inhibition of cough, experimentally induced by inhalation of irritating agents, is to be considered as the most suitable one in order to predict a therapeutic effectiveness.

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Potentiation of the Anti-reserpine Effects of Dihydroxyphenylalanine by Antidepressants and Stimulants

Dihydroxyphenylalanine (DOPA), the biological precursor of the catecholamines[1], causes stimulation of the central nervous system in normal mice[2] and mice treated with reserpine[3]. In the latter the effects of DOPA are dramatic; the mice are aroused from the reserpine-induced depression and become normal or hyperactive in behaviour[3]. Such arousal is thought to be effected through repletion of the reserpine-depleted catecholamines[3].

Inhibitors of monoamine oxidase (MAO) will enhance the repletion of amines by DOPA and will potentiate its central stimulatory effects in normal and reserpinized animals[3]. This potentiation probably depends on a sparing of the substrates of MAO. Other antidepressants, for example, imipramine ('Tofranil®', *N*-(γ-dimethylaminopropyl)iminbibenzyl hydrochloride) and amitriptyline ('Elavil®', 5-(3-dimethylaminopropylidene)-dibenzo [*a.d*] [1,4] *cyclo*-heptadiene hydrochloride), of proved clinical utility are not inhibitors of MAO. The latter compounds had the characteristics of tranquillizers when initially evaluated in experimental animals[4]. It has, however, been shown that imipramine has anti-reserpine activity[4–6]. It has also been demonstrated that imipramine enhances the ability of methamphetamine to increase the rate of rewarding self-stimulation in rats[7], and the facilitating activity of *d*-amphetamine on conditioned avoidance behaviour[6]. It has been suggested that the antidepressant effect of imipramine is the result of sensitization of central adrenergic receptors[4]. We have found that, like the inhibitors of MAO, the non-MAO-inhibiting antidepressants can potentiate the arousing effect of DOPA in reserpinized mice. In addition, the psychomotor stimulant *d*-amphetamine was found to have this activity in doses which cause no overt stimulation.

Female albino mice weighing 18–24 g were used in these experiments. Mice were reserpinized by the intraperitoneal administration of 10 mg/kg of reserpine (dissolved in acetic acid and diluted to the proper concentration with deionized water). Three hours after reserpine, at which time the animals were immobile with complete eye closure, *dl*-DOPA, in graded doses, was injected intraperitoneally and the mice observed for arousal. The criteria for arousal were induction of hyperactive behaviour and reversal of the eyelid closure. The percentage of animals responding at each dose was recorded, and a dose-effect curve constructed. Eight animals were used at each dose. The ED_{50} for DOPA-induced arousal is 1,080 mg/kg (19/20 confidence limits 963–1,264 mg/kg)[9]. The DOPA-potentiating potencies of the test drugs were evaluated as follows. The test drug was administered intraperitoneally in graded doses 2 h after 10 mg/kg of reserpine. One hour later the ED_5 (520 mg/kg) of DOPA, obtained by extrapolation from the dose-effect curve, was injected intraperitoneally. The incidence of arousal was recorded for each dose of the test drug. Dose-effect curves were constructed, and the dose of the test drug (ED_{50}) which caused 50 per cent of the animals to respond to the ED_5 of DOPA was estimated[9]. Neither the ED_5 of DOPA nor any of the doses of the test drugs used caused an arousal response alone. The drugs tested were nialamide ('Niamid ®'), imipramine ('Tofranil ®'), amitriptyline ('Elavil ®') and *d*-amphetamine sulphate. The arousal induced by the combination of the low dose of DOPA and the test drug was grossly identical to that caused by the higher doses of DOPA alone.

Table 1. ED_{50}'s for Potentiating the Arousal of Reserpinized Mice by DOPA

Drug	ED_{50} (mg/kg intraperitoneal)	19/20 Confidence limits
Nialamide	2·3	1·4–3·9
Amitriptyline	3·7	2·4–5·6
Imipramine	5·0	3·7–7·5
d-Amphetamine	0·55	0·33–0·93

Table 1 shows the ED_{50} values with 19/20 confidence limits for the drugs tested to potentiate the arousal of reserpinized mice by DOPA. The psychomotor stimulant *d*-amphetamine is more potent than the antidepressants.

In the case of the MAO inhibitor, nialamide, the effect is likely to be due to its anti-MAO activity and the consequent maintenance of high levels of the substrates of MAO, especially dopamine[3]. The results with imipramine and

amitriptyline reinforce the idea that many of the activities of the MAO inhibitors are possessed by substances which do not inhibit the enzyme. It is not established from our results that a similar mechanism of action is involved in the potentiation of DOPA by psychomotor stimulants and antidepressants. Since special tests[5–8] have shown that imipramine stimulates the central nervous system, it might be that the antidepressants, which do not inhibit MAO and the non-stimulating doses of psychomotor stimulants, exert their effect through a sub-threshold excitation (sensitization) of the central structures involved in the response.

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HÆMATOLOGY

Hæmoglobins of Two Elephant Shrews

The Macroscelididae, elephant shrews of the order Insectivora, have been considered close relatives of some animals often classified as Primates—the Tupaiidae. Certain authors consider that the tupaiids and macroscelidids are very closely related[1]. It is generally conceded to-day that the Tupaiidae—the tree shrews of Malaysia—are Primates and that the Macroscelididae are not[2]. A recent author, nevertheless, still refers the tupaiids to the order Insectivora[3]. However the Tupaiidae are classified, it is clear that the Macroscelididae are Insectivora and that they may well represent a link between the two orders.

Our laboratory has been investigating the evolution of the hæmoglobin molecule among the Primates. We recently received an opportunity to study the hæmoglobin of two East African elephant shrews, *Petrodromus sultani* and *Rhynchocyon chrysopygus*, species of Macroscelididae. Blood was obtained by heart puncture of tranquillized shrews; syringes rinsed with heparin were used. The cells and plasma were separated by centrifugation and hæmolysates prepared in the usual fashion. Electrophoresis of hæmolysates was carried out in vertical starch-gels as described previously[4]. Hæmolysates were dialysed against the same buffer as that used for the gels, 0·027 M *tris*(hydroxymethyl)aminomethane plus 0·020 M boric acid, *p*H 8·6. Tests for alkali-resistant pigments were carried out according to the method of Singer *et al.*[5].

A typical gel is illustrated in Fig. 1. Two bands appear in each of the samples from *Rhynchocyon*. The slower band moved somewhat farther than human hæmoglobin A. Samples of human A, S, and cord blood (F plus A) were run on the gel for comparison. The samples of *Petrodromus* are, in all cases, quite smeared. Examination of the gels with transmitted light showed that two bands appear to be present, both of which have mobilities markedly less than the mobility of human hæmoglobin A. However, in one *Petrodromus* sample (Fig. 1, sample No. 9) in which two bands are also evident, they do not have the same mobility as the two bands found in the other samples. Four samples from *Rhynchocyon* and 12 from *Petrodromus* were tested.

The alkali denaturation test on the samples from *Rhynchocyon* demonstrated a relatively large proportion of alkali-resistant pigment. The mean value for the four samples was 51·9 per cent, and the range was 47·7–54·0 per cent. We had extremely small samples from *Petrodromus* and only three of these were large enough for the alkali denaturation test. The mean value of these three was 5·2 per cent, the range being 5·0–5·6 per cent.

The amount of alkali-resistant pigment in *Rhynchocyon* and the presence of two distinct components after electrophoresis suggest that one of the two bands is the alkali-resistant pigment. There is no similar obvious relationship between the amount of alkali-resistant pigment in *Petrodromus* and the pattern after electrophoresis. All the animals tested were adults, therefore it is clear that these are not fœtal pigments, even should their structural properties prove to be similar to human hæmoglobin F.

The proportion of alkali-resistant pigments found in *Rhynchocyon* is higher than that found in *Galago*, for example, and is similar to the proportions found in *Nycticebus* and certain Lemuridæ[4,6]. A single hæmoglobin sample from *Tupaia glis*, a tree shrew from Malaya, captured near Kuala Lumpur, was examined in our laboratory. The proportion of alkali-resistant pigment was 9·1 per cent[6]. Electrophoresis of the sample showed a single band moving somewhat more slowly than human hæmoglobin A (ref. 7). The difference in proportions of alkali-resistant pigments between *Rhynchocyon* and prosimian Primates is less than the variation among the prosimians themselves. It is the structure and possible special functions of these alkali-resistant pigments that require elucidation. Conclusions with regard to specific problems in taxonomy are still premature.

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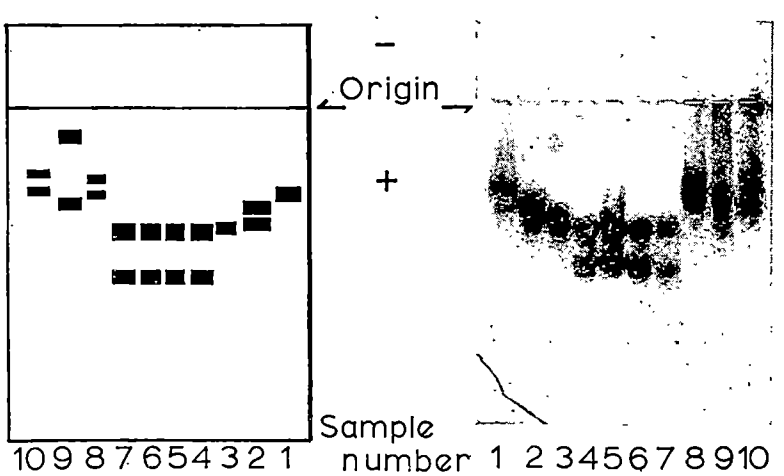


Fig. 1. Diagram (left) and photograph (right) of electrophoretogram of samples from *Rhynchocyon chrysopygus* (Nos. 4, 5, 6 and 7), *Petrodromus sultani* (Nos. 8, 9 and 10), and human hæmoglobins A (No. 3), S (No. 1), and A plus F (No. 2). The diagram was drawn from half the gel after it was stained with amido black. The photograph was made from the half the same gel after it was stained with benzidine. We find that better photographs, with greater contrast, are obtained from the benzidine-stained gels

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Sedimentation Characteristics of the Human Hæmoglobins A, F and E

IT is now well established that normal human hæmoglobin A consists of four polypeptide chains of two types named α and β (ref. 1). The normal fœtal hæmoglobin F also consist of four polypeptide chains of two types, namely, α and γ (ref. 2). Thus the difference between the Hb-A and Hb-F is the substitution of two complete γ-chains for the two β-chains. A number of physical and chemical differences between these two hæmoglobins have been discovered[3] which are undoubtedly associated with this replacement. However, no systematic study of difference, if any, in the sedimentation character of these two hæmoglobins appears to have been made as most of the previous sedimentation studies were concerned with Hb-A[4–8]. This communication describes comparative investigations of the sedimentation behaviour of two normal human hæmoglobins A and F. When the measurements were in progress, samples of pure hæmoglobin E were available from the blood of a homozygous subject (E. E.), whose blood contained 98 per cent Hb-E and 2 per cent Hb-F. Some sedimentation studies were also carried out with this abnormal human hæmoglobin E, which differs from hæmoglobin A by the replacement of a single amino-acid, glutamic acid, by lysine in the 26th residue of the β-chain[9]. No previous sedimentation examination of Hb-E has been carried out.

Hb-A and Hb-F samples were obtained from the blood of normal adults and from that of the cord of new-born babies. The Hb-F content of the different cord bloods, as determined by the method of Singer *et al.*[10], varied from 70 to 80 per cent. In all cases, red cells from the oxalated blood were collected by centrifugation and freed from the serum by repeated washing with isotonic saline. The serum-free red blood cells were hæmolysed with double distilled water and the hæmolysates were freed from the stroma by centrifugation at 3,000 r.p.m. for 30 min.

The concentrations of the hæmoglobin solutions were determined from the measured extinction at 540 mμ with a Zeiss spectrophotometer using $E^{1\%}_{1cm} = 8{\cdot}55$[11]. Rossi-Fanelli[7] showed both from light scattering and sedimentation experiments that the hæmoglobin molecules are easily dissociated. The degree of dissociation increases with increasing salt concentration from 0·1 M to 2·0 M sodium chloride and from 0·1 M to 0·5 M phosphate buffer. Hence, in the present series, all ultracentrifugal experiments were carried out in double distilled water at *p*H 6·8 with a Spinco analytical ultracentrifuge equipped with the schlieren optics and the standard 12 mm cell. The light source used was a General Electric *AH* 6 lamp and Kodak photomechanical films were used for recording the optical patterns.

The schlieren pattern (Fig. 1) in all experiments, characterized by the presence of one coloured symmetrical component, was found to travel as a single peak and no evidence of dissociation was found even at a concentration of 4×10^{-2} g/100 ml. Fig. 2 is a plot of sedimentation coefficient against concentration for Hb-E obtained in the present investigation. For comparison, the results of previous workers for Hb-A are also plotted on the same graph. One measurement on Hb-A carried out in the present experiments is also shown which agrees very well with previous measurements.

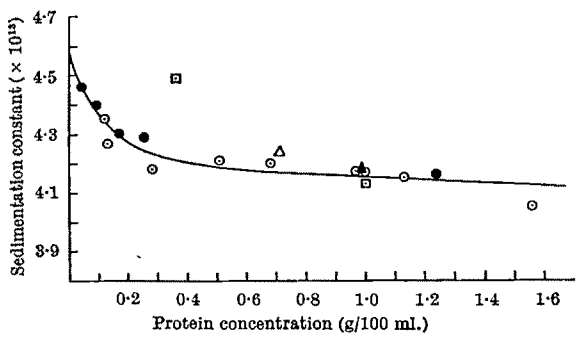


Fig. 2. Concentration dependence of sedimentation coefficient of hæmoglobins A and E. ●, Hb-E; ▲, Hb-A (present work); ⊡, Hb-A (ref. 8); ⊙, Hb-A (ref. 4); △, Hb-A (ref. 5)

It will be found that the sedimentation constants of Hb-E measured in the experiments described here as well as those of Hb-A obtained by previous workers fall on the same smooth curve. For concentrations above 0·3 g/100 ml., the results may be represented by a linear variation of S with C. However, for concentrations below 0·3 g/100 ml., the measured points deviate considerably from linearity and there is rapid increase of S with C. In fact, the present results fit better with two measurements of Kegeles and Gutter[4] at low concentrations than with the linear presentation given by them. The extrapolated value of $S^0_{20,w}$ for Hb-E (or Hb-A) was found to be 4·56. If this value of $S^0_{20,w}$ is combined with the diffusion constant of $6{\cdot}9 \times 10^{-7}$ for hæmoglobin A (ref. 4), one obtains a value of 64,150 for the molecular weight. The amino-acid compositions of the α- and the β-chains of Hb-A are now well established[12,13]. From these chemical studies the molecular weight comes out to be 64,450 in excellent agreement with that obtained from ultracentrifugal studies.

For a molecule with molecular weight 64,000, which is spherical and behaves in solution as ideal compact spheres, with smooth surface and which is not hydrated in solution, the sedimentation constant should have been 5·4 S. The discrepancy between the theoretical value of 5·4 S and the observed ones of 4·56 S (Hb-E and Hb-A) must be ascribed to the facts: (1) that the molecules are highly solvated while sedimenting, thus increasing the hydrodynamic volume and the frictional force; (2) that they do not behave as ideal, compact, hydrodynamic spheres. X-ray diffraction on hæmoglobin show clearly the presence of holes in the molecules and of folds and



Fig. 1. Schlieren pattern of hæmoglobin E, concentration 1 per cent at 56,100 r.p.m., recorded 40, 50 and 60 min after start

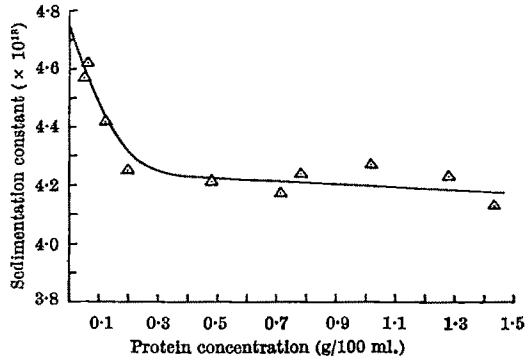


Fig. 3. Concentration dependence of sedimentation coefficient of hæmoglobin F

depressions on its surface where the different chains meet[14]. It is obvious that the hydrodynamic behaviour of such a particle will deviate considerably from that of the ideal compact sphere.

Fig. 3 shows the concentration dependence of S-values for Hb-F. The points are somewhat more scattered than in the previous case due to the fact that the measurements were made on hæmoglobin samples from the cord blood of six infants, where the per cent of Hb-A varied from subject to subject. The measurements on Hb-E, however, were all made with the blood from the same person. Again the results show that for $C > 0{\cdot}3$ g/100 ml. S varies almost linearly with C, while for $C < 0{\cdot}3$ g/100 ml. S increases more rapidly. The extrapolated value for $S^0_{20,w}$ for Hb-F was 4·74 S, slightly greater than what was found for Hb-E (or Hb-A). The difference in the sedimentation coefficient between Hb-A (or Hb-E) and Hb-F may be explained as follows. Different workers[6,7,15] have recognized that hæmoglobin in solution is possibly a heterogeneous mixture. However, a single boundary is observed due to a rapidly established association-dissociation equilibrium. On this ground the difference in S-values between Hb-F and Hb-A represents a difference in the weight average molecular weight of the two mixtures.

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PATHOLOGY

Effect of Thymectomy on the Induction of Skin Tumours by 3,4-Benzopyrene

Many tumours induced by chemical carcinogens[1] or viruses[2–4] have been shown to be antigenic in the syngeneic and even autochthonous host. The fact that antigenically 'foreign' tumours can grow progressively to kill their hosts suggests the existence of factors which compensate for the antigenic disadvantage of the tumour cells. Thus, perhaps, only tumours of weak antigenicity can progress whereas more strongly antigenic clones may fail to develop. Furthermore, tumour progression might be possible only when the host's mechanism for homograft immunity is either poorly developed, as in the new-born[5], or depressed as a result of irradiation[6] or of the chemical carcinogen itself. It is known that a wide variety of carcinogenic agents themselves depress various types of immune response, including the homograft reaction[6]. It has in fact been suggested that the biological *raison d'être* of homograft immunity may be to allow the body to eliminate antigenically foreign neoplastic clones as they arise and before they can spread[7].

An animal with a deficient immune mechanism might be a useful tool for the study of host resistance to spontaneous and experimentally induced tumours. Experiments performed in this laboratory have shown that mice thymectomized immediately after birth fail to reject skin grafts from foreign strains and even from rats[8]. Most of them, however, die from a wasting disease which develops within 4 months, the actual percentage of mice affected and the age at death varying with the strain. Thymectomy at 3 days of age is still associated with some reduction of the capacity for rejecting homografts but only when antigenic differences between donors and hosts are slight[8]. Furthermore, the number of mice dying from wasting disease is considerably reduced when thymectomy is performed later than 48 h after birth. We therefore decided to examine the susceptibility of mice, thymectomized at 3 days of age, to the carcinogenic activity of various hydrocarbons. Preliminary results in one system only are reported here.

Mice of a colony bred albino stock, derived originally from S. Schofield of Oldham, Lancs, were thymectomized or sham-operated 3 days after birth and painted from 5 weeks of age with 50 μg benzopyrene dissolved in 0·2 ml. acetone twice weekly for 20 weeks, the total dose being 2 mg. The solution was delivered on to the dorsal skin by graduated pipette after removal of hair by electric clippers. Other thymectomized and sham-operated mice were treated in an identical way but only with acetone. From 11 weeks onwards a weekly record was kept of the size and positions of all papillomas, and biopsies were performed on all tumours which reached a size of 12–15 mm in diameter. Survivors were killed at 40 weeks and all tumours more than 10 mm in diameter were examined histologically.

Table 1. Effect of Thymectomy on Incidence of Skin Papillomas induced by Benzopyrene

Group	No. of mice*	No. of mice with skin papillomas at following time (days) after first application of benzopyrene										
		70	80	90	100	110	120	130	140	150	160	170
Thymectomized	16	1	3	6	7	8	8	12	12	15	15	16
Sham-thymectomized	22	0	1	1	1	4	6	10	11	19	20	21

* Only papillomas which did not regress are shown.

The time taken for the mice to develop their first papillomas is shown in Table 1. It can be seen that by 100 days after the first application of benzopyrene almost 50 per cent of thymectomized mice had developed papillomas in contrast to only 5 per cent of mice in the sham-thymectomized control group. Table 2 indicates the number of papillomas which regressed and the number which became malignant in the two groups at various

intervals. Fewer papillomas regressed in thymectomized mice than in controls. On the other hand, more papillomas became malignant in thymectomized than in control mice. The total area of papillomas developing in each mouse at various intervals after painting is shown in Figs. 1–3. The histograms indicate that papillomas tend to develop and grow to a larger size earlier in thymectomized than in control mice. One mouse out of 12 thymectomized mice treated with acetone only developed papillomas. None developed in 12 sham-thymectomized acetone-treated mice.

TABLE 2. EFFECT OF THYMECTOMY ON PROGRESSION OF SKIN PAPILLOMAS INDUCED BY BENZOPYRENE

No. of days after first application of benzopyrene		No. of survivors	Total No. of skin tumours	No. of papillomas regressing	No. of tumours becoming malignant
120	Thymectomized	17	34	1 (3%)	—
	Sham-thymectomized	22	36	7 (20%)*	—
150	Thymectomized	16	123	6 (5%)	6 (5%)
	Sham-thymectomized	22	109	18 (16·5%)*	1 (1%)
180	Thymectomized	14	134	14 (10%)	16 (12%)
	Sham-thymectomized	20	189	33 (17%)	8 (4%)*

* $P < 0.05$

In considering these results, we are comparing carcinogenesis in two groups of mice: in one group the mice have had a depression of the homograft rejection mechanism from the neonatal period and may, therefore, be expected to allow neoplastic clones of altered antigenic constitution to grow freely; in the other group the mice initially had normal immune mechanisms but these may have become depressed progressively as a result of repeated application of the carcinogen. If we assume that some of the tumours arising in the present experiment are antigenic in their host and that benzopyrene depresses immunological capacity, we would expect thymectomized mice to show a higher incidence of tumours and a lower regression-rate, and differences between the two groups to become less with time. The results presented in fact indicate that tumours arise earlier in thymectomized mice, that the regression-rate is higher in the controls and that these differences are less later. These results are in harmony with the data obtained from rats thymectomized at birth and injected with polyoma virus[9]. Such rats are more sensitive to the oncogenic effect of the virus than are normal and sham-operated controls; they show a higher incidence of tumours and a prolongation of the neonatal period, during which they remain susceptible to

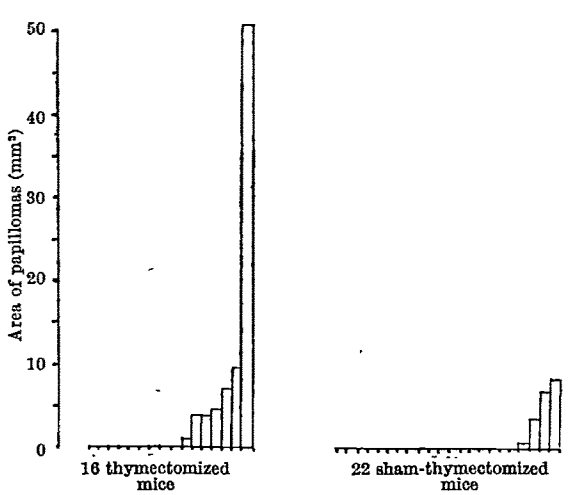


Fig. 1. Incidence and area of papillomas in mice 100 days after first application of benzopyrene. The difference in area of papillomas in controls and thymectomized mice is statistically significant ($P < 0.001$)

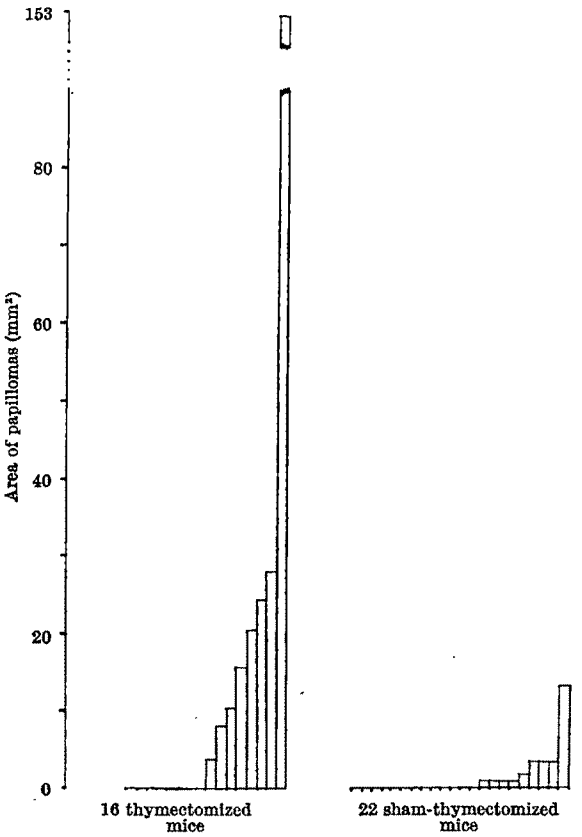


Fig. 2. Incidence and area of papillomas in mice 120 days after first application of benzopyrene. The difference in area of papillomas in controls and thymectomized mice is statistically significant ($P < 0.001$)

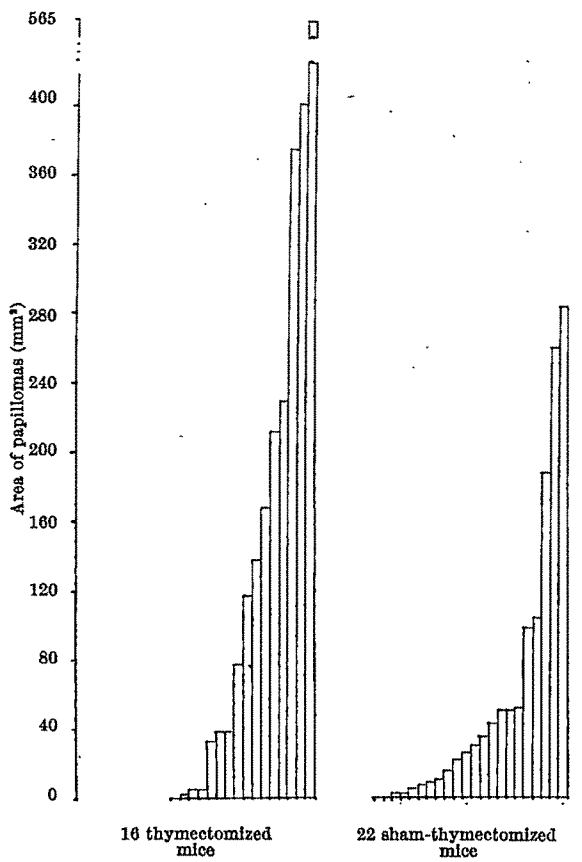


Fig. 3. Incidence and area of papillomas in mice 160 days after first application of benzopyrene

the oncogenic effect of the virus. It may be, therefore, that interference with the immune mechanism is an important pre-requisite for successful carcinogenesis.

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Wine as Source of Iron in Hæmochromatosis

The belief that there is increased absorption of iron from a normal diet in idiopathic hæmochromatosis is based on assumptions of normal dietary iron during the time the disease is developing. Studies of iron absorption late in the disease have led to conflicting conclusions[1,2]. This communication directs attention to wine as a possible source of increased dietary iron in hæmochromatosis.

A close association exists between the use of alcohol and hæmochromatosis; 20–80 per cent of hæmochromatotics are alcoholic. It may be inferred that additional patients consume alcohol in lesser quantities. Increased tissue iron has been found in 40–80 per cent of patients with 'alcoholic' or nutritional cirrhosis in several countries of the world[3–5]. The amount of iron may frequently be as great as in hæmochromatosis.

Investigations of the iron content of alcoholic beverages from various countries reveals that wine, in contrast to distilled beverages, is a rich source of iron, chiefly in the ferrous form, with an average of 4·4 mg/l. in U.S. varieties consumed most commonly by alcoholics in Boston; an average of 6·2 mg/l. was found in 22 French varieties from Burgundy, Bordeaux, and Romagne; and 10–16 mg/l. in cider and wine from Rennes, France, where iron diseases are especially common.

The iron appears to be derived chiefly from pipes and equipment used in preparation of wine, and is well known to the wine industries[6]. The copper content of French wines is approximately 1 mg/l., derived from copper sprays of the grapes and from equipment. Prior to the Second World War the iron content of U.S. wines was considerably higher, up to 35 mg/l. From about this time attention was directed in the wine industry to lowering the iron content because it is an important contributor to the formation of sediment, and the U.S. market prefers sediment-free wines. Chronic alcoholics in Boston consume large quantities of wine in preference to distilled spirits because it is cheap; 2 l./day is not unusual and is probably a minimum figure. Chronic alcoholics in France consumed an average of 2·5–5 l./day, and 50–116 non-alcoholic controls drank 1–2 l./day in an investigation[7].

The normal Western diet contains 10–15 mg/day of iron, of which approximately 10 per cent or 1–1·5 mg/day is absorbed and excreted. If iron is added to the diet it continues to be absorbed[8], resulting in increased body stores. Hæmochromatosis is a disease of middle age, and is characterized by a body store of 20 g or more of iron in contrast to the normal 4–5 g. Absorption of 1·8 mg. of iron a day over the normal amount for 30 years, or of 2·5 mg/day for 20 years accounts for the quantity of body iron in hæmochromatosis. With absorption of 17·8 per cent[8] of dietary iron, consumption of 2 l. of particular brands of present-day U.S. wines would result in absorption of 1·8 mg of iron. Non-alcoholic Frenchmen in Rennes consuming 2 l. of wine with 16 mg of iron/l. and with 15·1 per cent absorption[8] would absorb 5 mg. Alcoholic Frenchmen in Rennes consuming 5 l./day of the same wine, assuming 12·6 per cent absorption[8] would absorb 10 mg. It is possible that absorption would decrease with increased body stores, but even assuming 10 per cent absorption this would alter the foregoing figures to 0·8 mg iron for 2 l. of U.S. wine, 3 mg for 2 l. Rennes wine, and 8 mg for 5 l. of Rennes wine. Consumption of 2 l./day of U.S. wine prepared prior to the Second World War, with its iron content 30 mg/l., would result, with an absorption of 12·7 per cent[8] in absorption of 7·6 mg iron, and with 10 per cent absorption in 6 mg.

Ingestion of iron and copper containing wine or other beverages can explain the characteristic features of hæmochromatosis, and is consistent with the concept that it is a variant of nutritional or 'alcoholic' cirrhosis or of the factors that lead to cirrhosis[9]. The occasional familial occurrence of the disease, and of elevated serum or tissue levels of iron in relatives is commonly presumed to be due to genetic inheritance. Detailed dietary histories and personal dietary analysis have not been carried out during the developmental years of the disease, however, nor in family members. A familial occurrence of elevated serum or tissue iron in the presence of ingestion of a common dietary substance such as iron obviously need not be genetically determined. In as much as the source of iron in wine appears to be from equipment used in preparation, other alcoholic beverages, particularly from early years when iron equipment was more prevalent, or 'home made' alcoholic preparations, might be a rich source. Other factors in cirrhosis, such as diet, pancreatic disease, and anæmias, could further enhance iron absorption[9] but need not be invoked. Additional sources of oral iron are non-prescription medical preparations taken commonly in many countries, including the United States. The amounts of iron in wine and in medicines are not harmful, even when accumulated in large quantities in the liver, and so are not contra-indicated, but an understanding of the source of iron in hæmochromatosis will clarify many controversial beliefs about the disease, and about iron metabolism.

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RADIOBIOLOGY

A Fast-neutron Source for Radiotherapy

In a recent note, Lomer and Greene[1] have recommended the development of a 14-MeV fast-neutron source for radiotherapy. We feel that there are disadvantages to this approach. The relative biological effectiveness of fast neutrons is related to their linear energy transfer, which in turn varies inversely with the energy of the neutron[2,3]. It has been shown that cell-killing by 1–2-MeV neutrons (linear energy transfer, 50–60 keV/μ) is relatively independent of oxygen[3]. As the energy of the neutrons is increased, the effect becomes more oxygen-dependent[4]—30-MeV neutrons would have a linear energy transfer similar to 250-kV X-rays, and hence a similar oxygen-dependence. If one is to expect an improvement in the results of radiotherapy due to the relative oxygen-independence of the effect of fast neutrons, then one must attempt to use those neutrons the effects of which are relatively oxygen-independent; this cannot be claimed for 14-MeV neutrons (linear energy transfer 20 KeV/μ). A useful increase in effective linear energy transfer with depth of penetration in tissue has yet to be demonstrated: the neutrons will lose energy and become more densely ionizing but the γ-ray component of the total dose rises.

Lomer and Greene concede that in the *D–T* reaction, 14-MeV neutrons are produced isotropically and do not suggest how they intend to collimate a useful beam of neutrons for localized radiotherapy without introducing extensive γ contamination and lowering the average linear energy transfer of the beam. By contrast, the bombardment of a thick target in a cyclotron produces a beam of fast neutrons projected mainly forwards, and this demands less collimation[5]. Any desired neutron energy may be obtained by appropriate choice of cyclotron size to produce the required energy of bombarding particle. Since fast-neutron irradiation seems to offer the promise of improved tumour response[6], it deserves to be carefully tried using equipment which produces the energy of particle most likely to give this improved response. The choice of a machine which only produces the less-effective 14-MeV neutrons solely because it is less costly, would be a disservice both to the radiotherapist and to clinical science in general.

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Berry, Oliver and Porter have ably restated the facts we attempted to summarize in the second paragraph of our letter, but do not appear to have grasped the need for a compromise between the desirable high linear energy transfer associated with low-energy neutrons and the desirable penetrating power associated with high-energy neutrons. We do not suggest that 14-MeV neutrons give the best compromise of this type, but merely that since they are obtainable from a relatively cheap and compact source, their usefulness is worthy of experimental evaluation.

We do not dispute that a cyclotron is a more versatile neutron source than a *D–T* generator, but we do claim that the merits of low cost and compactness of the latter are very relevant to radiotherapy. We would certainly not agree that economic consideration can be entirely dismissed, as Berry, Oliver and Porter seem to suggest, though their imputation that cost is our sole consideration is, of course, incorrect.

It is perhaps worth enlarging a little on the importance of compactness. In radiotherapy accurate direction of the beam at the tumour is a primary consideration, and this is best achieved by using a flexibly mounted radiation source so that the beam may be moved with respect to a comfortably placed patient, rather than the patient 'lined up' to the machine: compactness is thus important because it facilitates accurate treatment.

We are, of course, well aware that designing a neutron collimator is a problem, and the solution of this problem is naturally an important part of our development programme.

Furthermore, even if 14-MeV neutrons prove to have no biological advantage over megavoltage X-rays, the *D–T* source may still be competitive with high-energy X-ray units, in so far as there are reasonable grounds for believing that they will produce similar dose distributions inside a patient.

For these reasons, to talk of a "dis-service to science" (as in their last lines) displays an emotional reaction which, when speaking of a research project, is scarcely justifiable.

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Lomer and Greene have recently proposed the development of a source of monoenergetic 14-MeV neutrons for radiotherapy[1]. Berry, Oliver and Porter have commented in the foregoing communication that such neutrons would have too high an energy to give the advantage over X-rays of independence of the biological effect on oxygen concentration which has been found with lower energy neutrons.

There are two distinct aspects to this discussion. One is the discussion of the 'biological' properties of such a beam compared with those of X-rays. The other is a possible 'economic' advantage if simple, cheap apparatus could produce a beam of penetrating radiation, even if the biological properties are not different from those of X-rays.

I agree with Berry, Oliver and Porter's comment that 14-MeV neutrons would have too high an energy, and consequently would give rise to proton tracks in tissue having too low a linear energy transfer, to give a significant advantage over X-rays in respect of the oxygen independence[2]. There is little doubt that the optimum neutron energy for obtaining the best compromise[3] between oxygen-independence[4–6], requiring low neutron energies, and good penetration of tissue[7], requiring high energies, is (giving wide limits) between 3 and 10 MeV mean energy. The small doubt that remains is due to the lack of information about the degradation of neutron energy with penetration into tissue, but rough estimates make this appear extremely unlikely to give a useful gain in ionization density with depth in tissue for neutrons of higher energies. There would be no 'biological' advantage in using neutrons which are too fast to give an oxygen-dependence significantly different from that of X-rays. Indeed, there may be some dangers in doing so until further investigations have been carried out on the effects of fast neutrons on cartilage, connective tissues, and other structures[8]: such work is in progress on the Medical Research Council cyclotron at Hammersmith.

It is true that an 'economic' advantage over cobalt-60 or linear electron accelerator sources would arise if a beam of radiation having penetration and output similar to that of 4-MV X-rays could be generated from simple, cheap

apparatus, as envisaged by Lomer and Greene, even if the oxygen-independence were no better than for X-rays. The main problem is to obtain adequate output. An associated problem is to keep the γ-ray proportion small. Both problems are difficult for 14-MeV neutrons, since an effective collimator would have to be very long.

Even if these problems could be solved, the advantage would be economic rather than biological, at these linear energy transfers. It should not be confused with the fundamental advantage of oxygen-independence in radiotherapy, which can be expected on radiobiological grounds[4,5,9] to give improved cure rates, and which may be approached by a number of methods[2]. High-pressure oxygen breathed by the patient during X-ray treatment[10] or a beam of fast neutrons of optimum energy[8,11] (3–10 MeV) are the only two methods immediately available, although a beam of negative π-mesons has been suggested[12]. There is no virtue in building more sources of radiation of a relatively inefficient type (low linear energy transfer) just because they are cheap; their existence may displace, or obscure the need for, more effective beams of radiation.

It would be most useful if Lower and Greene could design a neutron generator using a lower energy exoergic reaction and giving an adequate output with a small proportion of gamma rays. Pending this development, it is important to remember that small cyclotrons are now commercially available in the United States for about £60,000 for 4·5-MeV, £100,000 for 6-MeV and £300,000 for 8-MeV deuteron energy. These prices are not so far above the costs of linear electron accelerators which are at present used for radiotherapy—but which do not give the biological advantage of oxygen-independence—that cyclotrons can be ruled out of consideration for radiotherapy in the future.

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Influence of Fractionation on Clinical Response and Dosage in Radiotherapy

In general, the clinical and biological response to irradiation is directly proportional to the dose and inversely proportional to the length of time over which the total dose is administered—the so-called dose-time relationship.

In clinical radiotherapy by external irradiation, using beams of X- or γ-rays, the total dose has commonly been divided up into a variable number of fractions each of which is given daily (Sunday and often Saturday excluded), over a total period of several weeks.

By using these prolonged fractionated techniques, quantitatively larger doses can be administered to patients with safety than when the total dose is given in one treatment. Experimental and clinical observations in the 1920's, furthermore, led to the belief that such fractionation enhanced the greater differential destruction of malignant cells by irradiation, a conception open to some challenge in recent years[1].

Experimental work in mice has shown that in both a carcinoma[2] and a rapidly growing sarcoma[3] an interval of 48 h, between fractions of irradiation, gave better results than intervals of 12, 24, 72 or 120 h. Techniques reducing the number of fractions during courses of X-ray therapy, but maintaining the overall period of the treatment, have gradually been evolved in this Department since 1948. Initially this was applied to the treatment of the smaller skin tumours, for example, rodent ulcers, where even single treatments were found adequate in many instances[4,5]; with increasing experience the technique was extended to the treatment of some larger lesions and more extensive volumes of tissue, until a régime of three treatments a week, at 48-h intervals, was adopted in an appreciable number of patients.

Both in the experimental work in animals and in our clinical practice it was noted that a reduction had to be made in the total dose when it was given in a curtailed number of fractions if a similar biological effect was to be obtained. In pigs, doses given every other day require reduction by 15 per cent of the doubled daily dose if, over the same total period, the same degree of observable skin reaction is to be obtained as that found on daily fractionation[3]. Comparable results in the skin of pigs were found by Fowler *et al.*[6], who state that a reduction in dose of about 20 per cent is required when a change is made from 21 fractions to 9 fractions over a total time of 28 days.

The experimental results suggest that considerable improvement in clinical radiotherapy might be possible by adopting curtailed fractionation techniques. Before changing from a well-established method of treatment it is natural to look for any guide as to the best new method to adopt and, secondly, what the immediate and long-term consequences of such a change would be. Lack of information on these points has prompted us to report the following results at this stage, because we feel that they provide an adequate reassurance on which to base further clinical trials. A much fuller analysis of the cases is being undertaken and it is hoped to publish this at a later date.

We have found that reduction of about 15–20 per cent in the total dose is required when the number of fractions in which the treatment is given is reduced from five a week to three a week.

This conclusion is based on our observations in both small and large treatment areas, the former on the results of treating skin malignancies such as rodent ulcers. These represent small treatment areas, less than 4·5 cm diameter, irradiated at 60 kV, half-value layer 2·75-mm aluminium. The cases treated five times per week received a surface maximum dose of up to 6,000 r. in 10 fractions over 14 days and those treated three times a week received 4,800 r. in 6 fractions over the same 14-day period. These doses resulted in similar immediate skin reactions following treatment, and there was no significant difference in the late results between the two methods of treatment[5].

Consequent to the foregoing findings in small treatment areas, we extended the curtailed fractionation regime to larger treatment areas as required, for example, in radical irradiation following a mastectomy for carcinoma of the breast.

In this latter treatment our original method of irradiating the chest wall[7] entailed the use of beams of X-rays at 250 kV, half-value layer 1·75-mm copper, directed tangentially across the chest wall. The patients on the thrice weekly régime received an average tissue maximum dose of 3,600 r. in 12 treatments over 28 days, while those treated five times weekly received an average tissue maximum dose of 4,500 r. in 20 treatments over 28 days. These doses were arrived at, following adjustment, so that a similar immediate skin reaction resulted. Those receiving treatment five times a week were in-patients, whereas the others attended as out-patients and the difference in clothing may well have influenced the severity of the chest wall skin reaction resulting in the three times

weekly group receiving a slightly lower dose in order to maintain similar skin reactions in the two groups.

A large number of the cases assessed has been followed up for more than 10 years. At no time has there been any apparent difference in the late radiation effects, such as fibrosis, atrophy or telangiectasis, between the patients treated three times weekly and those treated five times weekly with the doses mentioned. Close observations at follow-up have not, so far, revealed that curtailed fractionation has apparently influenced survival times and recurrence free rates; it is hoped to publish with fuller details the results of some further investigations into this at a future date.

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BIOLOGY

Effect of Certain Soil Organisms on the Eggs of Parasitic Roundworms

The influence of biological factors on the development and viability of eggs of parasitic roundworms (geohelminths) in soil has not yet been sufficiently explored. It is known that some fungi are able to attack free-living Nemathelminths[1–3]. It has also been shown that some fungi can attack larvæ of *Strongyloides* from horses and larvæ of *Trichostrongylides* from cattle[3]. Some fungi are also capable of inhibiting the development of eggs of ascarids *in vitro*[4]. Gudzhabidze and Preobrazhenskaya[5] performed experiments on *Ascaris* eggs by influencing them with micro-organisms, which had been isolated from the soil specimens from sewage farms. Certain species of actinomycetes and soil bacteria were capable of exerting a depressing effect on the eggs of ascarids.

I have attempted to ascertain whether in open fields the eggs of human parasitic geohelminths are influenced by organisms which are living in the soil and to what extent. I carried out my experiments in the following way: on the specimens of the soil with fæcal pollution (human or animal) I placed small piles of fertilized and unfertilized eggs of ascarids from pigs. Organisms from the soil to which the *Ascaris* eggs produced favourable conditions of life broke from the soil through the piles of eggs and profusely multiplied there. These organisms either inhibited the development of *Ascaris* eggs or even destroyed them.

On some piles of *Ascaris* eggs Acari and Collembola multiplied considerably. Acari: *Rhyzoglyphus* sp. (Claparède 1869), *Sancassania tschernyschevi* (Zachvatkin), *Annoetus ferroniarum* (Dufour 1939) and *Fuscuropoda marginata* (Koch 1939); Collembola: *Hypogastrura (Ceratophysela) denticulata* (Bagnall 1941), *Hypogastrura* (Hypogastrura) *vernalis* (Carl 1901), *Folsomia candida* (Willem 1902) and *Folsomia candida* var. *distincta* (Bagnall 1939). Acari from the family Acaridae (Tyroglyphidae) were especially capable of consuming a large quantity of ascarid eggs. For example, in one experiment 5 adult individuals of Acari were put on 0·5 g ascarid eggs. At the end of this experiment, which lasted 32 days, these Acari multiplied in 58 adult individuals and a very large number of larval stages. In the course of this experiment the Acari consumed 0·38 g of eggs.



a *b*

Fig. 1. Eggs of ascarids broken through by the fungus *Fusarium redolens* Wr.

Besides these soil animals various species of fungi and soil bacteria penetrated into the *Ascaris* egg piles. Some of these organisms destroyed the morphological structure of the eggs. Numerous experiments were made with isolated clear cultures of these fungi. The eggs were destroyed especially by two species of fungi: *Fusarium redolens* Wr. and *Cephalosporium* sp. Both these fungi have already been described by other authors as parasites on free-living Nematoda in the soil. These fungi penetrated the eggs, consumed their contents so that only deformed thin eggshells remained (Figs. 1*a* and *b*). The eggs were also destroyed by other species of fungi and even bacteria. The isolated organisms, however, soon lost their biological activity after having grown for a short time in the artificial medium.

In this communication I wish to direct the attention of parasitologists who are engaged in the problem of cleaning soil of eggs of pathological nematodes to the fact that the development and the length of viability of these eggs in the soil are influenced not only by physical and chemical factors but also by some animals and fungi which live in the soil.

I thank Dr. O. Fassatiová, Faculty of Natural Sciences, Charles University, Prague, for her assistance in identifying the fungi, Mr. J. Nosek, Virological Institute of the Academy of Sciences, Bratislava, for his assistance in determining the Collembola, and Dr. K. Samšinák, Biological Institute of the Czechoslovak Academy of Sciences, Prague, for his assistance in identifying the species of Acari.

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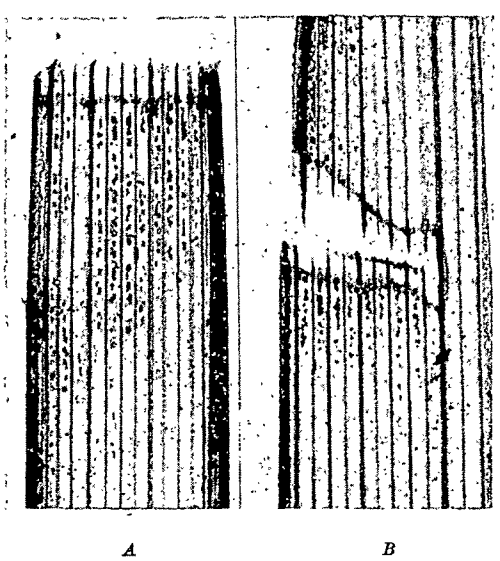
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Influence of Mechanical Damage on Opaline Silica Deposition in *Molinia caerulea* L.

In our microscopic investigations of opaline silica bodies in the leaves of a large number of British grasses[1], we have found that where leaves were already bruised or torn before being taken from the tiller or culm, the epidermal preparations made from them often showed abnormalities in the degree and pattern of deposition of silica. It was therefore decided to investigate the effects of various kinds of deliberate damage. *Molinia caerulea* was chosen for preliminary experiments because the silica pattern in this grass had been examined in some detail by us, and opal had been found in a variety of its epidermal

cells ranging in size from the intercostal short silica cells to the very large bulliform cells which are silicified erratically near the tips of some of the leaves[2].

Ten rooted clumps were potted singly in John Innes compost so as to provide thirty actively growing shoots, five to serve as controls and twenty-five for various damage treatments. The shooting leaves of actively growing tillers were subjected to five treatments: (i) pricking through the leaf blade with a fine needle at a number of points along its length; (ii) removing the apical portion by cutting off with scissors one-sixth of the blade; (iii) cutting off five-sixths of the blade; (iv) notching by making a transverse cut from margin to mid-rib in a position one-sixth of the way down the blade, and (v) notching in a similar manner five-sixths of the way down the blade. There were five replications of each of the five treatments.



A B

Fig. 1. Silica deposited in bulliform cells in damaged blades of *Molinia caerulea*. *A*, Treatment (ii); *B*, treatment (iv). (× 15)

After six weeks the treated leaves were removed and prepared for microscopical examination using successively ammonia and hydrogen peroxide[3]. None of the five treatments had produced any unusual development of the dumb-bell-shaped silica bodies characteristic of the costal regions nor of the small silica bodies of the intercostal areas. Both types are too small to be seen in Fig. 1. Three treatments, (i), (iii) and (v), produced no unusual development of silica in the bulliform cells, but the other two, where the damage was applied near the tip, produced deposition in these cells which was abnormal both in amount and distribution. The heavy silicification resulting from treatment (ii), namely removal of tip, was very pronounced in three, but completely absent from two of the replicates, and that resulting from treatment (iv), namely notching near the tip, was very pronounced in four but only slight in the remaining replicate. The phenomenon could be seen easily at low magnification. In Fig. 1*A* silicification of bulliform cells extends across the entire breadth of the blade. In Fig. 1*B* silicification is heaviest below the point of damage where it corresponds precisely with the width of the notch.

Fagan[4] working on *Dactylis glomerata* has shown that the silica content of the leaf increases by about 350 per cent in the withered tip as opposed to the lower green and non-withered portions of the blade. The present work suggests that certain types of mechanical damage can also be causes of excessive deposition of silica. These and other aspects of silica deposition are being investigated further by us. Among other considerations, they could well have a bearing on the recovery and subsequent palatability and digestibility of grass herbage after several partial defoliations such as would occur under any grazing system.

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MICROBIOLOGY

Unlinked Loci affecting Related Biosynthetic Steps in *Pseudomonas aeruginosa*

It has been shown by Demerec, Hartman and others[1], that in *Escherichia coli* and *Salmonella typhimurium* it is usual for loci controlling related functions to be closely situated to each other on the chromosome and even for the arrangement of such loci to be the same as the sequence of biosynthetic steps concerned. The chief exception to this rule is the arginine biosynthetic pathway in which the controlling loci are distributed widely over the *E. coli* chromosome[2]. Using the technique of joint transduction, we have obtained evidence that in *Pseudomonas aeruginosa* this latter type of marker distribution of loci controlling sequential biosynthetic steps occurs with all the pathways so far investigated.

Joint transduction by phage *F*116 for the markers str (streptomycin resistance or sensitivity) and try (a marker controlling tryptophan biosynthesis) has previously been described[3]. These two markers show about 15 per cent recombination in sexual crosses[4] and are jointly transduced at a frequency of about 11 per cent. A collection of auxotrophic mutants of *P. aeruginosa* strain 2 was obtained by treatment with either manganous chloride or ethyl methane sulphonate and the mutants broadly classified by their requirements for amino-acids, purines, or pyrimidines.

Two methods have been used to determine the linkage relationships of the various markers.

(1) Phage *F*116 is propagated on a given auxotrophic mutant and is then used to infect bacteria of a second mutant strain. The infected bacteria are plated on minimal medium supplemented with the nutrient required by the mutant on which the phage was propagated. When the colonies so formed are replicated on to unsupplemented minimal medium, the frequency of joint transduction of the two markers is indicated by the ratio of the transduced colonies which can grow on minimal to those that cannot.

(2) For cases where method 1 is not applicable, for example, with transductions between mutant strains having identical requirements, some indication of the linkage between the loci can be gained by comparison of the transduction frequency obtained with one of the mutants using phage propagated on the other mutant, to the frequency obtained with phage propagated on prototrophic wild-type bacteria. If the two markers are cotransduced, the transduction frequency in the former case will be lower than that of the latter.

The strains, techniques and media used are the same as those described previously by Holloway *et al.*[3] and are similar to those commonly used in transduction studies. The results of a variety of transduction tests are shown in Table 1.

It is seen that there is no joint transduction of markers controlling related biosynthetic steps. The joint transduction of these markers with other unrelated markers shows that linkage can be revealed by this type of experi-

Table 1. JOINT TRANSDUCTION IN *P. aeruginosa* STRAIN 2 BY PHAGE *F*116

Marker	Phenotype requirement	Percentage joint transduction with try-3A	try-3B	met-1	met-2	met-3	leu-1	str*
try-1	tryptophan	0	0	0	0	0	0	0
try-3A	tryptophan or indole, accumulates anthranilic acid		0†	0	0	0	0	11
try-3B	as for try-3A			0	40	0	70	0
met-1	methionine or homocysteine				0†	0	0	0
met-2	methionine or homocysteine					0	44	0
met-3	methionine, homocysteine or cystine						0	0
leu-1	leucine							0

* Marker for streptomycin resistance or sensitivity.
† Linkage relationships determined only by method 2.

ment. Similar results have been obtained with groups of mutants affecting other pathways, including mutants having requirements for histidine, leucine, arginine and isoleucine plus valine, in these cases our conclusions being based largely on data obtained by method 2.

It seems reasonable to conclude that in contrast to the Enterobacteria, this lack of grouping of related loci on the chromosome is of general occurrence in *Pseudomonas*. This finding may have interesting implications in relation to the mechanism of genetic control of enzymatic reactions in this organism[5].

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Polysaccharide Fractions and Transformations in *Rhizobium*

OUR interest in the composition of rhizobial gums[1,2] led us to attempt transformations using, first a preparation based on that of Ljunggren[3] and later the cruder, and therefore deoxyribonucleic acid-rich, fraction used by Lange and Alexander[4]. In the first case we aimed at restoring virulence to avirulent strains of clover *Rhizobia*, including two used by Ljunggren, but with *R. trifolii*, *SU*297/31 as the source of 'polysaccharide'. In the second we attempted a wider transformation, similar to those reported by Lange and Alexander, between clover and lucerne. In the latter we included a specially prepared deoxyribonucleic acid fraction as an additional treatment.

In no case have we been successful in obtaining transformation, and an experience we had in our attempt to repeat Lange and Alexander's method points to what we believe to be a possible weakness in their evidence. In that case an aliquot of the crude clover polysaccharide, when plated directly on yeast mannitol agar, yielded a number of *Rhizobia*-like colonies. These were able to nodulate the clover host and were serologically indistinguishable from the donor strain. A puzzling feature was that there were no nodules formed when parallel aliquots of the same polysaccharide solution were added to the clover host. We believe, however, that we have demonstrated that a few rhizobial cells were able to survive what appears to have been a drastic chemical treatment.

The element of doubt which this possibility introduces into work of this nature recalls the need to demonstrate a mixed genome in 'transformed' cells, partly derived from donor and partly from recipient (c.f. ref. 5). Evidence as to the persistence of genetic markers of the recipient strain in a culture, transformed in respect of its symbiotic capacity, is strangely lacking with *Rhizobium*. It is possible that the workers concerned already have direct evidence that will permit distinctive characteristics of the recipient to be identified in the symbiotically 'transformed' culture, or that they are in a position to investigate further the transformed cultures they already hold. Certainly such a demonstration can be regarded as a prime requirement for the validation of claims of this nature.

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ENTOMOLOGY

Penetration of Pyrethrin I labelled with Carbon-14 into Susceptible and Pyrethroid Resistant Houseflies

As part of a wider investigation into the causes of insecticide resistance in a strain of house flies (*Musca domestica*), which show resistance to both DDT and the pyrethroids[1], specifically labelled (^{14}C)-pyrethrin I has been used to examine penetration of this insecticide into these insects.

(^{14}C)-pyrethrin I, labelled in the *cyclo*propane ring adjacent to the ester linkage, was prepared by the esterification of (^{14}C)-(±)-*trans*-chrysanthemum monocarboxylic acid with (+)-pyrethrolone. The alcohol was isolated from natural sources by Elliott's method[2]. The (^{14}C)-acid was prepared from (2-^{14}C)-glycine by the general route of Harper[3] with suitable modifications to the semi-micro scale; in particular, the separation of the mixture of *cis* and *trans*-chrysanthemum acids was carried out by fractional precipitation (infra-red control). Because of the small quantity of material available the (±)-*trans*-acid could not be resolved and so the final product was a mixture of natural pyrethrin I and one of its diastereoisomers, henceforth referred to as (^{14}C)-'pyrethrin I'.

Sub-lethal quantities of (^{14}C)-'pyrethrin I' in acetone were then applied by topical application to batches of flies of three strains: first, a susceptible strain (Fig. 1 *A*), secondly, a Swedish resistant strain[1] (Fig. 1 *B*), and thirdly, a colony of the Swedish resistant strain which had been allowed to revert to 'almost susceptible' by removing the pyrethroid selection pressure (Fig. 1 *C*). At increasing increments of time, batches of the flies were killed and the unabsorbed insecticide was washed from the flies and the containers with light petroleum, b.p. 40°–60°, and the combined washings were concentrated to dryness *in vacuo* and the weight of (^{14}C)-'pyrethrin I' was determined by a scintillation counting technique. A control experiment showed that the washings did not remove (^{14}C)-'pyrethrin I' already absorbed.

The results from these experiments are shown in Fig. 1, curves *A*, *B* and *C*. It would appear from these results that penetration into the resistant strain is significantly slower than into the other strains. No attempt was made to measure directly the loss of 'pyrethrin I' by excretion. Radioactivity recovered from the containers alone prob-

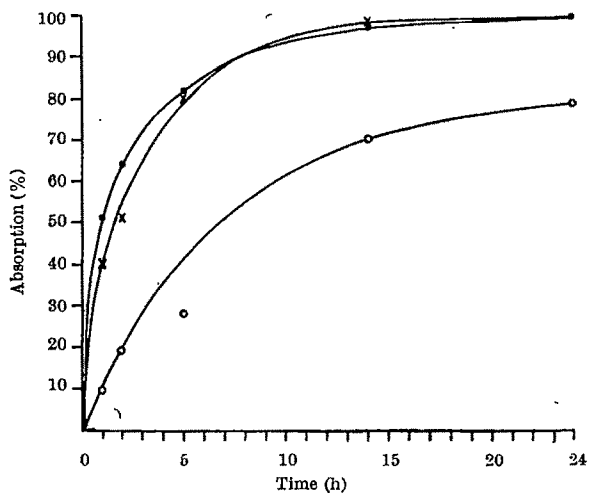


Fig. 1. ●, *A*; ⊙, *B*; ×, *C*

ably represents excreted (^{14}C)-'pyrethrin I' and its metabolites plus material lost from the flies by mechanical causes. In some cases this was assayed separately. The amounts recovered from the susceptible and reverted strains were negligible (about 1 per cent); however, in the resistant strain 9 per cent of the applied dose was in the containers after 14 h. This is probably due to mechanical loss, a function of reduced penetration which retains a large quantity of the insecticide on the outside of the flies for long periods. In any event, the amount recovered does not invalidate the differences shown in Fig. 1.

Both the susceptible and reverted strains were paralysed but later recovered after dosing, in contrast to the resistant strain which appeared unaffected. It may be argued because of this that penetration is facilitated by intoxication in the affected strains. To test this, susceptible and resistant flies were dosed with (^{14}C)-'pyrethrin I' at a very low level, where the susceptible strain was not outwardly affected, and it was found that after 2 h 65 per cent of the insecticide was absorbed by the susceptible strain and only 28 per cent by the resistant strain. This is in fairly good agreement with the results obtained using the higher dose-level (cf. Fig. 1).

It must therefore be concluded that there is a definite correlation between the rates of absorption of (^{14}C)-'pyrethrin I' and pyrethroid resistance in the strains studied here.

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Sound Signals and Alternation Behaviour in *Pholidoptera*

The classical work of Regen[1] on the effect of artificially produced sounds on the stridulation behaviour of *Pholidoptera* (= *Thamnotrizon*) *aptera* (Fab.) has prompted similar work on the related British species *Ph. griseoaptera* (De Geer), with the view of clarifying our understanding of the factors involved in the alternation 'singing' of males and the response to sound by females. There appears to be some confusion in the literature about the conditions under which Regen induced his insects to 'sing' in alternation with artificial signals. The widely accepted view[2,3] is that he was able to obtain this response only from newly moulted adults which had not already learnt to sing in concert with other males. In fact, Regen points out that young males do not alternate at all until they have 'sung' together for some time and his experiments did not begin until they had started to alternate for long periods. There seems to be little evidence that his earlier failures, with older males, were due to learning by the insect, and other work in this field[4,5] supports the view that any recognition of the specific 'song' is due to inborn mechanisms.

The alternation 'singing 'of *Ph. griseoaptera* males is simpler and less regular than that of Regen's insect and consists of single chirps of approximately 0·05-sec duration made up of three or four syllables (wing movements). The interval between alternate chirps is in the order of 0·2–2·0 sec. It is relatively easy to induce them to alternate with such varied sounds as the note of a Galton whistle, pure tones from a signal generator, and hissing sounds made between the teeth. In a series of experiments three males were kept in a cage with a number (4–7) of females. Between experiments, alternation appeared to be similar to that observed in the field. During experiments, it was quite usual for one male to alternate with the artificial signals for a time and then for another to take over.

Some of the most interesting results were obtained when the sound used was long in comparison with the chirp of the insect. If successive periods of sound and silence of approximately 1 sec in duration were used, the insects alternated with the sound and scarcely ever interrupted it.

Fig. 1 is an oscillogram taken from such a sequence in which there were 137 replies to 144 signals. A morse key was used in this experiment and transients were produced both at make and break. When the length of the signals and the interval between them was increased, the insects interrupted the signal more often. Nevertheless, even when the periods used were as long as 15 or 30 sec, stridulation occurred more often during the silent periods than during the signals (in all of 12 χ^2 tests $P < 0{\cdot}01$). The frequencies used varied from 10 to 30 kc/s and the intensities varied from 60 to 90 db (relative to 0·0002 dynes/cm^2). Fig. 2 shows the frequency distribution of chirps during 24 min of a trial using alternate periods of 15 sec and a sound of 15 kc/s and 85 db. It will be seen that a large number of the emissions occurred during the first second after the end of the signal and this result has been confirmed in seven other trials where the chirp distribution was recorded. In three of these, the silent periods were obtained by placing a cushion over the loudspeaker and in the others a morse key was used as make and break. Both methods gave similar results, in spite of the fact that the cushion method produces a signal without any sharp transients (Fig. 3). This is in contrast with the work of Busnel *et al.*[6] where transients were found to be important.

These preliminary results suggest that artificial sound signals function both as releasers and inhibitors of the stridulation of *Pholidoptera*. A similar inhibition has been noted with *Ephippiger*[6]. The tendency of the insects to

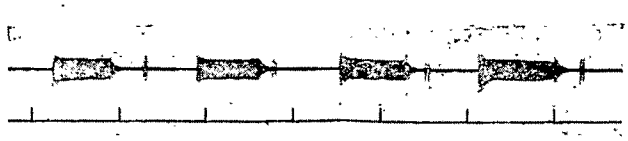


Fig. 1. Oscillogram of alternation with a 12-kc/s 85-db sound signal. The signal comes first in the record. Time marker 1 per sec

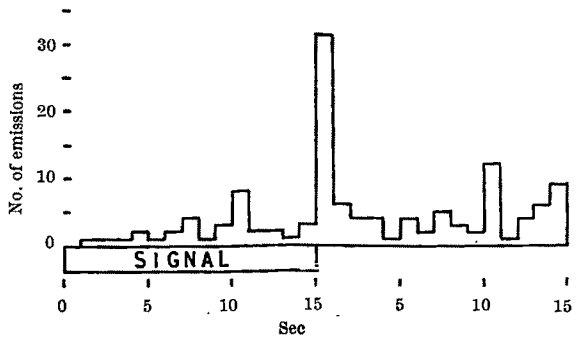


Fig. 2. Distribution of chirps during a 24-min trial using alternate periods of 15 sec and a sound of 15 kc/s, 85 db. 48 consecutive results are thus added together

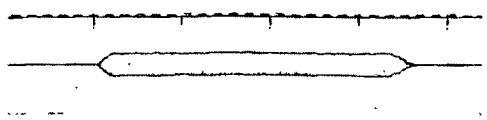


Fig. 3. Oscillogram of a 15 kc/s 85 db signal released and stifled by moving a cushion over the loudspeaker. Time marker 1 per sec

stridulate directly after the end of the signal may be due solely to the removal of the inhibition, or there may be, in addition, a direct response to some characteristic of the signal. Further work is being carried out to investigate these possibilities.

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CYTOLOGY

Corrected Chromosome Numbers for *Spartina* × *townsendii* and its Parent Species

Intensive cytological investigation of *Spartina*, carried out in the Department of Botany, University of Southampton, has revealed chromosome numbers which are not in agreement with those of Huskins[1] and Church[2,3].

In the classical case of *S.* × *townsendii* and its putative parents it will be recalled that chromosome numbers were previously given by Huskins as $2n = 56$ for *S. maritima* (Curt.) Fernald (formerly *S. stricta* (Ait.) Roth.) and $2n = 70$ for *S. alterniflora* Lois., the summation of these numbers, namely, $2n = 126$, being reported for the amphidiploid, then identified as *S.* × *townsendii*. Although these observations numerically accord well with each other in constituting the hybrid origin of *S.* × *townsendii* they nevertheless create an anomalous situation in the cytology of the Gramineae as a whole. The chromosomes of *Spartina* are very small and it is an established fact that small chromosomes and a basic number of 7 are not coincidental in the family[4,5]; small chromosomes are usually associated with $x = 9$, 10 or 12. In addition, the base of 7 is unusual in the tribe Chlorideae, in which *Spartina* is often included, where most genera have $x = 10$. This apparently paradoxical situation is resolved by the present results.

Counts of *S. maritima* from 6 localities on the south and east coasts of Britain show it to be a species with $2n = 60$. *S. alterniflora* Lois. plants, surviving as relics of a one-time much more extensive distribution of this species last century[6] in Southampton Water, have $2n = 62$ $(60 + 2)$. *S.* × *townsendii*, the sterile natural F_1 hybrid between these species, has been generally overlooked until recent years but still survives in large quantity at Hythe in Southampton Water. It has $2n = 62$. Some of the amphidiploid plants (previously known as *S.* × *townsendii*, but at present officially nameless) have $2n = 124$. Other plants of the amphidiploid type have $2n = 120$ and 122, respectively. Plants which appear by their morphology and chromosome number to be natural back-cross derivatives are found growing in the vicinity of the F_1 hybrid in Southampton Water, thus adding significantly to the evidence for hybridity. *Spartina glabra* Muhl. (another form of *S. alterniflora* sometimes known as *S. alterniflora* var. *glabra* (Muhl.) Fern.), introduced to Britain from the United States by F. W. Oliver in 1924, also has $2n = 60 + 2$ ($2n = 56$ according to Church[3]) and a regular meiosis.

Chromosome investigations have been extended to include the North American species of *Spartina* and the existence of a basic number of 10 is substantiated by counts of *S. pectinata*, which proves to have $2n = 40$ in two introductions from North America at Kew. *S. alterniflora*, received from Quebec, Canada (coll. Hamel), has $2n = 62$, similar to both the introduced forms of this species already in Britain. Other species are now being investigated. Gould's[7] count of $2n = 40$ in *Spartina spartinae* from Texas is further evidence in support of the base of 10.

It is clear that these data do not deny the hybrid nature of *S.* × *townsendii* or its precise parentage, although they make the cytological support for its origin slightly less acute than hitherto. *Spartina* × *townsendii* thus remains a plant of outstanding evolutionary significance by virtue of its natural origin by hybridization and chromosome doubling, resulting in a highly successful new 'species'. It is perhaps the most well-known and now substantiated example of natural species formation of this type and one of the few cases of its kind which has actually been observed to occur in a known locality rather than inferred entirely by indirect observations.

Spartina is certainly not unique in having wrong chromosome numbers reported for it. The *Chromosome Atlas*[8] reveals many genera in which numbers are uncertain, and doubtless many more anomalies exist but are as yet undetected. Perhaps the most well-known case of error in chromosome number is that of man, formerly thought to have $2n = 48$ chromosomes and, afterwards, from more intensive study and greater familiarity with the karyotype, shown clearly to have $2n = 46$ (ref. 9).

Because of its former chromosome numbers and supposed base number of 7, *Spartina* has not accorded satisfactorily with other genera of the Chlorideae and, indeed, its separation from the tribe has been proposed by various authorities[10]. This uncertain situation is now resolved by the new numbers, which bring *Spartina* into line cytologically with other members of the Chlorideae.

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Analysis of Tritium-labelled Human Chromosomes and Sex Chromatin

It has been shown[1,2] that differential DNA synthesis in tissue culture may be studied by the incorporation of tritiated thymidine, followed by autoradiography of the labelled chromosomes. Grain counts have been made on human chromosomes labelled at the end of DNA synthesis, but the high grain density over the late-replicating *X* often obliterated morphology[3]. Asynchronous DNA synthesis has been demonstrated within human interphase nuclei[4]. An area of differential labelling was shown where the sex chromatin body might be expected to be present; but it was reported that the heavy concentration of grains made it difficult to determine whether an underlying body was present.

A method is described here which enables detailed examination of those structures which are obscured by a heavy concentration of silver grains. This method has been used to demonstrate and study the late-replicating *X* chromosome, an 'isochromosome *X*', and the sex chromatin body in the interphase nucleus of cells grown in tissue culture.

Fibroblasts from human skin and leucocytes from human blood were cultured by modifications of the standard methods[5,6]. 'Flash-labelled' chromosome preparations were made by adding tritiated thymidine (specific activity 1·41 c./mM, Radiochemical Centre, Amersham) to give a concentration of 1 μc./ml. of medium. The culture was incubated at 37° C for 10 min and the cells washed with, and re-incubated in, medium which contained excess thymidine (100 times the molar concentration of the tritiated thymidine). 'Long' labelling was performed by adding tritiated thymidine to give a concentration of 0·2 μc./ml. of medium. After the usual 'Colcemid' (Ciba) and hypotonic treatment, the cells were fixed at intervals varying between 4 and 11 h after labelling. Chromosome preparations were made by the air-dry method and stained with lacto-acetic orcein. The slides were taken from the stain, through 'Cellosolve', to water. If they were not required immediately for autoradiography, they were left to air-dry.

Each slide was covered with a film of 'Vinalak', which is a synthetic resin dissolved in a volatile solvent immiscible with water. A small drop of the lacquer was allowed to fall on to the surface of clean, distilled water, where it spread and formed a very thin film of resin. The slide, with cells uppermost, was brought up under the film and withdrawn from the water, carrying the film with it. As the slide was lifted from the water it was tilted at an angle of about 20° to the water surface, so that one long edge emerged, allowing the water to drain from between the film and the slide. The slide was left to dry at room temperature. Kodak '*AR* 10' autoradiographic stripping film was applied to the slide at 25° C (ref. 7), and it was exposed for 7–14 days. During photographic processing it was important to prevent the stripping film from floating off the slide. This was done by placing a clean slide at the back of the prepared slide and two 1-in. glass squares at either end and in front of the prepared slide; the whole was held together by two rubber bands. During processing, the slide was kept horizontal. All photographic solutions were at 18° C and, after 5 min development in Kodak '*D*-19*b*' and a 5-sec rinse in water, the slide was placed in 'Ultrafix', diluted 1 : 4 with water and with 1 : 20 hardener added. It was fixed until clear and then for half the time again. The slide was then washed for 1 min in each of six changes of tap water, followed by one wash in distilled water. It was left to dry horizontally. These precautions limited displacement of the stripping film.

The dry preparation, after careful removal of the retaining glasses and rubber bands, was mounted in 'Euparal' for examination. After photographic record, the coverslip and film were floated off by soaking in 'Euparal' essence, leaving the lacquered preparation undamaged. The lacquer has a negligible effect on resolution but, if desired, it may be removed by taking the slide through 'Cellosolve' to xylol, and leaving it in xylol for 12–24 h. The cells may then be mounted in Canada balsam.

For the study of the sex chromatin body, fibroblasts from human skin were cultured on cover-slips[8]. Labelling was performed by adding tritiated thymidine to give a concentration of 0·2 μc./ml. of medium, and the cells fixed at intervals, varying between 30 min. and 5 h after labelling. Each coverslip was stained with cresyl fast violet acetate[8]. After staining, the cover-slip was destained for about 1 min in water. It was left to dry before being

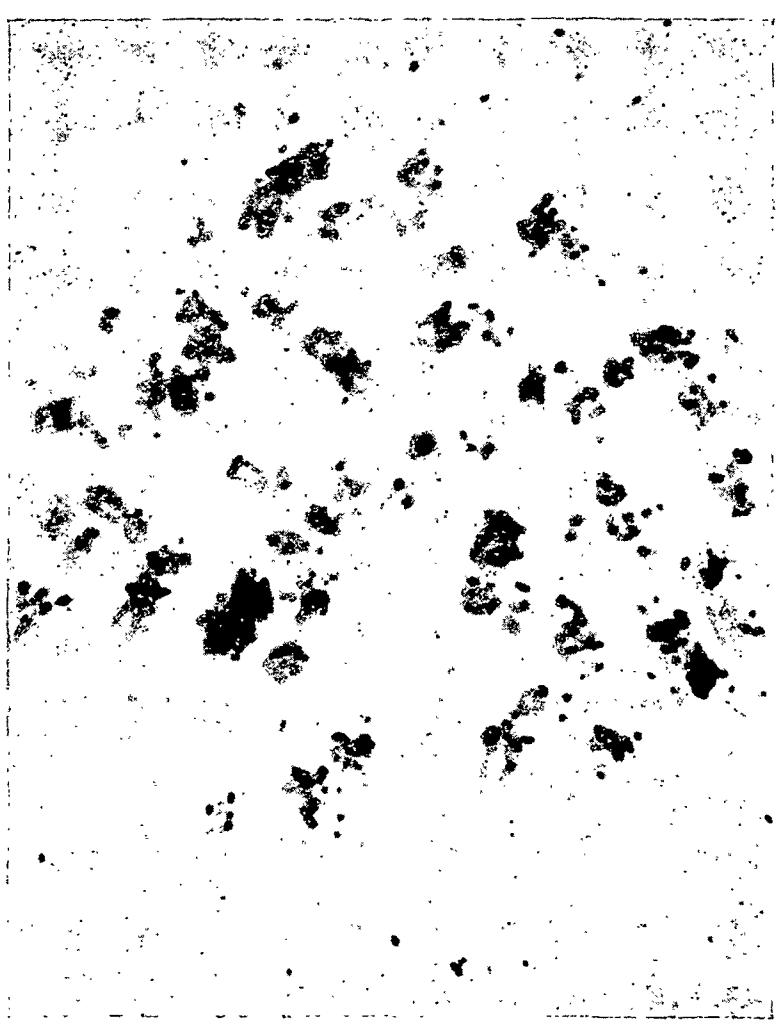


Fig. 1. Metaphase plate from human peripheral blood culture, after a 4-h 'long' labelling period in tritiated thymidine. This cell is from an individual with a normal female karyotype. Several chromosomes are obscured by a high grain density (× *c.* 2,500)

chemical composition of the DNA of the gene-groups with respect to the action of the mutagens.

The usual picture of the genetic material is that a gene contains a chain of about 10^3 nucleotide pairs, that there are 4 types of nucleotides, that DNA molecules of one type of organism do not differ grossly in the nucleotide ratio, and that most mutations change only one nucleotide pair in the DNA. If the mutability of a nucleotide type by a certain mutagen is determined by the structure of the base alone intergenic electiveness could scarcely appear since the fluctuations of the base ratio between genes or gene-groups would have to be very high. Therefore, this electiveness indicates that the mutability of a base depends very probably also on the neighbouring bases. If—as a minimum—only the 2 neighbouring bases are essential, the mutability of a site ('hot', 'warm' or 'cool' spot, etc.) would be determined by a base triplet.

Sixty-four such triplet types are possible, if the direction of the sequence along the DNA strand is essential, and 40 triplet types, if it is unessential for the mutability (for example, CAT = TAC). A gene of 10^3 nucleotides could therefore contain about (1,000 : 50 =) 20 triplets of the same type with large fluctuations of their numbers (and locations) between different genes depending on the ratio and pattern of the bases and the length of the genes. If the triplet types are different in their stability against mutagens a certain degree of electivity due to such fluctuations would then already be possible. But, in addition, mostly a considerable portion of mutations (base changes) is lethal or does not change the phenotype appreciably. In *Neurospora*, for example, 3/4 of the X-ray mutations of the ad-gene are found to be recessive lethals[8]; in phages the ratios of lethal to mutation hits by mutagens are high (Table 1). On the other hand, several mutations at different sites in the rII-region of phage *T*4 are reported to give wild phenotype[10]. Because of such lethal or intraphenic base changes, undetectable by the methods used, the number of hot spots for visible mutations would be further reduced to only a few per gene. This agrees with the finding of 2–5 strong hot spots per rII-gene of phage *T*4 (ref. 3).

Supposing this to be true for most genes the fluctuations in average mutation rate per gene or gene group are high when a mutagen attacks preferentially one type of base triplet. This would lead to the gene-group electivity observed.

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PSYCHOLOGY

Electroencephalogram Alpha-activity during Mild Vestibular Stimulation

Previous reports have indicated that nystagmus resulting from vestibular stimulation is significantly influenced by instructions given to subjects regarding their mental activity[1–4]. In addition, under some conditions, nystagmus appears to reflect the subject's state of alertness more clearly than electroencephalogram (EEG) patterns[2]. There has been recent interest in the relation of arousal to EEG activity[5] and in the relation of EEG patterns to vestibular stimulation[6]. This report, bearing on both these interests, presents EEG findings obtained under different sets of instructions during repeated rotational stimulation. In addition, these new EEG results are here correlated with previously reported[1] nystagmus scores.

Eight men, aged 18–25 years, were tested on each of 5 consecutive days. The first session consisted of 4 practice trials, each under a different set of instructions. The remaining 4 (experimental) sessions each consisted of 6 trials. For all experimental trials on one day, subjects were given a single set of instructions. Tasks included: (*a*) silent arithmetic calculation; (*b*) signalling subjective rotatory experiences; (*c*) temporal reproduction of sound stimuli; (*d*) engaging in reverie. A counterbalanced order of task presentation was established such that 2 subjects were assigned to each of 4 orders of presentation. Rotation trials were conducted in total darkness with 15-sec periods of acceleration and deceleration (4·5°/sec²) separated by 2 min at a constant velocity. More detailed explanations of the tasks, rotatory conditions, and the method of scoring nystagmus data may be found elsewhere[1].

Subjects selected had no history of labyrinthine disorder, and no previous experience in vestibular experimentation. Five electrodes were used with each subject: a parieto-occipital pair for the EEG; a pair located at the outer canthi for the horizontal component of eye movements; a ground electrode. Signals were transmitted through slip-rings to an Offner Type *T* electroencephalograph situated in the adjoining control room.

Eye-movement scores were obtained by summing the amount of slow-phase eye displacement for 30-sec periods beginning with the onset of the stimulus. For the same time period, the number of seconds occupied by alpha activity was tabulated. Analyses of variance indicated that the instructions produced no significant differences in alpha activity, but that nystagmus output was significantly influenced ($P < 0{\cdot}001$). Application of *t* tests to the nystagmus data showed that mental arithmetic tasks resulted in significantly more ($P < 0{\cdot}05$–$0{\cdot}001$), and reverie tasks in significantly less ($P < 0{\cdot}01$–$0{\cdot}001$) nystagmus than each of the other 3 sets of instructions[1]. Table 1 contains mean results: (*a*) for all subjects; (*b*) for those subjects with high alpha output. The results are presented according to tasks, sessions and trials.

In spite of gross changes in nystagmus output as a function of the tasks, there was no apparent relation between alpha activity and amount of nystagmus for any given subject. However, 2 of the 3 subjects who displayed very little alpha produced the least overall amounts of slow-phase eye movement.

Representative examples of obtained EEG and nystagmus tracings appear in Fig. 1. It should be noted that some subjects will yield even less eye-movement under the reverie condition than is evident in this figure[1].

Makarian and Koreshkov[6] have indicated that vestibular stimulation induced by vigorous rotation (up to 30 r.p.m.) caused EEG changes. They reported increased beta activity, a lowering of alpha amplitude, and the appearance of asynchronous waves sometimes accompanied by slower undulations. Repeated rotation resulted in adaptation, particularly in individuals with low vestibular sensitivity. Our results (which do not include amplitude measurements) show no increased slow activity. There was no relationship between vestibular sensitivity, as measured by nystagmus output, and alpha activity. Further, there was no significant trend toward change in the amount of alpha activity either within a given session of 6 trials, or from day to day. However, it should be noted that the lowest average alpha output

Table 1. Mean Slow-Phase Nystagmus Output and Mean Number of Alpha Seconds per Subject for 30-sec Periods beginning with the Onset of a Rotatory Stimulus (4·5°/sec² for 15 sec).
The same data are separately examined according to tasks, sessions and trials. *MA*, *KP*, *TR* and *RV* refer to mental arithmetic, key pressing, temporal reproduction and reverie, respectively

	All subjects (N = 8)						Alpha subjects (N = 5)					
	Nystagmus (deg)			Alpha sec			Nystagmus (deg)			Alpha sec		
	Acceleration	Deceleration	M	Acceleration	Deceleration	M	Acceleration	Deceleration	M	Acceleration	Deceleration	M
Task												
MA	564	561	563	9·6	11·6	10·6	596	632	614	15·1	18·2	16·7
KP	504	472	488	12·7	11·5	12·1	513	553	533	20·4	18·1	19·3
TR	415	454	435	13·4	13·5	13·5	476	561	519	21·2	21·0	21·1
RV	270	215	243	12·7	12·7	12·7	336	278	307	19·7	19·4	19·6
Session												
1	453	381	417	10·9	12·6	11·8	489	480	485	17·3	19·7	18·5
2	464	431	448	13·5	12·8	13·2	474	454	464	21·3	20·1	20·7
3	430	442	436	11·6	11·4	11·5	462	512	487	18·3	17·8	18·1
4	407	448	428	12·4	12·4	12·4	497	578	538	19·2	19·2	19·2
Trial												
1	518	426	472	11·8	13·1	12·5	535	479	507	18·6	20·6	19·6
2	458	435	447	12·3	11·7	12·0	487	502	495	19·2	18·4	18·8
3	449	482	466	12·4	12·6	12·5	489	547	518	19·5	19·7	19·6
4	411	420	416	11·5	11·8	11·7	465	530	498	18·1	18·3	18·2
5	387	390	389	12·1	11·9	12·0	438	505	472	19·1	18·5	18·8
6	406	400	403	12·8	12·8	12·8	467	473	470	20·1	19·7	19·9

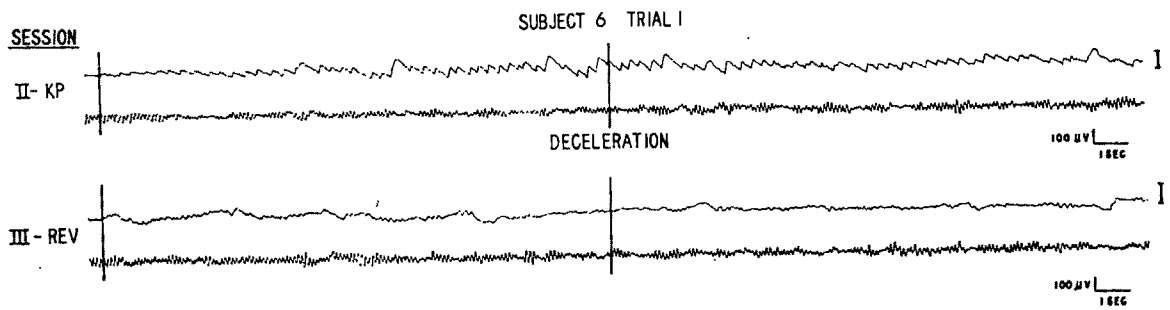


Fig. 1. Nystagmus and EEG tracings. Vertical bars through the records demarcate the stimulus period. *KP* and *REV* refer to key pressing (estimating subjective velocity) and reverie, respectively. Eye-movement calibrations (20°) appear at the right of each nystagmus tracing

occurred during the acceleration portion of the mental arithmetic trials (Table 1). The differences noted between our findings and those of Makarian and Koreshkov may be due to differences in electrode placement (they used temporal-occipital leads), in rotary velocity, or in subjects (instructions and prior experience).

Using caloric stimulation, Lidvall[7] recently reported no changes in EEG activity, although nystagmus output varied. In addition, he noted that alpha-blocking rarely occurred, and was never marked, when the vestibular stimulus was introduced. Our results and an earlier report[2] are in agreement with his observations.

That the tasks produced striking behaviour differences is clear from the nystagmus data. However, whether subjects were relaxed, attending to rotatory sensations, attending to external auditory signals, or doing mental arithmetic, alpha activity continued at the same rate during periods of sensory stimulation (acceleration and deceleration). These results lend support to the findings of Mulholland and Runnals[5] who, using attention-sets and "recurring complex alerting" (responding to a recurring signal by giving a report), indicated that " . . . (alerting) processes showing little relation to alpha suppression were continually activated from *within* (after an initial starting signal) and were *enduring*". Alpha suppression appeared when subjects were given an external signal and were required to present a verbal report. That the subjects in this investigation showed no alpha suppression to the tonal stimuli (external signal) might be accounted for by their set (estimating signal duration), uninterrupted by verbal reports. Mulholland and Runnals suggest the need for a qualification of the classical alpha-attention hypothesis "in terms of a particular kind of attention process or behaviour," and that " . . . the classical . . . hypothesis refers to a special case, that is, transitory alerting to an external signal".

The results obtained in this investigation also suggest that: (*a*) under some conditions, EEG monitoring is a less sensitive index of a subject's state of alertness than are vestibularly induced eye movements. This confirms a previous indication[2] and is similar to results obtained from new-born infants by Pendleton and Paine[8]. The latter have observed that " . . . the absence of vestibular nystagmus seems one of the most sensitive signs (of some alteration in the state of consciousness) in the newborn. It changes earlier with approaching sleep than do electroencephalograph tracings or respiratory rates and rhythms". (*b*) In addition, the simple monitoring of eye movements in given situations (for example, during air- or space-vehicle manœuvres) without some knowledge of the mental activity-level of the subject may give "false negatives" in judging the presence or degree of possible vestibular involvement. The marked differences in vestibular response depicted in Fig. 1 underline the importance of the mental state of the subject.

The results were obtained while we were at the U.S. Army Medical Research Laboratory and Ireland Army Hospital, Fort Knox, Kentucky.

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FORTHCOMING EVENTS

Sunday, September 1—Wednesday, September 4

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at Aberdeen)—Continuation of 125th Annual Meeting.

Sunday, September 1

At 11 a.m.—Service in the West Church of St. Nicholas, Union Street. Minister: The Reverend Anderson Nicol, M.A., J.P. Preacher: The Reverend Prof. John M. Graham, D.D.

Monday, September 2

At 10 a.m.—Prof. J. H. Taylor, F.R.S.: "Some Aspects of Diagenesis", Presidential Address, Section C).

At 10 a.m.—Prof. T. Wilson: "The Price of Growth" (Presidential Address, Section F).

At 10 a.m.—Mr. Peter Opie: "The Tentacles of Tradition" (Presidential Address, Section H).

At 10.10 a.m.—Prof. H. M. Steven: "The Beneficent Forest" (Chairman's Address, Section K*).

At 2 p.m.—Dr. J. N. Murrell: "Colour and Chemical Constitution" (Kelvin Lecture).

Wednesday, September 4

At 10 a.m.—Dr. L. Wolpert: "Growing in a Definite Shape" (Darwin Lecture).

Wednesday, September 4

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. R. Farley and Mr. D. J. Ray: "The Design and Operation of the Pilot Scale Plant for Hydrocarbon Synthesis in the Slurry Phase".

Wednesday, September 4—Friday, September 6

INSTITUTE OF PHYSICS and the PHYSICAL SOCIETY (at the University of Manchester)—Conference on "Low and Medium Energy Nuclear Physics".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT (with practical experience in freshwater biology or a degree or equivalent qualification) to Dr. T. B. Reynoldson, Reader in the Department of Zoology, to work on the ecology of freshwater triclads—Dr. T. B. Reynoldson, Department of Zoology, University College of North Wales, Bangor, North Wales (September 7).

LECTURER IN THE DEPARTMENT OF SOCIOLOGY—The Secretary, The University, Edinburgh (September 7).

ASSISTANT LECTURER, Grade III (with a degree in biochemistry) IN BIOCHEMISTRY—The Registrar, The University, Senate House, Bristol 2 (September 11).

LECTURER, Grade II (with a degree in engineering and at least five years approved industrial experience) IN MECHANICAL ENGINEERING at the School of Mines and Industries, Ballarat, Victoria, Australia—Council for Technical Education and Training for Overseas Countries, 12 Lincoln's Inn Fields, London, W.C.2 (September 13).

SCIENTIFIC OFFICERS/SENIOR SCIENTIFIC OFFICERS (with an honours degree and postgraduate experience in entomology, botany or chemistry) AT THE TROPICAL STORED PRODUCTS CENTRE based at the Pest Infestation Laboratory, Slough, to spend part of the time overseas to undertake original investigations into problems associated with the handling and storage of foodstuffs in tropical climates, to evolve methods of improving quality and reducing losses, and to assist in the training of local personnel—The Research Department, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S.W.1 (September 13).

ASSISTANT LECTURER or LECTURER IN SOCIAL PSYCHOLOGY—The Registrar, University College of Swansea, Singleton Park, Swansea (September 14).

SENIOR LECTURER IN CHEMICAL PATHOLOGY—The Secretary, St. Thomas's Hospital Medical School, London, S.E.1 (September 14).

RESEARCH ASSISTANT or RESEARCH FELLOW (with a university degree or equivalent qualification), for research on control of food intake in animals—The Secretary, Marischal College, The University, Aberdeen (September 14).

LECTURER IN BIOCHEMISTRY (Veterinary Biochemistry)—The Secretary of University Court, The University, Glasgow (September 15).

LECTURERS or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF GEOGRAPHY, University of Malaya—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, September 15).

ASSISTANT LIBRARIAN—The Secretary, The University, Aberdeen (September 16).

LECTURER (preferably candidate with medical qualifications registrable in the United Kingdom) IN PHARMACOLOGY—The Registrar, The University, Manchester 13 (September 16).

RESEARCH ASSISTANT (graduate) IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY AND SOCIOLOGY in the Faculty of Economic and Social Studies—The Registrar, The University, Manchester 13 (September 16).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (graduate of Ph.D. standing or equivalent experience) IN THE DEPARTMENT OF VIROLOGY, to specialize in serology of animal viruses—The Secretary, Institute for Research on Animal Diseases, Agricultural Research Council, Compton, near Newbury, Berkshire (September 16).

ASSISTANT LECTURER (preferably with a special interest in physical geography) IN GEOGRAPHY—The Secretary to the Trustees, Magee University, Londonderry, Northern Ireland (September 17).

TECHNICIAN (male, preferably with experience in mycology) AT RUAKURA ANIMAL RESEARCH STATION, Agriculture Department, New Zealand, for work concerned with the study of mycotoxicoses of grazing animals—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B.13/2/22/2227 (September 18).

LECTURER IN ELECTRICAL THEORY AND MEASUREMENTS; a LECTURER IN ENGINEERING PHYSICS; and a LECTURER IN MATHEMATICS at Malta College of Arts, Science and Technology—The Council for Technical Education and Training for Overseas Countries, 12 Lincoln's Inn Fields, London, W.C.2 (September 20).

LECTURER or ASSISTANT LECTURER IN SOCIAL ANTHROPOLOGY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 20).

RESEARCH FELLOW AT THE UNIVERSITY OF LONDON COMPUTER UNIT, to develop research in a branch of numerical analysis in collaboration with senior staff of the Unit—The Secretary, University of London Computer Unit, 44 Gordon Square, London, W.C.1 (September 20).

CHAIR OF ADULT EDUCATION to be held in association with the DIRECTORSHIP OF EXTRA-MURAL STUDIES—The Registrar, The University, Manchester 13 (September 23).

CURATOR (preferably with the N.D.H. or an equivalent qualification, and trained in and possessing a certificate from a botanic garden) OF THE BOTANIC GARDEN, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, for duties which will include a preliminary detailed survey, and planning and clearing the area—The Assistant Registrar, Kwame Nkrumah University of Science and Technology, 15 Gordon Square, London, W.C.1 (September 23).

LECTURER IN PATHOLOGY at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 25).

LECTURER IN MATHEMATICAL STATISTICS at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 27).

SENIOR LECTURERS/LECTURERS (preferably with research interests in the fields of invertebrate histology or embryology) IN ZOOLOGY AND COMPARATIVE PHYSIOLOGY at Monash University, Victoria, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 27).

CHAIR OF PSYCHOLOGY at the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 30).

LECTURER (with some experience of hospital laboratory work, and preferably some teaching experience) IN THE DEPARTMENT OF CLINICAL CHEMISTRY—The Secretary, The University, Edinburgh (September 30).

MEDICAL LIBRARIAN (graduate with library qualifications and experience) for the Barr Smith Library, University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

PROFESSOR OF ANIMAL PHYSIOLOGY at the Waite Agricultural Research Institute, University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Adelaide, September 30).

STANLEY ELMORE RESEARCH FELLOW IN EXTRACTION METALLURGY, including MINERAL PROCESSING, for work in the Department of Metallurgy of a University or similar Institution in the United Kingdom—The Secretary, The Institution of Mining and Metallurgy, 44 Portland Place, London, W.1 (September 30).

TEMPORARY LECTURER IN EDUCATIONAL PSYCHOLOGY—The Registrar, The University, Manchester 13 (September 30).

ASSISTANT LECTURER (honours graduate in physics and preferably an interest in electronics) IN THE DEPARTMENT OF PROSTHETICS, Turner Dental School, for duties mainly in research on electro-physiological measurements in the dental field—The Registrar, The University, Manchester 13 (October 2).

LECTURER IN MATHEMATICS at the University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 4).

SENIOR LECTURER and a LECTURER IN APPLIED MATHEMATICS at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Australia, October 8).

LECTURER (preferably specialized in the psychology of education, including mental measurement) IN EDUCATION at the Institute of Education—The Registrar and Secretary, The University, Bristol (October 15).

SENIOR LECTURER or LECTURER IN PHYSICS at the University of Otago—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, University of Otago, Dunedin, New Zealand (New Zealand and London, October 15).

ASSOCIATE PROFESSOR (with high academic qualifications in science, engineering or textile technology, and preferably research experience in the field of textile physics) IN TEXTILE TECHNOLOGY at the University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (October 31).

FOUNDATION CHAIR OF PSYCHOLOGY at Monash University, Melbourne, Australia—The Secretary, The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (Australia and London, October 31).

READER or LECTURER (with a university degree in civil or agricultural engineering and with teaching and research experience preferably in soil and water engineering as related to agriculture or to conservation) IN AGRICULTURAL ENGINEERING at Lincoln College, University of Canterbury, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

SENIOR LECTURER/LECTURER (with an honours degree in science or engineering and preferably with experience in the application of problems to digital computers) IN THE DIGITAL COMPUTING LABORATORY, School of Engineering Science, University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W.C.2; and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (London and Australia, October 31).

AGRICULTURAL OFFICER (male, under 40, with a university degree in agriculture) IN THE DEPARTMENT OF AGRICULTURE, Bechuanaland Protectorate, for pasture research, vegetation surveys and soil conservation—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S.W.1, quoting Ref. C.1/RC.213/11/04.

ASSISTANT (with special interests in inorganic or physical chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Professor, Department of Chemistry, University College, Upper Merrion Street, Dublin 2, Republic of Ireland.

CHIEF LIBRARIAN (suitably qualified librarian, graduate of a university, and preferably some experience in a library of science or technology)—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 24/G.

EXPERIENCED MEDICAL LABORATORY TECHNICIAN (or Senior Technician) FOR THE DEPARTMENT OF CHEMICAL PATHOLOGY—Prof. F. T. G. Prunty, William Dunn Laboratory, St. Thomas's Hospital Medical School, London, S.E.1.

FELLOW (preferably post-doctorate, with interest and ability in optics and electronics), to take part in the optical maser programme of the Department of Physics, with special emphasis on harmonic generation and non-linear optical effects—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey.

NEUROCHEMIST (with a Ph.D. in biochemistry with postgraduate research experience in neurochemistry, or an interest therein), to participate in ongoing research programmes—The Director, Missouri Institute of Psychiatry, 5400 Arsenal Street, St. Louis, Missouri, U.S.A.

PHOTOGEOLOGIST (with a first- or good second-class honours degree in geology, about five years postgraduate experience in regional mapping and field geology, a good knowledge of photogeology, and preferably a knowledge of Spanish) with the Government of Peru, for the preparation of geological maps by means of photo-interpretation; some field work, and the training of locally recruited assistants—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S.W.1, quoting Ref. RC.218/117/02.

PHYSICAL CHEMIST (with an interest in, and preferably experience of, surface chemistry), for studies of pigment particles and their behaviour in paint media; a PHYSICIST or PHYSICAL CHEMIST, for fundamental work on the rheology of pigment and polymer latex dispersions; and an ANALYTICAL CHEMIST, to take charge of a small laboratory for devising qualitative and quantitative techniques applicable to paint components, pigments, polymers, surface active agents, etc., and for analytical studies of paint blemishes and the causes of defects—The Director, Paint Research Station, Research Association of British Paint, Colour and Varnish Manufacturers, Waldegrave Road, Teddington, Middlesex.

RESEARCH ASSISTANT (graduate chemist or biochemist) IN THE ARTIFICIAL KIDNEY UNIT—The House Governor, London Hospital, Whitechapel, London, E.1.

RESEARCH ASSISTANT or TECHNICIAN (graduate or non-graduate), to work in the field of chemistry in relation to medicine—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

RESEARCH ASSISTANTS and FELLOWS, for full-time research on polymer problems—Head, National College of Rubber Technology, Holloway, London, N.7.

SENIOR LABORATORY TECHNICIAN (Grade II) (preferably with a knowledge of optical instruments and their maintenance) IN THE DEPARTMENT OF OPHTHALMIC OPTICS, to be responsible for the clinical laboratories in the Department—Head, Department of Ophthalmic Optics, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

SENIOR TECHNICIAN (preferably with a knowledge of glass-blowing) IN THE DEPARTMENT OF PHYSICS, to assist in a research programme on large-gap semiconductors—Dr. R. H. Tredgold, Department of Physics, University College of North Wales, Bangor, North Wales.

TECHNICIAN or GRADUATE ASSISTANT, for biochemical research on root growth and morphogenesis involving cell and organ culture—K. W. Fuller, Botany School, The University, Oxford.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Institute of Seaweed Research. Annual Report for 1962. Pp. 23. (Inverask, Midlothian: Institute of Seaweed Research, 1963.) [17

Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization. Fire Note No. 4: Tests on Roof Constructions Subjected to External Fire. Pp. iii+24. (London: H.M. Stationery Office, 1963.) 2*s*. 6*d*. net. [17

Privy Council. Medical Research Council Monitoring Report Series, No. 6: Assay of Strontium-90 in Human Bone in the United Kingdom—Results for 1962, Part 1, with some Further Results for 1961. Pp. iii+15. (London: H.M. Stationery Office, 1963.) 1*s*. 6*d*. net. [17

Cement and Concrete Association. Report for the year 1962. Pp. 102. (London: Cement and Concrete Association, 1963.) [17

Annual Report of the Council of the Yorkshire Philosophical Society for 1962. Pp. 32. (York: Yorkshire Philosophical Society, 1963.) [17

Department of Scientific and Industrial Research. Torry Research Station Annual Report 1962 on the Handling and Preservation of Fish and Fish Products: Report of the Torry Research Station Steering Committee and Report of the Director of the Torry Research Station. Pp. vi+38+4 plates. (London: H.M. Stationery Office, 1963.) 4*s*. [17

Woman, Work and Age: To Study the Employment of Working Women Throughout Their Middle Lives. By F. Le Gros Clark. Pp. 111. (London: The Nuffield Foundation, 1963.) 7*s*. 6*d*. net. [27

Federation of British Industries. Civil Research Policy: a Report of an F.B.I. Working Party. Pp. iii+11. (London: Federation of British Industries, 1963.) 2*s*. 6*d*. [27

Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization. Fire Research Technical Paper No. 8: Fires Involving Liquefied Petroleum Gas. By Jane M. Hogg. Pp. vi+9. (London: H.M. Stationery Office, 1963.) 1*s*. 9*d*. net. [27

Ministry of Education. Statistics of Education, Part 2: 1962. Pp. 115. (London: H.M. Stationery Office, 1963.) 20*s*. net. [27

The International Nickel Company (Mond), Ltd. Automatic Plating Plant. Pp. 32. (London: The International Nickel Company (Mond), Ltd., 1963.) [57

Department of Scientific and Industrial Research; and the British Council. Scientific Research in British Universities and Colleges, 1962–63. Pp. xxv+669. (London: H.M. Stationery Office, 1963.) 32*s*. 6*d*. net. [57

Department of Scientific and Industrial Research. New Ways With Ceramics. Pp. 12. (London: Library, Department of Scientific and Industrial Research, 1963.) [57

White Fish Authority. Annual Report and Accounts for the year ended March 31, 1963. Pp. iv+55. (London: H.M. Stationery Office, 1963.) 3*s*. 6*d*. net. [57

Department of Scientific and Industrial Research. Research for Industry 1962: a Report on Work Done by Industrial Research Associations in the Government Scheme. Pp. iv+74+22 plates. (London: H.M. Stationery Office, 1963.) 7*s*. net. [57

Honours Courses in Mathematics in the Universities of Great Britain. By A. P. Rollett. Pp. ii+61. (London: The Incorporated Association of Head Masters; and the Association of Head Mistresses Incorporated, 1963.) 3*s*. 6*d*. [57

Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 60: The Riding Quality of Concrete Roads. By Dr. R. H. H. Kirkham. Pp. iv+87+4 plates. (London: H.M. Stationery Office, 1963.) 6*s*. 6*d*. [57

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SCIENTIFIC AND TECHNICAL AID FOR COMMONWEALTH COUNTRIES

IN replying for the Government in the debate on science in the House of Commons on July 15, the Secretary for Technical Co-operation, Mr. R. Carr, referred more especially to the role of Britain's scientific effort in the future of the Commonwealth. He pointed out that expenditure on overseas research, mainly in the Commonwealth, amounted in the current year to some £2 million. Of the figure of £2·3 million, given in a written answer by the Parliamentary Secretary for Science the following day, £2·16 million is through the Department of Technical Co-operation, but although Mr. Carr mentioned specifically a number of home-based units concerned with the problems of the developing countries, mainly in the Commonwealth, such as the Anti-Locust Research Centre, the Tropical Products Institute, the Tropical Divisions of the Road Research Station and the Building Research Station, the Tropical Store Products Centre and the Tropical Medicine Research Board, he gave no indication of the expenditure of any of these units.

All such information has been lacking since the publication of the report *Colonial Research* was terminated. Mr. Carr did, however, mention the very important work of the Land Use Section in the Directorate of Overseas Surveys, which was being strengthened further, and where soil scientists were doing very valuable scientific work in assisting the development of the natural resources of many of these countries. It was estimated that at present Britain was sending abroad to these countries more than 2,000 scientists, but part of her substantial contribution to scientific development in Commonwealth and in some other under-developed countries lay in the training of some 4,300 students of science at her universities and about 8,600 students at technical colleges.

Mr. Carr welcomed the reference of Sir Harry Legge-Bourke to the importance of this effort, but his speech did little to fill the gap left by the discontinuance of the reports on *Colonial Research*. By implication, however, his speech seemed to point to a continuing and increasing need for such a survey of overseas research, and this appears to have been in the background of recent debates on the report of the Colonial Development Corporation. The Corporation itself is not concerned with research, although there is a reference in the report to continued metallurgical research, partly at the Warren Spring Laboratory, with the view of developing an economic flotation process for a pyrochlore deposit, and the development projects for which the Corporation provides money could often not be possible apart from the results of earlier research. It also, however, brings in skilled management and skill in training local people as the managers of the future, and the benefits of its skilled management are not only reserved for the Corporation's own projects. As often as possible these benefits are placed at the disposal of small farmers in the countries in which the Corporation operates, and in this way the Corporation has not only done much to develop agriculture but has also contributed to the advances in understanding which make scientific and technical advance elsewhere possible.

When the Commonwealth Development Bill received a second reading in the House of Commons on June 19, there was considerable reference to the Corporation's report. In introducing the Bill, Mr. J. Tilney, the Under-Secretary of State for Commonwealth Relations, pointed out that the Corporation's operating surplus had risen from £1,869,000 in 1959 to £4,361,000 in 1962, when the profit was £1·6 million; since 1952 only £250,000 had been lost, although by that date £12 million had been lost out of the then investment of £21 million. The total investment now approaches £100 million and is handled by a head office staff of 157 and a regional staff of 78, compared with a head office staff alone at the end of 1951 of 325. In 1962, the Corporation's investment in manufacturing and processing enterprises alone was associated with a turnover of £38 million, of which goods worth £23 million were exported from the territories concerned, and these activities last year generated more than £7 million of British exports. Mr. Tilney emphasized that on the successful establishment of a project the Corporation was ready to dispose of its investment at a satisfactory price, particularly to buyers resident in the country where the project was located, and the proceeds of such a sale were available for use in the development of some other project.

Mr. Tilney explained that the Bill amended the Overseas Resources Development Act 1959 and the Colonial Development and Welfare Act 1959 so as to put back into the sphere of activity of the Corporation, now to be renamed the Commonwealth Development Corporation, all Commonwealth territories which were within its scope when it was set up in 1948. The Corporation would, however, be instructed to seek the prior approval of the Secretary of State for Commonwealth Relations before starting operations in an independent country and that approval would be given only with the agreement of the Government of that country. The Corporation would still be excluded from India, Ceylon and Pakistan, and while the late Mr. John Strachey strongly challenged this in the debate, holding that the Corporation should not be stopped from initiating a project anywhere in South-east Asia if it wished, the Under-Secretary of State for the Colonies, Mr. N. Fisher, maintained that the Corporation could already exercise advisory functions in those countries. The Government view that to do more would seriously disturb the balance of the Corporation's activities was maintained in the subsequent debates at the Committee stage and in the House of Lords.

Mr. Tilney added that there was no intention of raising the present limit of £130 million in the amount the Corporation could borrow from the Treasury, and, turning to the colonial development and welfare provisions of the Bill, he said that the Bill extends the period for making and approving such schemes from March 31, 1964, to March 31, 1966, increasing from £315 million to £340 million the amount which could be spent on schemes under Section 1 of the Act. Since by March 31, 1963, about £43·5 million was unexpended, about £68·5 million would be available for the three years 1963–66. While previously research and technical assistance had been provided under the Colonial Development and Welfare Acts, this expenditure was now financed by the Depart-

ment for Technical Co-operation, but the change did not imply any decrease in the amount of research carried for the benefit of the colonial territories. When the Bill was introduced into the House of Lords on July 16, the Minister of State for Colonial Affairs, the Marquess of Lansdowne, repeated that the change did not mean that any less importance was attached to research activities or technical assistance.

While the Bill was generally welcomed in the House of Commons and some warm tributes were paid to the work of the Colonial Development Corporation, the chief criticism was in respect of the exclusion of the Corporation from India, Ceylon and Pakistan and on the limitation on its Treasury borrowing. Mr. T. Dalyell made a useful suggestion that the Corporation could assist in supplying some printing and paper-making apparatus which would enable the developing areas to cope with their critical shortage of paper, writing paper and text-books—a suggestion which Mr. Fisher promised to note particularly—but Mr. P. Walker challenged the Bill as failing to meet the need of potential investment in the Commonwealth. Mr. G. M. Thomson, directing attention to the anomalous position of the Corporation with reference to the Colonial Office, the Commonwealth Relations Office and the Department of Technical Co-operation, thought that the Corporation should now come under the last and that it might usefully play some part in the administration of Colonial Development and Welfare funds.

Criticism of the placing of the Colonial Development Corporation under the Commonwealth Relations Office was again voiced in the House of Lords on July 16 when the Earl of Listowel again advocated the merging of the Colonial Office and the Commonwealth Relations Office and urged the appointment of a new 'minister for overseas development' to watch the increasing importance of this function of Government. Such a Minister, with a seat in the Cabinet and responsible for the whole of Britain's overseas aid, would be responsible for all non-military Government expenditure in developing countries. Besides his concern with an adequate share of Government expenditure and as the spokesman of British aid, he would determine priorities relative to the limited amount of capital and technical aid available and would co-ordinate both money and technical skill. He would also ensure that representatives of recipient countries and of international bodies giving aid were fittingly received.

The Bill was generally welcomed in the House of Lords also, but the Marquess of Lansdowne was very guarded concerning Lord Listowel's suggestion of a single minister for overseas development. Neither in the Lords nor in the Commons, however, was there any further specific reference to research and it would seem that a further opportunity has been missed of directing attention to a conspicuous omission over the past two years or more. Despite the importance of overseas research, both in relation to economic development and to educational development, and the alarm in particular quarters over the threatened closure of important research institutes, Britain is not being given the information on which to base a realistic opinion as to the scale and disposition of her effort in that field, or as to whether the existing arrangements are appropriate and efficient. This is a matter to which the Department of Technical Co-operation should be giving urgent and increased attention with the full support of all scientists working in this field.

A GREAT MATHEMATICIAN

The Mathematical Works of J. H. C. Whitehead

Edited by I. M. James. Vol. 1: Differential Geometry. Pp. xxxiii+361. Vol. 2: 'Complexes and Manifolds', Pp. xiii+435. Vol. 3 : 'Homotopy Theory', Pp. xiii+451. Vol. 4: 'Algebraic and Classical Topology', Pp. xiii+347. (Oxford, London, New York and Paris: Pergamon Press, 1962.) 84*s*. per volume; £15 per set.

THE question whether the publication of the collected works of a mathematician is justified is one on which there can be more than one opinion. It can be argued that the labour involved in editing the papers and the cost of the collected edition are scarcely justified in these days when almost everyone has access to a good library of mathematical journals, and there are many efficient reference books. This is indeed a valid argument if the publication is simply an act of piety; and it is even more true if most of the author's papers, though of importance at the time when they were written, are of ephemeral interest. But there are cases when the publication of the collected works of a mathematician can be a real service to the mathematical community; as, for example, when the mathematician in question has been at the centre of one or more of the major developments of his time. Then it can be of great value to those who come after him to see the subject unfolding itself, and to re-capture the spirit behind the original development.

The publication of the collected papers of Henry Whitehead is fully justified. When Whitehead first began his work on topology, he and Prof. M. H. A. Newman were the only two British mathematicians who were making any significant contribution to the subject. While Prof. Newman, though he has maintained his interest in topology and has made significant contributions to it, has extended his interest in mathematics beyond what is strictly topological, Whitehead devoted all his energies to tackling major problems in topology, and so establishing himself as a world figure in this field. Many of Whitehead's contributions are fundamental in topology, and some of his methods are still the most powerful available: thus, for example, the proof of the generalized Poincaré hypothesis given by Dr. Stalling just after Whitehead's death is based on the foundations laid by him in 1940. There is now a large and distinguished British school of topology; many of its members owe everything to Whitehead, and all owe him a great deal. To all of them his collected works will be invaluable. Whether they can all afford to purchase the four volumes is perhaps a doubtful question, but for mathematical libraries they are 'a must'.

Dr. James, the editor, is to be congratulated on the speed with which he has brought the volumes out; the value of the collected works would have been greatly reduced had we had to wait for them as long as we have had to wait for the collected works of some others. In order to achieve this, Dr. James has had to work to a very simple plan which has resulted in some loss of elegance. The papers are fairly easily classified. In the main, Whitehead's work on any particular topic was done at one time, and the order is broadly, though not completely chronological. Other publications, such as the Cambridge Tract on the *Foundations of Differential Geometry* (written jointly with Veblen), and the very informative biographical memoir of Elie Cartan, written for the Royal Society are easily fitted into their proper place. The first volume contains all Whitehead's writings on differential geometry and Lie groups, and covers the first phase of his mathematical activity; the second volume deals with his work on complexes and manifolds, and contains some of the papers which established him as a world figure; the third volume contains the papers in which Whitehead made his massive contribution to homotopy theory and the final volume deals with fibre spaces and the

Spanier–Whitehead *S*-theory, and ends with his final papers on classical topology. Only a limited amount of editing has been done; a very few corrections have been made, and the headings of papers have been reduced to uniform style. A small criticism is that the papers are not numbered, and the original place of publication is not given with the papers; this slightly complicates the task of following up cross-references. The reproduction has been by a photographic process from reprints; the result is at first a little disconcerting, due to the varieties of type used by the different journals in which the papers originally appeared.

Each volume contains a complete bibliography of Whitehead's works, and the table of contents of all four volumes, as well as an extremely good portrait (the same in each volume). In addition, the first volume contains a delightful personal memoir by Prof. M. H. A. Newman and Mrs. Whitehead, and a critical appreciation of Whitehead's contributions to mathematics by Prof. John Milnor of Princeton University.

Prof. Milnor's appreciation is so just, and so illuminating, that it would be an act of supererogation to attempt another here. Both this, and the personal memoir, recall vividly to my mind my own relations with Whitehead. I do not think I ever met anyone who so obviously enjoyed mathematics. I was very seldom in his company when the conversation did not turn, sooner or later, to mathematics, not because he had no other subjects to talk about, or because he wanted to talk of what he himself was doing. He usually talked about the mathematics that interested his audience, and if he could find a relation between this and what he himself was doing, so much the better. But it was no monologue; everyone was drawn in and enjoyed the discussion, usually much more than he normally enjoyed talking shop. Whitehead's enthusiasm was infectious, and this was one of the reasons why his lectures were so stimulating. I never heard him deliver a formal course of lectures; but on many occasions I have been present when he gave a talk to a mathematical society, or a seminar, or a students' club—and he was most generous in giving such talks. Viewing them from the narrow standpoint of lecturing technique, I suppose one would say they were not good lectures: he often gave the impression of feeling his way while he was speaking, so that at first one might think that the acknowledged expert did not know much about his own subject, but he carried his audience with him, and aroused their interest, and at the end one was surprised to realize how much had been achieved. His style was most informal: he usually lectured with a piece of chalk in one hand and a cigarette in the other, and not infrequently there was an interruption when he got their functions interchanged. But for ability to provoke enthusiasm, he had few equals.

Whitehead was that *rara avis*, a mathematician who grew in stature as he grew older. His first work, in differential geometry, was done under the influence of Veblen, and his early work in topology showed the influence of J. W. Alexander and Lefschetz. In this, while he achieved some valuable results, and seemed, in a way, to be moving towards some of the great achievements of later years, he still seemed to be groping for something to give him a real chance. The big break came at the International Congress of Mathematicians held in Oslo in 1936, when discussions held with other mathematicians present stimulated his interest in the problems of homotopy. His achievements in this field came much later, but from then on his work seemed to have more direction. He was just getting into his stride when the War interrupted his career.

He was forty when the War ended, and the task of getting back into the stream would have daunted many of his age. But he never faltered, and he took up where he had left off, and when, in 1947, he became Waynflete professor at the University of Oxford, he was ready to take full advantage of the opportunities which the appointment offered. From then, he went on from strength to strength, and although he was fifty-five when he died there was never the slightest sign of any decline in his powers. The frequent visits that he paid to Princeton had, I think, much to do with his success. His easy manner with all with whom he came in contact enabled him quickly to absorb the latest trends, and refreshed by these visits he returned to Oxford to stimulate the growing school of topologists there, which he left without a rival in the country. Every topologist of any standing has at some time been either his pupil or his colleague, and he had an unrivalled gift of getting the best out of anyone with whom he came in contact. The number of distinguished pupils who freely acknowledge what they owe to Whitehead is an even more fitting memorial to him than these four excellent volumes of his mathematical works.

W. V. D. HODGE

COMMUNICATIONS SATELLITES

Communications Satellites

Proceedings of a Symposium held in London, 12 May 1961, organized by the British Interplanetary Society. Advisory Editor: L. J. Carter. Pp. ix + 202. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1962.) 42*s*.

WITHIN the last two years there have been held, in Great Britain, three conferences concerned with the use of satellites for world-wide radio-telephone and television communications. The first of these was organized by the British Interplanetary Society in London in May 1961, the second by the British Institution of Radio Engineers at Oxford in July 1961 and the most recent by the Institution of Electrical Engineers in London in November 1962. The great interest in the subject arises because satellites appear to provide the only means of bringing about a major expansion in communications capability to meet global needs. Communications satellites cannot, however, be a success unless use is made of the very latest scientific knowledge in a number of different fields. These include the design of radio receivers with the lowest possible internal noise, the design of very-high-power transmitter valves, the design of electronic circuits which are insensitive to radiations occurring in space and the development of communication—theories on how best to modulate radio waves in order to transmit the maximum number of telephone channels with given equipment.

This book contains eight collected papers which were read at the first of the three symposia. Since then, much has happened. The *Telstar* and *Relay* satellites have been successfully launched, and organizations such as the General Post Office have obtained a wealth of practical experience on the use of satellites and the associated ground terminal equipment for television and other communications. The book, therefore, cannot be regarded as completely up to date. In addition, the papers contain relatively little on the various problems of designing low-noise receivers, large aerials and transmitters for the ground terminals and little on the detailed design of relay equipment for satellites.

In spite of these limitations, however, the book does contain some very useful theoretical papers on the general design of communication systems involving satellites. Such features as the optimum shape of orbit, height of satellite and operating frequency are considered in several papers and it becomes clear that to be commercially successful the satellites must be active repeaters, not passive reflectors such as the *Echo* balloon, and that they should be controlled in their relative positions and attitude with respect to the Earth. One particularly difficult matter to resolve is the optimum type of radio

modulation to use. This problem, which has to be considered in the light of the required services and satellite technology, is dealt with in two papers, and the conclusion drawn is that a form of pulse code modulation should be used. In contrast, both the *Telstar* and *Relay* systems actually use wideband frequency modulation. A minor point is that one paper (p. 177, Fig. 31), which discusses the overall cost of satellite systems, appears to treat the ground terminals and the satellites as separate items. Thus as the satellite height is increased, the satellite cost increases rapidly while the terminal cost is shown at a constant figure. Perhaps with high altitude orbits it would have been preferable to consider smaller satellites, in order to minimize launching costs, and to accept larger ground terminals than would be needed in the low altitude case. T. W. G. Dawson

RADIO WAVE PROPAGATION

Monograph on Radio-Wave Propagation in the Troposphere

Proceedings of Commission II on Radio and Troposphere during the XIIIth General Assembly of URSI, London, September 1961. Edited by J. A. Saxton. Pp. viii+200. (Amsterdam and New York: Elsevier Publishing Company, 1962.) 48*s*.

Monograph on Ionospheric Radio

XIIIth General Assembly of URSI, London, September 1960. Edited by W. J. G. Beynon. Pp. vi+264. (Amsterdam and New York: Elsevier Publishing Company, 1962.) 65*s*.

Monograph on Radio Noise of Terrestrial Origin

XIIIth General Assembly of URSI, London, 1960. Edited by F. Horner. Pp. vii+202. (Amsterdam and New York: Elsevier Publishing Company, 1962.) 48*s*.

Space Radio Communication

A Symposium held under the Auspices of the International Scientific Radio Union in Paris in September 1961. Edited by G. M. Brown. Pp. xii+630. (Amsterdam and New York: Elsevier Publishing Company, 1962.) 140*s*.

The Measurement of Characteristics of Terrestrial Radio Noise

(URSI Special Report No. 7.) Pp. vii+58. (Amsterdam: Elsevier Publishing Company, 1962.) 25*s*.

Radio Wave Propagation and the Ionosphere

By Ya. K. Al'pert. Authorized translation from the Russian. Pp. x+394. (New York: Consultants Bureau, 1963.) 22.50 dollars.

THE first five volumes listed here are reports from the Union Radio Scientific Internationale (URSI), one of the Unions of the International Council of Scientific Unions. Three are reports of the meetings of separate Commissions at the thirteenth General Assembly held in London in 1960, one is a report of a special symposium on "Satellite Communications" held in Paris in 1961, and one belongs to a series of special reports prepared by URSI, from time to time, on radio topics of particular interest.

Radio-Wave Propagation in the Troposphere is a report of the 1960 meetings of Commission II which were devoted to the subjects of experimental data on tropospheric propagation, physical characteristics of the troposphere, theories of tropospheric propagation, and radio-meteorology and climatology. It contains twelve papers on these subjects.

Ionospheric Radio is a report of the meetings of Commission III. It contains sixteen papers dealing with the electron-density/height profiles $N(h)$, $F2$ region ionization, sporadic E ionization, rocket and satellite data, very-low-frequency emissions and hydromagnetic waves, aurorae, ionospheric scattering, and ionospheric drifts.

Radio Noise of Terrestrial Origin presents edited reports of the meetings of Commission IV and covers sources of noise in lightning, properties of natural noise, International Geophysical Year whistler data, very-low-frequency emissions and hydromagnetic waves, whistler theory, the exosphere, man-made noise, very-low-frequency propagation, together with ten invited papers on some of these subjects.

Each of the volumes contains an edited account of the discussions which took place at the meetings. The accounts for Commissions II and III are given more or less verbatim.

The appearance of these three volumes prompts one to ask how far publication of the proceedings of meetings of this kind is justifiable, or valuable. In this context it must be remembered that the more formal *Proceedings* of the General Assembly are fully reported in the publications of URSI itself, the volumes here reviewed are concerned only with scientific aspects of the proceedings. They might serve one or more of the following purposes: (*a*) to provide an account, for historical reasons, of what was said at the meetings; (*b*) to provide good critical surveys of the subject; (*c*) to give summarized, but non-critical, accounts of work done by different groups or countries; (*d*) to present new results. It is thus worth asking how far these purposes are accomplished and how far their accomplishment is desirable.

The basic subject-matter of the three volumes is not new, it has been studied for several years, and there is inevitably, in all the papers, much that is well known. Where there is something new it has to be sought out from among the older material. For meetings of this kind it is seldom that authors have time, or inclination, to prepare really penetrating new critical surveys, and the 1960 General Assembly was no exception. The most useful are probably those by Briggs on ionospheric drifts and by Storey on whistlers. The other survey papers are more accurately described as summaries, often of work in one particular country. The 'edited discussions' are tedious to read, with important and trivial remarks intermingled; one would scarcely expect to look there for considered opinions.

Although I myself appreciate and admire the large amount of effort and time that has been put, by authors and editors, into the production of these volumes, I believe that it was mistaken policy to publish them, and that they represent an unfortunate waste of time for the writers, editors, and (perhaps even more important) for the readers. If they contained a really good critical review, or original paper, then it should, in my opinion, have been published in the usual way in a recognized scientific journal. The only purpose which the volumes achieve satisfactorily is to provide an account, for historical reasons, of what was discussed at the meetings; but a printed book is not required for that purpose. It is to be hoped that URSI will consider carefully its policy for publishing the results of the next General Assembly to be held in Tokyo in September this year.

Space Radio Communication is the report of a symposium organized by URSI. It contains 38 papers divided under the headings "Introduction", "Launching, Altitude Control and Tracking of Satellites", "Frequency Allocation, Interference and Propagation Problems", "Satellite Equipment", "Ground Equipment", "Modulation Systems", "Specific Communication Systems", and "Miscellaneous Aspects". Because the subject is a very new one the report is not open to the foregoing criticisms. There is little repetition of well-known material and most of the papers present what to many readers will be new and interesting ideas. The book as a whole provides a stimulating introduction to the subject. As a minor

criticism, it might be asked whether there was any value in including the three introductory formal addresses.

The Measurements of the Characteristics of Terrestrial Radio Noise was prepared by a working party set up at the eleventh General Assembly to discuss the problem of measuring and specifying the characteristics of radio noise in a manner useful to the design of a communication system. It describes how the noise is received on narrow-band receivers tuned to different parts of the radio spectrum and observations are made on the amplitude distribution and the time variations of the envelope of the output E.M.F. The different methods of measuring are discussed and recommendations are made for observations on a world-wide scale. The question of which characteristics of the noise are important for different types of communication is discussed. The problems involved are complicated, and it is perhaps not surprising that no very clear conclusions are reached.

The well-known book on *The Propagation of Radio Waves* by Al'pert, Ginzburg and Feinberg was published in 1953 and when the time came for re-writing it was split into three parts, each written by one of the authors. Al'pert wrote *Radio Wave Propagation and the Ionosphere*, which was published in Moscow in 1960, and it now appears in an authorized translation published in the United States. The book is divided into two parts, "The Ionosphere", and "Propagation of Radio Wave of Different Frequencies". The first part deals with the structure of the ionosphere, the theory of its formation, its regular and irregular variations, and the collision frequency of electrons. The second part deals, in succession, with ground waves, long, medium, short, ultra-short and micro-waves. The theory of tropospheric propagation is dealt with when the shorter waves are discussed. There is a bibliography of 243 key papers, a large portion of them in languages other than Russian. It is noticeable that the text contains a large proportion of diagrams copied direct from well-known papers in other languages. The book as a whole presents a clear account of the subject, somewhat out of date in some particulars, but of considerable interest when it deals with the author's own work.

J. A. RATCLIFFE

GEOLOGY OF IRELAND

Historical Geology of Ireland

By Prof. J. K. Charlesworth. Pp. xxiii+565 (140 illustrations). (Edinburgh and London: Oliver and Boyd, Ltd., 1963.) 84*s*.

THE aim of this book is to produce an advanced and comprehensive account of Ireland's geology. This is a major undertaking and one which has not really been attempted until now, for Cole and Hallissey's small volume of forty years ago was scarcely an advanced treatise. Clearly, no Irish geologist is more fitted to undertake this work than the illustrious author who, as head of the Department of Geology in the Queen's University of Belfast for more than thirty years, has vigorously worked in his special field of Irish glacial geology, and in many other topics as well. His earlier work—an elementary study of Irish geology in the nature of a school's or general reader's reference book—will have served as a preliminary to the present volume.

This book fully merits the title 'comprehensive'. In its bibliography of more than 750 references the reviewer could note only one or two very minor omissions. Nearly all the items receive some consideration in the text. In addition, it is fully up to date. In fact, several of the works to which the author has had access have not yet appeared in print. Such has been the development of Irish geology that a comparable work, if written a decade ago, could not have had much more than half the present coverage.

But the day must be at hand when an advanced work by a single author, covering the whole field of any science, is a practical impossibility. One wonders if—relative to the present work, for example—the advantages of a uniform style, treatment, and outlook can really counterbalance the difficulty in acquiring a first-hand, specialist, acquaintance with every actively developing field in geology.

The book achieves a great deal: its limitations are those which are to-day inherent in a treatment of this sort. It is, strictly speaking, a compilation. As such, it is a thorough one and an invaluable source of Irish geological knowledge. It treats all the major works on Ireland's geology equitably and carefully, the coverage is sometimes uneven, and for areas not recently investigated may be decidedly thin. The enumeration of local rock successions and rock groups *in extenso* can make for difficult reading, and in the accounts there is seldom any distribution of emphasis to help the general reader. Correlations of rock groups are often taken from old works and may not please the modern specialist. One would often wish for something of the more lively background of rock genesis and structural evolution. In particular, one misses any attempt to relate the country's geology to the broader setting of that of north-western Europe.

But, all in all, it is a most important and carefully compiled work, and will hold the field as a standard reference for many a day. It is a book designed, not for easy reading, but as a source in which the advanced student or professional worker can obtain accurate information on any aspect of Irish geology. In this way it fulfils a major requirement in Irish science, and should in later editions acquire the status of a great scientific work. This reviewer would wish, in particular, to see co-operation of the author and specialist workers achieving comprehensive syntheses of the geology treated in each chapter of the work, with the country's setting clearly depicted in relation to the general European background. Clearly, too, the work demands a series of specially drawn illustrations, laborious and costly though their preparation may be. The present figures are adequate in number and scope, but they are an irregular hotch-potch culled from original papers over the past sixty years or more. Each one was designed for a particular purpose, and it is seldom really suitable for the present work. Sometimes inaccuracy is thus introduced. Fig. 56, for example, though it has appeared in both the authors' works, has not yet resolved the confusion between reef-knolls and volcanic rocks. On a technical point, more attention might be given to misprints, which are common in the case of technical terms and place-names. To the expert they are no more than blemishes in a finely produced book, but the tyro may find them confusing.

J. C. BRINDLEY

GEOLOGY OF A NATURE RESERVE

The Geology of Moor House

A National Nature Reserve in North-East Westmorland. By Dr. G. A. L. Johnson. With contributions by Dr. K. C. Dunham. (Monographs of the Nature Conservancy, No. 2). Pp. xviii+182+14 plates. (London: H.M. Stationery Office, 1963.) 55*s*. net.

THIS volume follows the first monograph on *The Plant Communities of the Scottish Highlands* and is in sharp contrast in scope.

The Moor House National Nature Reserve comprises some 10,000 acres of high-lying, remote mountain and moorland, straddling the Pennine Watershed a little south of Cross Fell. This comprises a comprehensive account of the solid geology and the vitally important superficial modifications of glacial and post-glacial times and includes a detailed account of the soils.

The Conservancy wisely welcomed the proffered co-operation of the Geology Department of the University of Durham, and a detailed survey of the solid rock, drift deposits and soils was carried out by Dr. G. A. L. Johnson under Prof. K. C. Dunham's direction.

Detailed stratigraphy and palæontology of the Carboniferous rocks and associated Older Palæozoic and younger igneous intrusions occupy rather more than one half of the volume. There is also a new geological map in colour, on a scale of three inches to one mile. There are minor changes in correlation in the classification of the North Pennine Carboniferous. The retention of the 'Upper Limestone Group' formational name for the Namurian strata present and the elimination of the term 'Carboniferous Limestone Series' may be noted.

Clay mineralogy investigations, mainly by differential thermal analysis, have confirmed the dominance of illite in marine sediments and the restriction of the kandite clays to the deltaic and terrestrial part of the prevalent rhythmic or cyclothermic depositional pattern. The differential thermal analysis graphs and the commentary are a valuable contribution.

Prof. Dunham's two chapters deal succinctly with geological structure and mineral deposits. The latter comprise the important lead and barytes-bearing Dun Fell and Henrake Vein Complex. A short chapter on geomorphological setting could have been included here with advantage.

The last third of the book, which deals fully with the Quaternary, has perhaps the widest appeal. It is based on a detailed survey, the results of which are embodied in a new soil map. Glacial boulder clay of local origin is widespread beneath the peat. The great importance of solifluction processes, particularly in the distribution of mineral soils, is emphasized. Sandstone blockfield type of accumulation often overlies boulder clay, and stone polygon structures beneath the peat testify to the fossil character of much of these movements; but at these high altitudes solifluction processes are still active.

Laboratory investigations of soils have indicated the increasing importance of mixed-layer chlorite–illite at the expense of illite in the progressive conversion of bedrock to glacial drift, solifluxion deposited mineral soils. Typical profiles are given for a range of gleys, podsols, brown earths and peat soils and their variations according to physical environment described. Detailed profiles of thick bog and blanket peats are accompanied by full pollen diagrams which confirm and amplify the results of earlier workers.

The change from maximum spread and growth of the peat in Atlantic times to pronounced erosion is linked with the onset of cold, wet conditions of the Sub-Atlantic phase following the warm, dry, Sub-Boreal phase. In view of the widespread association elsewhere in peat stratigraphy of erosion intervals during warm dry phases, is it not perhaps more likely that localized drying out and cessation of peat growth were associated with weathering and local erosion in the Sub-Boreal ?

Subsurface channelling and surface collapse in peat bogs is said to be exactly paralleled with the erosion cycle in limestone country—surely this is heightening of what Is an interesting analogy in which solution and joint control factors in limestone imply very different processes. But the peat process is worthy of further study.

Generally topics are introduced at an elementary level as befits a work with a wide range of potential readers. Some stricter editing could have eliminated a number of minor repetitions. There are a good bibliography and index. The whole volume is an admirable beginning for a series of geological monographs for nature reserves which set the fundamental background for developing biological studies. The author and the Nature Conservancy are to be congratulated on a well-produced, clearly illustrated and scholarly contribution to knowledge.

S. E. Hollingworth

ZOOLOGICAL MICROPALÆONTOLOGY

Principles of Zoological Microplæontology

Vol. 1. By Dr. Vladimir Pokorny. Edited by John W. Neale. Translated by K. A. Allen. (International Series of Monographs on Earth Sciences, Vol. 10). Pp. xiv+652. (London and New York: Pergamon Press, 1963.) 84*s*. net.

THIS long-awaited English translation is a valuable and welcome contribution to the basic literature available to micropalæontologists.

The book, originally compiled from the author's lectures for students at the University of Prague, was first published in Czech in 1954, and afterwards published as a revised and expanded German edition in 1956. The new English edition, which is a direct translation from the German, does not contain any significant additional subject-matter.

Principles of Zoological Micropalæontology is intended as a hand-book of instruction for the advanced student or research worker, and as a reference work for the commercial micropalæontologist. It deals with seven different groups of microfossils, but principally with the Foraminifera. More than half out of a total of five hundred and nineteen pages, exclusive of the bibliographies, etc., are devoted to this order of the protozoa. Three short introductory chapters deal with micropalæontological definitions, methods, techniques, and microstratigraphical principles ; these are interesting and useful but general in approach. In six brief chapters the Radiolaria, Thecamoebae, Tintinnina, Chitinozoa, Hystrichospheres and certain problematic microfossils are discussed. The principal facts relating to the morphology, biology, structure and ecology of each group are given together with an outline of the classification. There is also a short account of the geological distribution and the history of research on each group of organisms.

As a preface to the taxonomy of the Foraminifera the author discusses the principles underlying a natural system of classification for the order. This section is Pokorny's main philosophical contribution, and consists of a critical appraisal of contemporary Russian thought on evolution, and its application to the classification of the Foraminifera.

It is strange to find that the author in his interesting historical survey of foraminiferal research does not refer to the systems of classification proposed by such workers as Cushman and Glaessner, although his own classification is a natural development of the scheme proposed by Glaessner in his *Principles of Micropalæontology* (1945). Pokorny recognizes nine superfamilies, compared with Glaessner's seven, and he subdivides these into sixty families and forty-two subfamilies. The generic descriptions are usually very brief, and most are illustrated by line drawings or photographs. In some instances, particularly among the larger Foraminifera, these are completely inadequate; the family Miogypsinidae is a case in point. There is only one line drawing of a schematic equatorial section for the genus *Miogypsina*, and the other genera in the family are not illustrated. Perhaps the most serious criticism which can be levelled against a book of this kind is that many well-known and quite important genera, for example, *Kathina*, *Brotzenia*, *Cycloloculina*, *Nubeculina*, *Praerhapydionina* and *Sherbornina*, to name only some, have been omitted, while other genera of doubtful validity, for example, *Epistomina*, have been included. It appears that the commonly occurring European genera are well represented, but the author's treatment of those found elsewhere is less adequate.

The section dealing with the geological distribution of the Foraminifera is useful since the records of the range of the Palæozoic genera have been brought up to date and recent Russian work has been included. A short glossary of terms in English, French, German and Russian

completes the chapter on the Foraminifera. This should be useful to research workers dealing with original papers, but the transliteration of the Russian terms mean that it has lost some of its value. The other microfossils are discussed in a much less exhaustive manner than the Foraminifera, for example, only the orders, suborders and superfamilies of the Radiolaria are described, and only the Tintinnina and the Hystrichospheres are treated generically.

The complete bibliography of seventy-five pages is generally adequate up to 1956, but since the more recent literature is not included, the book is already seven years out of date. One strong point, however, in favour of the work is the inclusion of numerous Russian references in the bibliography. These are not to be found in any comparable text-book in English.

In comparison with the German edition, the present edition is rather poorly bound and more bulky, with an extra seventy-two pages; typographical errors are frequent and there is a loss of quality in the half-tone illustrations. In spite of the fact that there are defects and omissions the book reveals the author's careful attention to his principal object, which is the lucid presentation of a large amount of factual material.

D. D. Bayliss

MEDICAL BIOCHEMISTRY

Medizinische Biochemie

Lehrbuch für Studierende und Ärzte. Von Dr. S. M. Rapoport. Pp. xvi + 992. (Berlin: Veb Verlag Volk und Gesundheit, 1962.) 53 D.M.

THE author of this book is a professor in the Humboldt University of Berlin and is also the director of the Institute of Physiological Chemistry of the Faculty of Medicine. The publication, which is from East Germany, is well printed on good paper and is written in German. The contents of the publication shows meticulous care in preparation and there is a liberal supply of tables, graphs, illustrations and photographs.

The book is divided into four sections. The first section, consisting of 200 pages, is concerned with the dynamic biochemistry of the living organism. As an introduction, the significance of physiological chemistry is discussed and a short historical background is included. The remainder of the section deals with physicochemical problems, the function of water and ions in the organism, proteins, enzymes and the chemistry of the cell. This section contains numerous diagrams, graphs, tables, illustrations and photographs.

In the second section, 336 pages are devoted to the intermediate metabolism of carbohydrates, lipids, steroids, amino-acids and proteins, nucleic acids, and porphyrins and hæm compounds. The subject-matter is particularly well illustrated with diagrams, graphs, etc. Reference is made to disease in those cases in which a disorder of metabolism is involved. In the third section, 246 pages are devoted to functional biochemistry. Salt and water metabolism and the influence of hormones are discussed. There are sub-sections on acid-base equilibrium, the composition of blood and the biochemistry of renal and liver functions. The biochemistry of muscle, connective and nervous tissues is discussed. There are also sub-sections on the biochemistry of reproduction, lactation and immunochemistry. The section is well illustrated with diagrams, tables, etc. Reference is made to disease in discussion of some of the biochemical phenomena.

The fourth section deals with problems very largely concerned with nutrition such as mineral metabolism, for example, iron, calcium and phosphorus. There is a large sub-section devoted to the vitamins. The section also includes a list of journals and review books that are related to the subject and are published throughout the world. Russian periodicals are included in this list. It is unfortunate that *Clinical Chemistry*, *Clinica Chimica Acta* and *Recent Advances in Clinical Chemistry* are not included in this very comprehensive list. The section concludes with the syllabus of the subject as taught in the author's department. Practical exercises are not included but there are frequent references to and descriptions of specialized techniques such as used in the examination of the structure of proteins, etc. Each sub-section is followed by a series of questions which the reader at his discretion may attempt to answer. The applications of biochemistry to the study of disease, usually considered under the headings of clinical chemistry or chemical pathology, have not been discussed except in the explanation of certain biochemical phenomena.

The subject-matter is rather more than required of the student of medicine in Britain and is probably more suitable for those reading for a degree in biochemistry with a bias towards medicine. The book would be of great value to the student of biochemistry conversant with the German language or one wishing to become conversant not only with biochemistry but also with the German language.

S. L. Tompsett

BEHAVIOUR OF THE RAT

A Study in Behaviour

Principles of Ethology and Behavioural Physiology, Displayed mainly in the Rat. By S. A. Barnett. Pp. xvi + 288 + 14 plates. (London: Methuen and Co., Ltd., 1963.) 45*s*.

IN this book Dr. Barnett restricts himself almost entirely to the behaviour of his favourite experimental animal, the rat. It is very useful to have such a study since the rat is by far the commonest item of the fauna of the modern biological laboratory—particularly that of the physiologist and psychologist. But, so far as wider objectives are concerned, the choice of the rat is unfortunate. Although the annual output of papers on this animal, or on some parts of it, is quite overwhelming, it does not provide in itself very convenient material for setting forth the principles of ethology. This is partly because it shares with many mammals the disadvantage of possessing relatively little of the fixed, species-specific, unlearned (instinctive) type of behaviour. Moreover, the author is so careful not to commit himself to any particular series of concepts or definitions of ethology that he tends at times to be confused by his own caution; so one begins to doubt what it is, in a given set of observations or experiments, that he finds interesting or important, and why. Of particular interest are those fields to which the author himself has most contributed, namely the behaviour of the wild Norway rat, the exploratory learning of rats and its psychological significance, and studies on the physiology of the stress which results when a rat is caged in the territory of a rival. Indeed, apparently perfectly healthy rats may die of shock in such circumstances; this lethal shock involves a whole series of neural and endocrine changes associated particularly with the sudden and severe drain on the secretion of the adrenal glands. As to exploratory behaviour, this is a phenomenon found very widely in the animal kingdom, and Barnett has made the particularly important point that exploratory learning in rats, as in the cautious sniffing and exploration of a new cage and new territory, gives the maximum of information about the environment in the safest possible way and thus has important survival value. Perhaps the success and ubiquity of the rat depends as much on this as on any other one factor.

It is easy nowadays to criticize any book of moderate size for its omissions. But in this case the limited size and the restriction to the rat is doubly unfortunate in that several topics basic to the modern study of ethology find no place. Thus, although releasing stimuli are mentioned, there can be no discussion of the relation of these to the filtering processes in the sense organs and in the central nervous system. Again, the significant work of von Holst and von St. Paul on the effects of brainstem stimulation in the domestic fowl, whereby a considerable series of fully co-ordinated and unpractised items of innate behaviour (instinctive acts) are elicited, finds no place. Further, the problem of imprinting and the sensitive period can scarcely be dealt with adequately in a book which is centred around the rat. Yet again it is unfortunate that all mention of the considerable volume of recent work, mostly in the U.S.S.R., which deals with interoceptive conditioning and its relation to conscious and unconscious processes, is omitted. Interoceptive conditioning is in fact classical conditioning in which either the conditioned stimulus or the unconditioned stimulus or both are delivered directly to the mucosa of some internal organ. Interoceptive stimuli are, by their very nature, closely bound up with the life of the organism and they tend to be much more recurrent and regular than do many of the stimuli which impinge from the external world. The study of such stimuli is probably as yet in its infancy and is likely to produce results of great importance in the future. It would have been interesting to have had it related to what is known of the learning powers of the rat as studied by other techniques. However, most of these criticisms derive from the title (reinforced by the sub-title) by which we are led to anticipate a general text-book on the principles of ethology and of behavioural physiology only to find that those subjects for the study of which the rat is not outstandingly good tend to be glossed over or omitted. This is a pity, because the book will be that much less successful in the very important task of presenting ethology to the behavioural psychologists. On the other hand, the work has many good features and will be a useful work of reference. It is attractively produced and the illustrations are excellent, though the price seems somewhat high in relation to the size.

W. H. Thorpe

PLANT HISTOCHEMISTRY

Botanical Histochemistry

Principles and Practice. By William A. Jensen. Pp. vi+408. (San Francisco and London: W. H. Freeman and Company, 1962.) 70*s*.

MICROSCOPIC histochemistry, it is stated, can be regarded as having been founded by the French botanist Raspail, who published work at his own expense after its rejection by a committee consisting of a physiologist, a chemist and a botanist. Raspail stated that the physiologist was ignorant of chemistry, the chemist of microscopy and the botanist of both! Zoologists presumably were uninterested.

Botanists contributed much of the early developments, but latterly 'botanical histochemistry' has not shown the striking advances of 'zoological histochemistry'. Dr. Jensen's book is thus welcome as a stimulant.

It combines the functions of a text on micro-analytical methods with one on methods available for investigations of localization of constituents within tissues. The term histochemistry may possibly include any analytical method but to me (the reviewer) it suggests a chemical technique revealing localization of specific materials within a tissue, rather than straight micro-analysis.

A section of each chapter is devoted to localization technique (termed here microscopic histochemistry): these sections are useful. Many of the methods developed by animal and medical histochemists have been applied to the special structural and other peculiarities of plant tissues. The sections on freeze-drying and freeze-substitution of plant tissues in which Dr. Jensen's own work is significant are timely and useful.

Successive sections deal with general histological procedures and with certain micro-analytical techniques. Techniques for isolation of mitochondria, plastids and nuclei are dealt with. The section on 'histochemical techniques' has chapters dealing successively with carbohydrates and cell walls; proteins; nucleic acids; lipids; minerals; enzymes; autoradiography. 'Minerals' is possibly an odd word to apply to a chapter where microchemical methods applicable to organic phosphate osters are dealt with. Each chapter has a section on localization (staining) procedures and also a section on micro-analytical procedures. It is convenient that these should be compiled together, but the micro-analysis sections do not in several cases compare very favourably with standard texts on microanalysis.

The book is rather obviously designed for the American market as it lists American dealers in equipment and reagents in the United States, materials which in most cases are very easily obtained elsewhere. Local trade names are an inconvenience in scientific literature. Canada balsam is international probably but 'permount' and 'drierite' are local jargon. Misprints occur, such as nitrate instead of nitrite, and uricil for uracil; hyposulfite is presumably dithionite (it is not thiosulphate).

This book should prove to be a valuable stimulant to botanical groups to use some of the techniques proving useful in dealing with animal tissues.

TOWARDS FOOD SCIENCE

Advances in Food Research

Vol. 11. Edited by C. O. Chichester, E. M. Mrak and G. F. Stewart. Pp. ix+454. (New York and London: Academic Press, 1962). 103*s*. 6*d*.

A SCIENTIST who works in the food industry is soon compelled to recognize that his thinking must be done at two levels. Part of it is required to be strictly scientific—the structure of protein, the mechanism of enzyme reactions, the intermediary metabolism of micro-organisms, the determination of the chemical configuration of vitamin B_{12}—these are all examples of topics that would interest a scientific investigator wherever his laboratory might be situated. Other topics, however, for example, continuous dough-making for the plant bakery, the manufacture of potato crisps, the utilization of natural polysaccharide gums in food manufacture need scientific understanding, to be sure, but they also require more diverse talents as well.

The last subject, namely, the use of natural gums in food manufacture, is dealt with by Dr. Martin Glicksman as one of the five long, detailed and well-documented monographs of which *Advances in Food Research* is composed. Dr. Glicksman's article exemplified the second aspect of food research. In it he discusses the use of agar-"structurally . . . still a mystery"—in chiffon pie, doughnut glaze, pickled tongue and sherbet; of carrageenan as a component of eggless 'custard', for the preparation of artificial meat-like products and for converting a mixture of tomato juice, lemon juice and vodka into "high quality Bloody Marys". An equally catholic variety of uses is described for alginates, furcellaran, locust bean gum, guar gum, gum arabic, gum tragacanth and gum karaya.

W. H. Stahl's article on the chemistry of tea and tea manufacture is an admirable survey of the state of knowledge of the subject. It contains a wide review of the literature, including papers from Japan and the Soviet

Union. There are pages of structural chemistry and tables of analyses covering scores of components which, combined, contribute to the flavour, aroma and stimulating action of tea. Figures for vitamin content are listed mainly, it seems, in deference to the Russians, whose view it is that, besides being a pleasant beverage, tea also has significant nutritional and physiological value. Yet in the end, the author is forced to admit that tea tasters and graders tend to be rigid and conservative, to cling to conventional qualities, such as the 'tippyness' of prepared tea, which have little relevance to the quality of what is drunk. Perhaps, indeed, one of the most useful features of this contribution is the definition of such terms as 'brisk', 'tired', 'weathery', 'hungry' and 'common' to which those who carry out research on tea have to give scientific meaning.,

The dichotomy in food research is most clearly apparent in the chapter by S. E. Charms from the Massachusetts Institute of Technology on "The Nature and Role of Fluid Consistency in Food Engineering Applications". This paper is pitched at a high intellectual level. Much of the treatment is mathematical. The concept of fluid consistency is developed theoretically in terms of liquid viscosity and the molecular structure of water. Consistency is then expressed in relation to shear stress and the rate of shear in fluid-flow. This allows different fluids to be classified theoretically as pseudo-plastic, Bingham plastic, dilatant, Newtonian and non-Newtonian. There is a full theoretical treatment of the practical methods for studying such problems of food manufacture as the handling of chocolate couverture and bread dough, the pumping of diverse liquids and the measurement of heat-transfer characteristics. This is indeed the proper application of scientific thinking to practical matters. Yet, at the end of seventy-eight pages, underlining at every point the importance to the food engineer of expressing consistency in terms of fundamental units, there comes the sentence, "at present, little information is available on the fundamental classification of the consistency of food materials."

Of the remaining articles, that by Ludwig Acker deals with enzymatic reactions in foods of low water content, that by Ingmar Bosund with the action of benzoic and salicylic acids on the metabolism of micro-organisms, and that by M. A. Joslyn on the chemistry of protopectin. These, like the rest of the book, are able and comprehensive and of a high standard. Joslyn's long paper, particularly, is a rich pile of ore, mined from the mountain of the literature from which yet more workers must extract the pure metal of the ultimate chemical understanding of a complex large molecule, and, having acquired the knowledge, find out how to use it.

Today, when men of affairs are looking more critically than they did before at the usefulness of scientific research into practical problems, a book like *Advances in Food Research* gives its readers a good opportunity to reflect on how the two ways of approaching food research sometimes complement each other, but sometimes do not.

MAGNUS PYKE

MICROCHEMICAL TECHNIQUES

Proceedings of the International Symposium on Microchemical Techniques

University Park, Pennsylvania, August 13–18, 1961. Edited by Nicholas D. Cheronis. (*Microchemical Journal* Symposium Series, Vol. 2). Pp. xviii+1181. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1962.) 300*s*.

THIS publication includes the four plenary lectures, the 123 original papers, and the six contributions on the teaching of microchemistry which were presented at the international symposium on "Microchemical Techniques" sponsored by the International Union of Pure and Applied Chemistry at the Pennsylvania State University during August, 1961.

In early July, 1962, analytical chemists of all countries heard, with a deep sense of personal loss, of the tragic death in a car accident of Dr. N. D. Cheronis, of Brooklyn College, N.Y., who had just completed the editing of the present volume, and whose excellent plenary lecture on "The Characterization of Organic Compounds at the Microgram and Submicrogram Ranges" will be found in Part 2 of this book.

In the same plenary section, D. D. Van Slyke authoritatively discourses on gasometric methods of analysis; E. Sawicki reviews methods for the determination of trace amounts of aldehydes, and R. Belcher describes the work on the development of sub-micro methods for the analysis of organic compounds which is proceeding in his department at the University of Birmingham. The original papers are grouped into sections covering the techniques of (*a*) microscopy, (*b*) inorganic elemental analysis, (*c*) organic elemental analysis, (*d*) functional group analysis, (*e*) chromatographic techniques, (*f*) electro-chemical and radiochemical studies and (*g*) molecular weight determinations and physical properties.

It is obviously impossible in a brief review of this nature to mention more than a small fraction of the investigations detailed by the many well-known contributors. Some papers tend to confuse trace analysis with micro-analysis, but this contentious subject need not be argued here.

E. Wiesenberger presents a fascinating paper on the use of electron microscopy techniques to identify minute amounts of precipitated or electro-deposited materials. Some excellent electron micrographs illustrate this paper. Thermo-micro methods, following the techniques of Kofler and Ludwig, are described by a number of research workers. Chronometric methods are developed by L. Erdey and G. Svehla for molybdenum or tungsten using catalysis of the hydrogen peroxide–iodide reaction. Following the work of J. Haslam in the United Kingdom, J. M. Corliss and C. A. Rush recommend the use of the oxygen flask technique for qualitative organic analysis.

M. Bobtelsky deals with microheterometry, a technique studied in but few laboratories; E. G. Wollish reviews briefly thin-layer chromatography, and H. M. Mongold and R. Kammereck develop its application in lipid radiochemistry. J. L. Brownlee presents a detailed paper on the determination of oxygen in organic compounds using the well-known triton-oxygen interaction to produce fluorine-18. S. Jacobs's method for the quantitative determination of nitrogen in organic compounds by the indanetrione method appears to have advantages over the classical methods. Barbara A. Thompson determines the composition and thickness of thin films by neutron activation followed by γ-spectrometry.

These are but few of the researches described in the present publication. Micro-analysis by combustion, Weiz ring oven techniques, thermo-electric molecular weight determinations, radiometric absorption measurements, Kemula's 'hanging drop' electrode, automated processes, crystalline inclusion compounds of urea and the paraffins. These, and a host of others, are all topics which fall within the ambit of this voluminous survey.

Teachers of microchemistry will find much of interest in Part 10, where the teaching of this subject is exhaustively discussed by experts of long practical experience. The introductory portion of the book, with addresses by Steyermark, Belcher, Malissa and historical contributions by Lieb and Benedetti-Pichler also have an obvious and fundamental educational value.

Despite its high cost, most progressive analysts and research workers will consider it a *sine qua non* to have this book on their reference shelves.

D. T. LEWIS

Advances in Clinical Chemistry

Vol. 5. Edited by Harry Sobotka and C. P. Stewart. Pp. xiv+329. (New York: Academic Press; London: Academic Press, Inc. (London), Ltd., 1962.) 86*s*.

NUMBER 5 is one of the best volumes which have so far appeared in this useful series. As usual the contents cover a wide range of totally unrelated subjects all of which are of present-day importance and the treatment varies from general theoretical discussion to severe practical detail.

The article in Volume 4 on mellituria has been followed up in Volume 5 by an exhaustive account of galactosæmia by L. I. Woolf covering not only the clinical features and the biochemical and genetical aspects but also the details of the laboratory diagnosis and the treatment. This is an excellent example of a molecular disease which lends itself to rational treatment now that the biochemical basis is properly understood. It is proposed to deal with other examples of molecular disease in future volumes.

Another clinical entity which is lending itself to rational therapy now that the nature of the causative factors is becoming less obscure is the malabsorption syndrome which is discussed by A. C. Frazer with special reference to the recent work on the mechanism of the effects of wheat gluten.

Two chapters are devoted to less familiar topics. Haptoglobins are dealt with by C. B. Laurell and C. Grönvall, and peptiduria, a much less well-recognized condition than aminoaciduria, by the late B. Skarzynski and M. Sarnecka-Keller.

The final two chapters are severely practical. H. Baker and H. Sobotka give full experimental details for the microbiological assay of the vitamins of the B group in biological materials, while Bruns and Werners describe the methods for estimating several enzymes, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, methæmoglobin reductase and sorbitol dehydrogenase. Although the treatment is exhaustive, not all clinical biochemists will find the experimental details completely adequate at the bench.

The editors have chosen well for this volume. It is an essential tool in every clinical chemistry laboratory.

J. N. Davidson

Semiconductor Reliability

Edited by John E. Shwop and Harold J. Sullivan. Pp. ix+309. (Elizabeth, N.J.: Engineering Publishers, 1961. Distributed by Reinhold Publishing Corporation, New York; and Chapman and Hall, Ltd., London.) 68*s*. net.

THIS book is based on the 1961 conference on reliability of semiconductor devices and contains twenty-five papers.

We are now reaching a state when the reliability of the newer types of semiconductor devices promises to be so good that it will no longer be a major consideration to the equipment designer. The limiting factors in the reliability of equipment are much more likely to be the interconnexion systems, and methods of incorporating the semiconductor devices into the circuits.

This high reliability of semiconductor devices also brings with it difficulties in measuring failure rates since these are so low. In fact it is almost inevitable that reliability information obtained by ordinary methods of life test will relate to out-of-date devices. This tendency is evident in this book since it is generally agreed that planar semiconductor devices are likely to be the most reliable, and only one paper of the twenty-five deals specifically with planar transistors. However, some of the contributions in the book are important in that they point the way to new methods of life-testing components which may give indications of very long life expectancy from life tests of much shorter duration carried out under overstressed conditions. The paper by D. S. Leck, for example, foreshadows the now widely accepted method of 'step-stress' testing.

An interesting speculation is whether useful information may be obtained by recording small parameter changes during step-stress testing as a barometer of production quality which will enable reliability to be improved even more.

A. A. Shepherd

Compound Semiconductors

Edited by Robert K. Willardson and Harvey L. Goering. Vol. 1: Preparation of III–V Compounds. Pp. xxii+553. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1962.) 25 dollars; 200*s*.

THIS book is a compilation of the work of many authors of original papers on the preparation and properties of III/V compounds. Although the book is largely based on original papers, each chapter has been specially written by the author concerned, and a considerable amount of hitherto unpublished material is included.

Its 53 chapters are grouped into ten sections, dealing with crystal structure, purification of the elements, impurity detection, compound preparation, single crystal growth, thin films, diffusion, segregation, surfaces, and thermodynamic properties of the compounds.

The authors of the book were selected on the basis of a bibliography of more than 1,000 papers on III/V compounds (which is included), and it is obvious that this method of selection has produced one of the most comprehensive and well-written works to date on this subject.

In addition to a thorough theoretical study of the III/V compounds, the book places considerable emphasis on the technology of purifying the component elements and preparation of the compounds in both polycrystalline and single crystal form.

It is extremely well produced by the Reinhold Publishing Corporation, and can be recommended as a necessity for any workers in the field of III/V compounds.

A. A. Shepherd

Proceedings of the International Conference on the Physics of Semiconductors held at Exeter, July 1962

Edited by A. C. Stickland. Pp. xi+909. (London: The Institute of Physics and The Physical Society, 1962.) 210*s*.

IT is a measure of the rate at which semiconductor problems are being worked out that this conference attracted over 130 papers suitable for publication.

The conference, which is the third in the series, the previous ones having been held at Rochester in 1958 and Prague in 1960, has undoubtedly contributed much towards the further understanding of physical phenomena in semiconductors. The papers are divided into sections dealing with transport, disordered semiconductors, effective mass determination, optical properties, lattice vibrations, band theory, magnetic properties, materials, surface effects, and recombination.

It is probably true to say that the sections on transport and optical properties contain much of the work which is providing basic information on electronic states in solids. Although many of the papers are concerned with the properties of germanium and silicon, the range of interest has now been widened; much of the material presented is concerned with what might be described as "second order" properties of metals.

It seems that the diversification of work which is occurring as solid-state physics enters this new phase will lead to a larger number of small, specialized groups working in the various parts of the subject. One fears that as time goes on, unfortunately, such groups will have less and less common ground.

The book has been excellently and promptly produced.

A. A. Shepherd

MAGNETIC ANOMALIES OVER OCEANIC RIDGES

By F. J. VINE and Dr. D. H. MATTHEWS
Department of Geodesy and Geophysics, University of Cambridge

TYPICAL profiles showing bathymetry and the associated total magnetic field anomaly observed on crossing the North Atlantic and North-West Indian Oceans are shown in Fig. 1. They illustrate the essential features of magnetic anomalies over the oceanic ridges: (1) long-period anomalies over the exposed or buried foothills of the ridge; (2) shorter-period anomalies over the rugged flanks of the ridge; (3) a pronounced central anomaly associated with the median valley. This pattern has now been observed in the North Atlantic[1,2], the Antarctic[3], and the Indian Oceans[4,5]. In this article we describe an attempt to account for it.

The general increase in wave-length of the anomalies away from the crest of the ridge is almost certainly associated with the increase in depth to the magnetic crustal material[1]. Local anomalies of short-period may often be correlated with bathymetry, and explained in terms of reasonable susceptibility contrasts and crustal configurations; but the long-period anomalies of category (1) are not so readily explained. The central anomaly can be reproduced if it is assumed that a block of material very strongly magnetized in the present direction of the Earth's field underlies the median valley and produces a positive susceptibility contrast with the adjacent crust. It is not clear, however, why this considerable susceptibility contrast should exist beneath the median valley but not elsewhere under the ridge. Recent work in this Department has suggested a new mechanism.

In November 1962, H.M.S. *Owen* made a detailed magnetic survey over a central part of the Carlsberg Ridge as part of the International Indian Ocean Expedition. The area (50 × 40 nautical miles; centred on 5° 25′ N., 61° 45′ E.) is predominantly mountainous, depths ranging from 900 to 2,200 fathoms, and the topographic features are generally elongated parallel to the trend of the Ridge. This elongation is more marked on the total magnetic field anomaly map where a trough of negative anomalies, flanked by steep gradients, separates two areas of positive anomalies. The trough of negative anomalies corresponds to a general depression in the bottom topography which represents the median valley of the Ridge. The positive anomalies correspond to mountains on either side of the valley.

In this low magnetic latitude (inclination −6°) the effect of a body magnetized in the present direction of the Earth's field is to reduce the strength of the field above it, producing a negative anomaly over the body and a slight positive anomaly to the north. Here, over the centre of the Ridge, the bottom topography indicates the relief of basic extrusives such as volcanoes and fissure eruptives, and there is little sediment fill. The bathymetry, therefore, defines the upper surface of magnetic material having a considerable intensity of magnetization, potentially as high as any known igneous rock type[6], and probably higher, because it is extrusive, than the main crustal layer beneath. That the topographic features *are* capable of producing anomalies is immediately apparent on comparing the bathymetric and the anomaly charts; several have well-defined anomalies associated with them.

Two comparatively isolated volcano-like features were singled out and considered in detail. One has an associated negative anomaly as one would expect for normal magnetization, the other, completely the reverse anomaly pattern, that is, a pronounced positive anomaly suggesting reversed magnetization. Data on the topography of each feature and its associated anomaly were fed into a computer and an intensity and direction of magnetization for each obtained. Fig. 2 shows the directions of the resulting vectors plotted on a stereographic projection. Having computed the magnetic vector by a 'best fit' process, the computer recalculated the anomaly over the body, assuming this vector, thus giving an indication of the accuracy of fit. The fit was good for the case of reversed magnetization but poor for that of approximately normal magnetization. The discrepancy is scarcely surprising since we have ignored the effects of adjacent topography, and the interference of other anomalies in the vicinity. In addition, the example of normal magnetization is near a corner of the area where the control of contouring is less precise. The other example is central where the control is good. In both cases the intensity of magnetization deduced was about 0·005 e.m.u.; this is equivalent to an

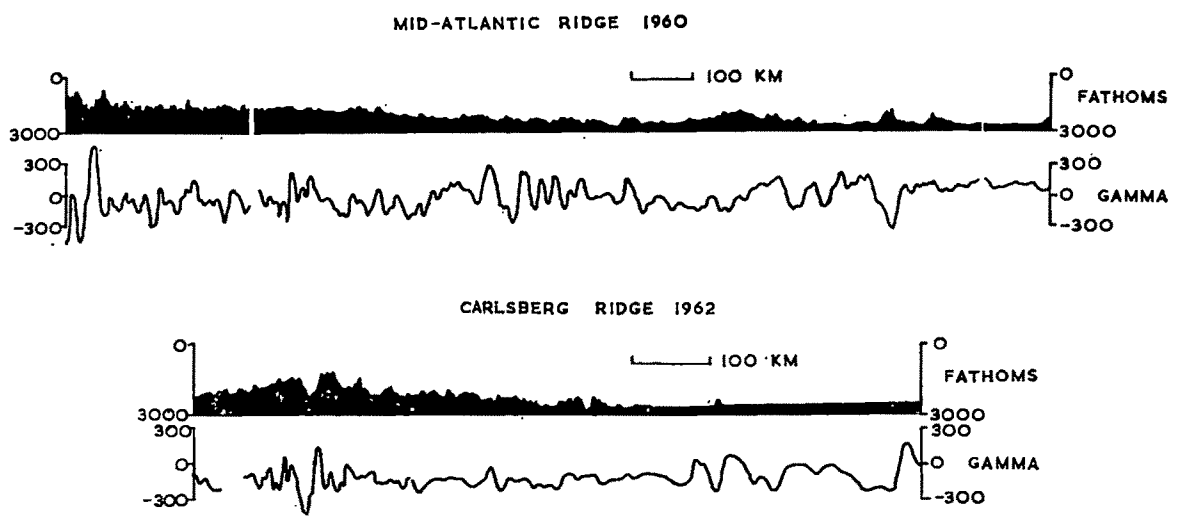


Fig. 1. Profiles showing bathymetry and the associated total magnetic field anomaly observed on crossing the North Atlantic and the north-west Indian Oceans. Upper profile from 45° 17′ N. 28° 27′ W. to 45° 19′ N. 11° 29′ W. Lower profile from 30° 5′ N. 61° 57′ E. to 10° 10′ N. 66° 27′ E.

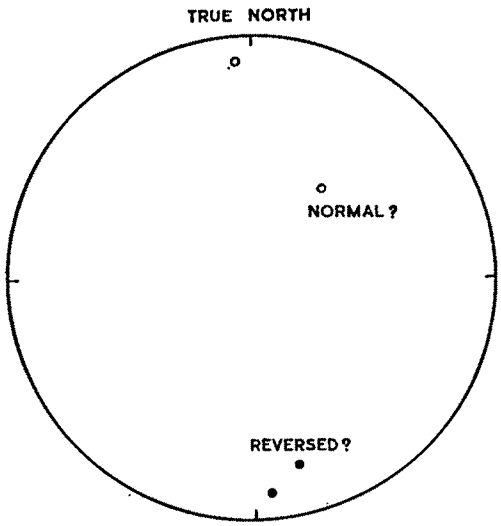


Fig. 2. Directions of the magnetic vectors obtained by the computer programme plotted on a stereographic projection, together with the present field vector and its reverse. Bearings and inclinations: present field vector 356°; −6° (up); computed vectors 038°; −40° (up); 166° 30′; +13° (down)

effective susceptibility of ± 0·0133: (effective susceptibility = total intensity of magnetization (remanent + induced)/present total magnetic field intensity : mean value for basalts of the order of 0·01).

In addition, three profiles, perpendicular to the trend of the Ridge, have been considered. Computed profiles along these, assuming infinite lateral extent of the bathymetric profile, and uniform normal magnetization, bear little resemblance to the observed profiles (Fig. 3). These results suggested that whole blocks of the survey area might be reversely magnetized. The dotted curve in Fig. 3 *B* was computed for a model in which the main crustal layer and overlying volcanic terrain were divided into blocks about 20 km wide, alternately normally and reversely magnetized. The blocks were given the effective susceptibility values shown in the caption to Fig. 4 (3).

Work on this survey led us to suggest that some 50 per cent of the oceanic crust might be reversely magnetized and this in turn has suggested a new model to account for the pattern of magnetic anomalies over the ridges.

The theory is consistent with, in fact virtually a corollary of, current ideas on ocean floor spreading[7] and periodic reversals in the Earth's magnetic field[8]. If the main crustal layer (seismic layer 3) of the oceanic crust is formed over a convective up-current in the mantle at the centre of an oceanic ridge, it will be magnetized in the current direction of the Earth's field. Assuming impermanence of the ocean floor, the whole of the oceanic crust is comparatively young, probably not older than 150 million years, and the thermo-remanent component of its magnetization is therefore either essentially normal, or reversed with respect to the present field of the Earth. Thus, if spreading of the ocean floor occurs, blocks of alternately normal and reversely magnetized material would drift away from the centre of the ridge and parallel to the crest of it.

This configuration of magnetic material could explain the lineation or 'grain' of magnetic anomalies observed over the Eastern Pacific to the west of North America[6]

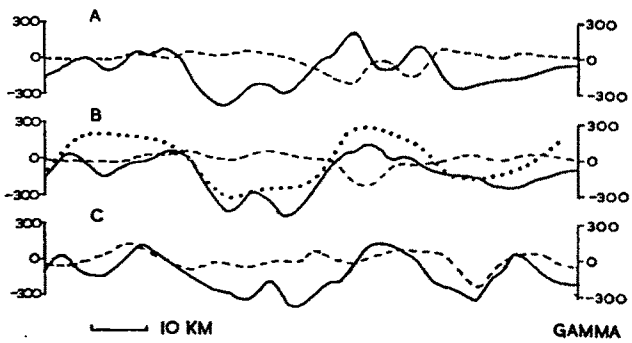


Fig. 3. Observed and computed profiles across the crest of the Carlsberg Ridge. Solid lines, observed anomaly; broken lines, computed profile assuming uniform normal magnetization and an effective susceptibility of 0·0133; dotted line, assuming reversals—see text. The computed profiles were obtained assuming infinite lateral extent of the bathymetric profiles

(probably equivalent to the long-period anomalies of category (1)). Here north–south highs and lows of varying width, usually of the order of 20 km, are bounded by steep gradients. The amplitude and form of these anomalies have been reproduced by Mason[9,10], but the most plausible of the models used involved very severe restrictions on the distribution of lava flows in crustal layer 2. They are readily explained in terms of reversals assuming the model shown in Fig. 4 (1). It can be shown that this type of anomaly pattern will be produced for virtually all orientations and magnetic latitudes, the amplitude decreasing as the trend of the ridge approaches north–south or the profile approaches the magnetic equator. The pronounced central anomaly over the ridges is also readily

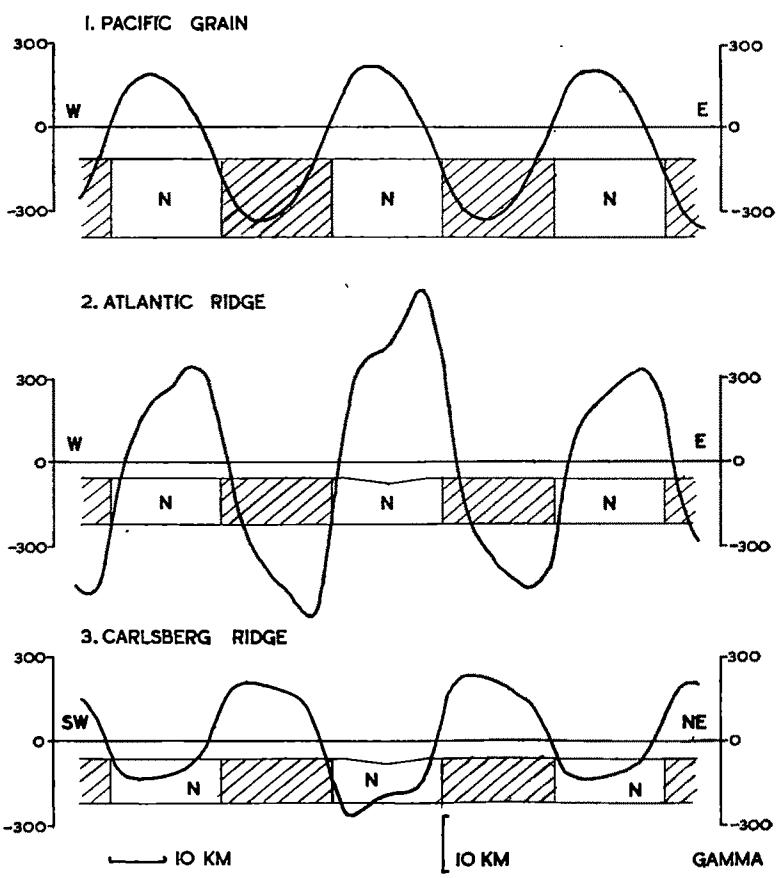


Fig. 4. Magnetic profiles computed for various crustal models. Crustal blocks marked *N*, normally magnetized; diagonally shaded blocks, reversely magnetized. Effective susceptibility of blocks, 0·0027, except for the block under the median valley in profiles 2 and 3, 0·0053

(1) Pacific Grain. Total field strength, T = 0·5 œrsted; inclination, I = 60°; magnetic bearing of profile, θ = 073°. (2) Mid-Atlantic Ridge, T = 0·48 œrsted; I = 65°; θ = 120°. (3) Carlsberg Ridge, T = 0·376 œrsted; I = −6°; θ = 044°

explained in terms of reversals. The central block, being most recent, is the only one which has a uniformly directed magnetic vector. This is comparable to the area of normally magnetized late Quaternary basics in Central Iceland[11,12] on the line of the Mid-Atlantic Ridge. Adjacent and all other blocks have doubtless been subjected to subsequent vulcanism in the form of volcanoes, fissure eruptions, and lava flows, often oppositely magnetized and hence reducing the effective susceptibility of the block, whether initially normal or reversed. The effect of assuming a reduced effective susceptibility for the adjacent blocks is illustrated for the North Atlantic and Carlsberg Ridges in Fig. 4 (2, 3).

In Fig. 4, no attempt has been made to reproduce observed profiles in detail, the computations simply show that the essential form of the anomalies is readily achieved. The whole of the magnetic material of the oceanic crust is probably of basic igneous composition; however, variations in its intensity of magnetization and in the topography and direction of magnetization of surface extrusives could account for the complexity of the observed profiles. The results from the preliminary Mohole drilling[13,14] are considered to substantiate this conception. The drill penetrated 40 ft. into a basalt lava flow at the bottom of the hole, and this proved to be reversely magnetized[13]. Since the only reasonable explanation of the magnetic anomalies mapped near the site of the drilling is that the area is underlain by a block of normally magnetized crustal material[14], it appears that the drill penetrated a layer of reversely magnetized lava overlying a normally magnetized block.

In Fig. 4 it will also be noticed that the effective susceptibilities assumed are two to five times less than that derived for the isolated features in the survey area described. Although no great significance can be attached to this derived intensity it is suggested that the fine-grained extrusives (basalts) of surface features are more highly magnetized than the intrusive material of the main crustal layer which, in the absence of evidence to the contrary, we assume to be of analogous chemical composition (that is, gabbros). This would appear to be consistent with recent investigations of the magnetic properties of basic rocks[6].

The vertical extent of the magnetic crust is defined by the depth to the curie-point isotherm. In the models this has been assumed to be at 20 km below sea-level over the deep ocean but at a depth of 11 km beneath the centre of the ridges where the heat flow and presumably the thermal gradient are higher. These assumptions are questionable but not critical because the amplitude of the simulated anomaly depends on both the thickness of the block and its effective susceptibility, and, although the thickness is in doubt by a factor of two, the susceptibility is in doubt by a factor of ten. Present magnetic declination has been assumed throughout the calculations: it would probably have been better to have ignored this, as in palæomagnetism, assuming that true north approximates to the mean of secular variations; but this is unimportant and in no way affects the essential features of the computations.

In order to explain the steep gradients and large amplitudes of magnetic anomalies observed over oceanic ridges all authors have been compelled to assume vertical boundaries and high-susceptibility contrasts between adjacent crustal blocks. It is appreciated that magnetic contrasts within the oceanic crust can be explained without postulating reversals of the Earth's magnetic field; for example, the crust might contain blocks of very strongly magnetized material adjacent to blocks of material weakly magnetized in the same direction. However, the model suggested in this article seems to be more plausible because high susceptibility contrasts between adjacent blocks can be explained without recourse to major inhomogeneities of rock type within the main crustal layer or to unusually strongly magnetized rocks.

We thank Dr. R. G. Mason and K. Kunaratnam of the Imperial College of Science and Technology, London, for details of the three-dimensional programme used in this work. The programme was originally devised by K. Kunaratnam for a Ferranti *Mercury* Computer. It has been rewritten for use on *Edsac* 2. We also thank the Director of the Cambridge University Mathematical Laboratory for permission to use *Edsac* 2, and Sir Edward Bullard for his advice and encouragement throughout.

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FORMATION OF STABLE SHELLS FROM PROTEIN SUB-UNITS AS A MODE OF VIRUS SYNTHESIS

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THE structure of virus particles is at present interpreted in terms of the generalized model originally proposed by Crick and Watson[1]. The basis of this model is an outer shell or 'capsid' of individual protein sub-units or 'capsomeres', arranged in a symmetrical manner and surrounding a core of nucleoprotein.

In electron micrographs, virus particles have been found to be either rod-shaped or approximately spherical. The spherical forms in some cases approximate very closely to polyhedra with regularly orientated faces. Given a sub-unit basis for the shell, the solution of virus particle structure depends on the determination of those symmetrical arrangements of individual units which will generate surfaces of spherical, polyhedral or cylindrical form.

The original model postulated by Crick and Watson insisted on a regular arrangement of sub-units in the shell, so that every unit position in the surface is geometrically equivalent. It follows that the forces exerted on each sub-unit by its neighbours are equal throughout, leading to a stable complete shell. The original proposal suggested that these forces might be physical or chemical but made no specific predictions about them. Crick and Watson[1] pointed out that spherical or near spherical shells

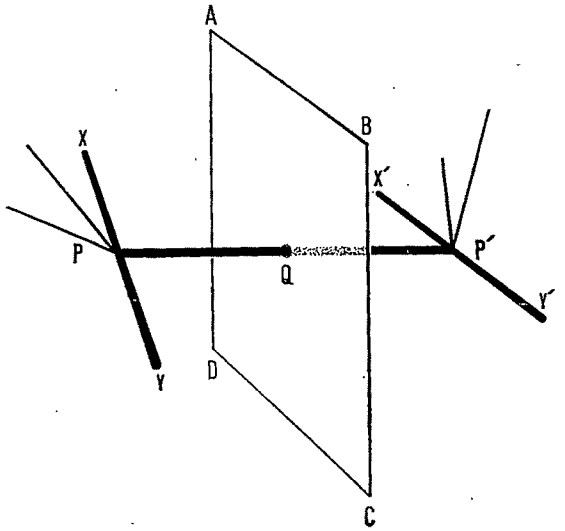


Fig. 1. Linkage of sub-unit axes (P, P') by bond vectors at point Q in common tangent plane ($ABCD$) of sub-unit surfaces

of sub-units probably have cubic point group symmetry which limits the form to structures of 12 or a multiple of 12 sub-units. If more than 60 sub-units are assembled into a shell with cubic, 532 symmetry, the environment of each sub-unit will not be identical and therefore the sub-units cannot be identical.

Negative contrast techniques of electron microscopy have yielded evidence that the particles of many viruses are near spherical or polyhedral shells containing up to 250 sub-units. Such structures cannot be harmonized with the original simple shell model based on 532 symmetry. Kellenberger[2] claims that electronmicrographs of phage-infected bacteria show the formation of a nucleoprotein core of bacteriophage which is later surrounded by a protein shell as if the protein units were joined to the core as to a template. The assumption of a primary linkage between the sub-units and the inner core disposes of any necessity for symmetry in the sub-unit arrangement.

It is well established, however, that tobacco mosaic virus (TMV) rods can be polymerized *in vitro* in the absence of the ribonucleic acid normally incorporated into the hollow cylindrical particle[3]. This resynthesis has not yet been accomplished with any spherical virus, but chemical analysis and electronmicroscopy show that particles of some viruses, for example, polyoma[4] and turnip yellow mosaic virus (TYMV) (ref. 5), occur without the central core of nucleoprotein. The presence of inhibitors of nucleic acid synthesis has caused formation of virus low in nucleic acid in the case of TYMV, an RNA virus[6] and pseudorabies virus, a DNA virus[7], and electronmicroscopy suggested that both preparations contained a high proportion of shells low in, if not actually free from, the nucleoprotein core. This evidence is against the formation of the protein shell on a nucleic acid template, and some other mechanism must be concerned.

The most recent solution of this problem of virus morphology is the 'quasi-equivalence' model put forward by Caspar and Klug[8]. To avoid the necessity of a nucleic acid template, the quasi-equivalence model again stabilizes the shell by the interaction of neighbouring sub-units through the agency of binding forces localized in the form of specific bonds. The essential feature of this concept is the acceptance of bond 'strain' and deformation as an intrinsic property of the model. On these terms each sub-unit does not necessarily have the same environment of sub-units as every other one, but the interrelationship between sub-units is a function of their position in the surface. The variation in mutual sub-unit symmetry may not be very great, but Caspar and Klug were able to show that polyhedral shells with a considerable range of sub-unit number could be assembled using this basic principle.

The quasi-equivalence principle can harmonize the apparent sub-unit morphology and symmetries deduced for a number of virus particles from electronmicrographs, but it introduces a high element of uncertainty into the possible sub-unit arrangement of shells. In purely physical terms, it is difficult to accept a shell built of individual sub-units as a stable structure where large numbers of interconnecting bonds are in a 'strained' or deformed state. It would seem more reasonable to suppose that the fewer the number of strained bonds, the more stable the structure, and such structures are demonstrably much fewer in number than those made possible by the principle of quasi-equivalence.

Random aggregation mechanism of virus assembly as a guiding principle in the interpretation of small virus structures. A consideration of the possible mechanisms of intracellular virus assembly can be a useful guide to the formulation of fundamental structural principles. The nucleic acid coding hypotheses, as understood at present, explain only the synthesis of the protein macromolecules or sub-units from which the viruses are built without any indication of how the morphological units can be assembled in the cell into virus particles. The simplest basic hypothesis applicable in the most general way is the assumption that the synthesized protein sub-units come together in the cell by random collision. The principal merit in this assumption is that this is the basic process of interaction of most particle systems.

The second assumption is that each protein sub-unit carries specific combining sites on its surface, and, if the relative orientation at collision is such as to appose two such sites, binding will occur. The dependence of the resultant shell structure on the shape and size of the sub-units and the surface distribution of their combining sites can be intuitively recognized. The problem is: what variations of sub-unit shape and concurrent arrangement of surface bonding sites will permit the formation of either hollow rods or hollow spheres ? The solution to this problem must be compatible with the circumstances of a random collision process.

Essential parameters defining the form of the stable shell. If two sub-units are bound by the apposition of specific bond sites, the tangent plane common to their surfaces at the point of contact will contain the two linked bond sites. Clearly, the normal to this plane drawn to pass through the point of contact would intersect the respective axes of symmetry of the bond sites in the two sub-units (Fig. 1). This normal may be termed the 'bond vector'.

The essential postulates adopted for the random aggregation model are: (1) That the bond vectors are unstrained throughout a stable shell. (2) That the number of bond vectors and their orientation around the symmetry axes is identical for each sub-unit. (3) That all bond sites are satisfied for every sub-unit in a stable shell. (4) That the number of bond sites on each sub-unit is finite and small.

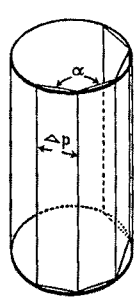


Fig. 2. Arrangement of plane surfaces containing bond vectors of an approximately cylindrical surface

The provision of postulate (1) is equivalent to regarding the sub-unit as being defined by a rigid, non-deformable surface. As the sub-units are protein molecules it may be questioned as to whether this basic assumption regarding the rigidity of surface is reasonable. In fact it is only the location of the bond site in space which is regarded by postulate (1) as being rigidly defined, and this assumption simplifies the overall provisions of the model, and may be acceptable as a reasonable physical property of a polypeptide chain under given conditions.

Postulate (2) ensures that every bound sub-unit is mutually interchangeable with every other in the completed shell: in other words, every sub-unit has an identical environment as required by the original Crick and Watson model.

To ensure that stable shells are built by random collisions, it is necessary that the most stable overall structure (in other words, the most probable structure) is the one in which every active site of every sub-unit is bound, and with approximately equal energy. Such structures will clearly accumulate in the system at the expense of less stable ones. Postulates (1), (2) and (3) make provision for such circumstances.

The restriction of bond site number introduced by postulate (4) is important in so far as it imposes greater limits on the possible relative orientations of the sub-units. As has already been said, the relationship between the number and orientation of bond vectors and the resultant shell structure is the essential problem to be solved. The probable form of the required solution can be deduced from certain geometrical considerations.

It is evident that the regular polyhedra will satisfy all the postulates enumerated here provided that the axes of symmetry for the sub-unit bond vectors are located radially through the vertices, and the corresponding bond vectors coincide with the face margins. The number of face margins meeting in each vertex gives the minimum number of bond vectors per sub-unit necessary for the formation of a given closed surface. This number is equivalent to the number of faces shared by each vertex. For regular polyhedra, excepting the octahedron, this number is 3 or 5 (Table 1). As the polyhedra are regular, each sub-unit is represented by the same number of bond vectors, and the n vectors are orientated in n-fold symmetry around the axes. Other non-regular polyhedra have vertices which share equal numbers of faces, but although these correspond to sub-units with identical symmetries, the n bond vectors are not arranged in exact n-fold symmetry around the bond vector axes. These polyhedra can also be built by aggregation of the appropriate sub-units.

Table 1. Basic Parameters of the Regular Polyhedra

Type of regular polyhedron	No. of vertices	No. of faces	No. of faces shared by vertex
Tetrahedron	4	4	3
Cube	8	6	3
Octahedron	6	8	4
Dodecahedron	20	12	3
Icosahedron	12	20	5

In the case of cylindrical surfaces the appropriate distribution of bond vector axes can be deduced from the fact that any symmetrical planar net can be wrapped around a cylinder to form a continuous cylindrical pattern. If the pattern is to be synthesized from individual units carrying identically orientated bond vectors, linked according to the postulates discussed earlier, certain important restrictions arise. The nature of these restrictions can be indicated geometrically.

A cylindrical surface of radius r may be approximated by the arrangement of p parallel intersecting plane surfaces, so that their intercepts are parallel to the axis of the cylinder and the interval Δp between successive intercepts and the angle between adjacent planes (α) are both constant (Fig. 2). If the intercepts lie on the surface of the cylinder, it is evident that:

$$p.\Delta p < 2\pi r$$

and:

$$\lim_{p\to\infty} p.\Delta p = 2\pi r$$

Similarly it can be shown that:

$$\alpha^0 = 180\ (1-2/p)$$

so that:

$$\lim_{p\to\infty} \alpha^0 = 180^\circ$$

Let a sub-unit be located so that some of its q bond vectors lie in the p^{th} and $(p-1)^{th}$ plane, and the symmetry axis of its bond vectors is coincident with the radial vector of the cylindrical surface at the point of location. If the q bond vectors intersect at a point on their common symmetry axis, then, if they are symmetrically orientated around this axis, all the vectors must lie somewhere on the surface of a cone, whose axis is the symmetry axis of the bonds, and whose apical angle is α'. Each bond vector will then sub-tend an angle of $\alpha'/2$ with the symmetry axis measured in a radial plane. It can be seen (Fig. 3) that for all α' such that $180^\circ > \alpha' > \alpha$ the surface of the cone will intersect the adjacent p^{th} and $(p-1)^{th}$ planes in four intercepts, provided the cone apex lies in the intersection between the planes. Thus only four of the q vectors can link symmetrically with identical sub-units lying on the cylindrical surface. It can also be seen that for $\alpha' = 180^\circ$ and $\alpha' = \alpha$, only two vectors can be formed, disposed in the vertical and circumferential planes respectively. It is clear that in this situation additional bond vectors cannot lie at the same angle to the symmetry axis around which the basic four vectors are disposed. Thus in a six-bond sub-unit, the additional two bond vectors must lie either in the vertical plane parallel to the cylinder axis, or in the circumferential plane. In neither case can they be made completely coincident, by rotation around the six-fold symmetry axis, with the other four bond vectors. If cylinders are made of sub-units with three symmetrically placed bonds, the axes of the sub-units have to be tilted from the radial plane of the cylinder with consequent deformation of bond vectors[9].

It is clear from these geometrical considerations that only a four-vector sub-unit can yield a completely symmetrical unstrained arrangement in a cylindrical surface.

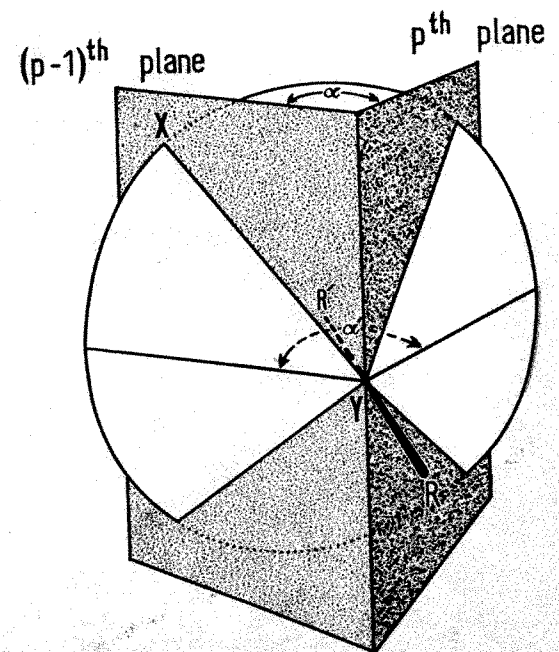


Fig. 3. Arrangement of symmetrical bond vectors forming a cylindrical surface

Conversely the random interaction of sub-units with a complete four-fold symmetry of their bond vectors will yield a cylindrical surface as their most probable configuration. The fact that the vertices of a regular octahedron can be made to lie on a cylindrical surface by placing the centre of symmetry of the octahedron on the cylinder axis confirms the generality of the geometrical deductions discussed above.

It is apparent that the bond vector number must be restricted to 3, 4 or 5, if identical sub-units are to aggregate into uniform spherical or cylindrical surfaces, taking into consideration the basic postulates which have been assumed to govern the aggregating process. The geometrical constructs which have indicated the nature of these restrictions also make it clear that the bond vectors on each sub-unit must be orientated so that they all lie uniformly to one side or the other of a plane which passes through the bond vector origin and is normal to the bond-vector axis of the sub-unit. This would imply that the bond site distribution confers a specific polarity on the sub-unit, a situation which does not seem completely incompatible with an asymmetrically folded polypeptide chain.

Confirmation of the general deductions by geometrical models. Confirmation of the general properties of bond-vector linked configurations of sub-units deduced above has been obtained by the construction of rigid geometrical models[10].

For practical model construction, it was convenient to use plastic skittles, the necks of which were cut off, the bond vectors being represented by large-headed pins thrust through from the inside with the large circular head bearing up against the inside surface. All the 'sub-units' of a set were given identical bond vectors, each pair of which was linked by putting the two shanks of the pins into a sleeve of fine-bore rubber tubing, one from each end. This gave a reasonably rigid link and enabled the requisite number of identically orientated 'bond-vector units' to be built into a complete model. For additional rigidity sleeves of narrow brass tubing could be used. In the completed structure every bond site was satisfied and each sub-unit was identical in bond-vector number and symmetry. The original generalizations could be demonstrated directly from the models, namely: (1) Sub-units carrying 4 bond vectors with suitable orientation in strict four-fold symmetry would result in the assembly of a hollow cylinder (Fig. 4). (2) Sub-units with 3 or 5 bond vectors formed hollow spheres (Figs. 5 and 6). It is important to appreciate that the various types of shell structure produced were determined solely by the bond-vector distributions, as in each case the 'sub-units' were constructed first, and then assembled unit by unit into the completed shell structure.

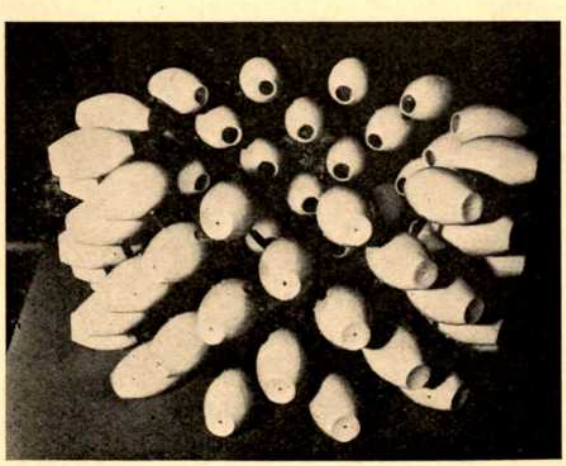


Fig. 4. Model of cylinder made from sub-units with four symmetrical bond vectors

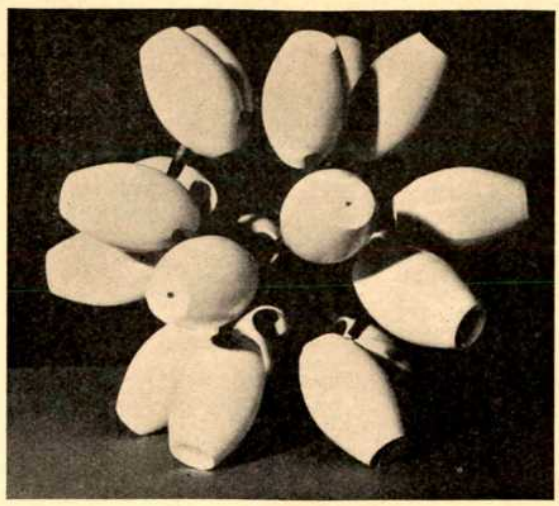


Fig. 5. Model of dodecahedron made from sub-units with three symmetrical bond vectors

Practical experience with this structural approach has emphasized the critical nature of the number of bond vectors and their angular orientation. It is clear, therefore, that if either spherical or cylindrical shells are randomly assembled by the interaction of bonding sub-units according to the enunciated postulates, the probable manner of sub-unit arrangement in the shell is sufficiently restricted in each case to yield a small number of mutually exclusive alternative structures easily compared with experimental observation. It has been possible by these means to deduce structures for certain virus particles which accord extremely well with both electron microscopic and X-ray diffraction data[9].

Nature of the combining sites. Although present theories envisage the virus sub-unit as a solid body with a well-defined and unique surface, these are only convenient representations of a complex macromolecule. Bonding sites, therefore, must be regarded as spatial regions of the macromolecule containing specific chemical configurations. It must be emphasized that the models built according to the postulates of our hypothesis do not in any way lay down *a priori* the shape and form of the sub-units, but only their relative positions in the virus shell and the number and direction of the bond vectors linking them. Thus the position of the bond sites in space is not explicitly defined, but if the particles are symmetrical about the axis intersected by the bond

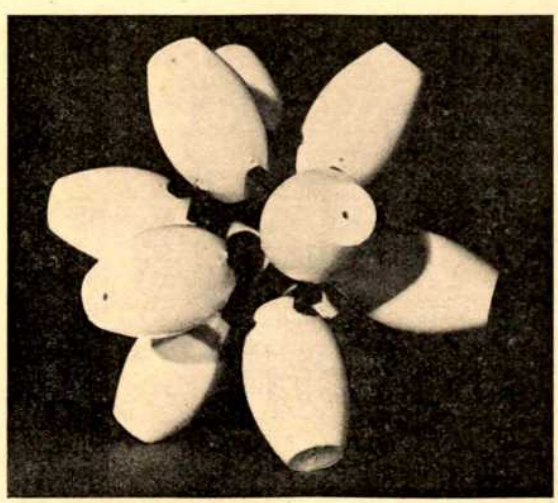


Fig. 6. Model of icosahedron made from sub-units with five symmetrical bond vectors

vectors, the bond sites will lie at the mid-points of the respective bond vectors. No inkling can be obtained from this as to the nature of the bonds but they are probably of a physical kind, such as hydrogen or hydrophobic bonds involving certain specific amino-acid residues in the protein chains of the sub-units.

General scope and consequences of the hypothesis. The random aggregation hypothesis presented in the preceding paragraphs predicts explicitly the relationship between the distribution of specific bond sites on the sub-units of the virus particle and the form of the shell into which they can be aggregated, provided certain postulates are satisfied. The most satisfactory aspects of the proposed hypothesis are: (1) that it can predict the formation of either cylindrical or spherical shells on the basis of common assumptions; (2) that the clear indication of the most probable bond-vector numbers and their orientation for these two types of shell allows a relatively objective formulation of possible sub-unit arrangement. The most important limitation of the hypothesis is that with respect to the spherical or near spherical viruses, the possible sub-unit arrangements that can be deduced from the postulates are not applicable to shells containing more than 60 sub-units, because this is the largest number of identical sub-units which can be arranged symmetrically. Furthermore, strictly polyhedral particles cannot be built as aggregated hollow shells, because the bond-vector orientations of sub-units in plane faces must be different from those at vertices, or face margins. We may ask, therefore, whether a generalized structure model applicable to all viruses can be valid. The most significant observation in this connexion is the size distribution of the spherical or near spherical viruses. The small RNA viruses, including the ARBOR viruses, enteroviruses, and many spherical plant viruses, fall within the narrow size range of 20–40 mμ. The DNA viruses, including several of the typically 'icosahedral' viruses such as the adenovirus[11] and tipula iridescent virus[12], are distributed through an extensive size range from about 60 mμ up to 250 mμ. The extremely restricted size range of the small RNA viruses would suggest a regular shell structure with an upper limiting size, a situation fully consistent with a spherical shell structure as proposed, limited to a maximum of 60 sub-units in the shell. On such a basis, a different structural mode must operate for the formation of the larger viruses.

The hypothesis under discussion has a second element which imposes certain interpretative limitations but which may be of the profoundest significance with respect to the size factors considered in the preceding paragraph. It is evident that the sub-units defined in the shell structure by the bond-vector axes of symmetry are primarily geometrical abstractions. While there can be no doubt that they must constitute the framework on which the actual morphological structure of the related virus particle must be based, their relation to structures seen in the electronmicroscope is not clear. It seems reasonable to assume that the geometrical unit defines the location of a discrete structural element which physically may be no more than a part of a single polypeptide chain. In this respect, it is possible that the geometrically defined sub-unit of the small hollow spherical particle may not be homologous with the morphological capsomere of the large virus. Thus the 'sub-units' of the larger viruses may be aggregates of small protein units which form by random collision, as envisaged in the hypothesis. The units thus formed may be assembled into the virus particle by some other process, such as regular polymerization on to an internal core. It is significant that electron micrographs suggest that many giant protein molecules are assembled from geometrical sub-units into the form of either hollow polyhedra or hollow cylinders[13]. Thus there seems some indication of a structural dichotomy between the large protein molecules and 'small' viruses on one hand, and the 'large' viruses on the other.

One of us (E. R.) is in receipt of a Nuffield Research Grant.

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MANAGERIAL AND COMMERCIAL EDUCATION*

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THE profound changes taking place in post-war Britain have at last reached the very much neglected field of managerial and commercial education. Although extensive pockets of public indifference and traditional muddle appear to dominate the scene, some progress has already been made. There is, for example, an increasing public interest in education in the field and a growing awareness of the need not only for a managerial *élite* similar to that trained at the Harvard Business School or at some of the French polytechnics but also a recognition that the national interest demands a fundamentally new approach to managerial and commercial education at various levels. In the words of the *Report on Management Studies in Technical Colleges* (H.M.S.O., 1962), "the need is for managers who are able to make intelligent use of various skills and techniques . . . and to use to best advantage the human and other resources at their disposal". The emphasis is on wide horizons, flexibility, planning ability and foresight.

The progress in this educational sphere has been more rapid and extensive in some other countries than in the United Kingdom. In the United States, for example, in 1958, nearly 50,000 students each year took the bachelor's degree, and more than 5,000 the master's degree in business studies, while the number of doctorates in this field was of the order of 100. Western Europe as a whole

* The views expressed in this review are not necessarily those of the Coventry Education Authority.

has accepted management training as a national responsibility at the highest levels of education. In Britain a customary Advisory Council has been set up under the chairmanship of Mr. J. W. Platt and has produced its Report on 1962, a slim but thoughtful document, which might become the basis for a new approach, though it has been very much criticized and somewhat maligned in certain teaching circles. On May 23, Lord Hailsham stated in the House of Lords that management studies are being developed at many universities. Twelve universities are offering courses in management, eight of their post-graduates full time for a period of about a year. Nine universities are to be given special help to expand their facilities. The Foundation for Management Education and the Government are prepared to support these universities as well as one of the colleges of advanced technology and one of the regional colleges of technology in this field. These grants are expected to rise eventually to £100,000 per annum in 1966–67.

Despite these successes progress in the main has, however, been slow, and the impact made on the business world slight. The McMeeking Report in 1959 came out with a devastating indictment of the deplorable situation in the domain of managerial education and commercial education at all levels, citing the 'sitting next to Nellie' complex, the profusion of professional bodies in a given sector of business, their insistence on the adherence to a syllabus set in every detail, and the tragic failure by the banking, insurance and finance firms to grant day-release to even 1 per cent of their young employees (the corresponding figure for the chemical industry is 48 per cent). The Urwick Report on Education for Management in 1947 recommended the establishment of courses which became the basis for commercial and managerial training at technical colleges, but which brought very little improvement. It must also be noted that the technical colleges appear to play a major part in the field of managerial and commercial training in Britain, while the contribution by the universities has not been, so far, impressive.

Commercial and managerial training at technical colleges. A student with passes in three Ordinary Level subjects of the General Certificate of Education can proceed via a part-time-day two-year course, if he is employed, to an Ordinary National Certificate in Business Studies (O.N.C.); a full-time two-year course of studies leads to the Ordinary National Diploma (O.N.D.). A successful student can then continue by part-time study for two years to a Higher National Certificate (H.N.C.) or by full-time study also for two years to a Higher National Diploma (H.N.D.), both being equivalent to a pass degree of a university. In May 1962 there were 8,699 students in the O.N.C. stream, 1,011 in the O.N.D. stream, and 1,450 in the H.N.C. stream. This represents a notable advance over the corresponding figures for the 'fifties.

The neglect of the business studies in the 1950's was mainly due to two reasons: (1) industry and commerce were not interested in this type of training; (2) the professional institutions would not recognize the National Certificates or Diploma as conferring exemption from any of their examinations. Lately, some change has occurred in the attitude of these bodies, and there is a gradual but slow trend towards the recognition of a common course with a flexible syllabus as giving exemption from certain parts of professional examinations. Certain firms—unfortunately few in number so far—are also beginning to take an interest and even support courses leading to higher qualifications, for example, the British Oxygen Co., British Petroleum, Unilever, the Metal Box Co., etc., are sponsoring students for sandwich courses at a number of colleges. At the Regent Street Polytechnic, for example, the five-year sandwich course supported by the General Electric Co. combines business studies with engineering. The Austin Motor Co. is supporting a three-year sandwich course at the Bromsgrove College of Further Education designed to train managers and supervisors. At the Lanchester College of Technology, Coventry, there are two separate H.N.D. courses in business studies, one with special reference to industrial sales and the other with special reference to industrial accountancy; of the 26 students enrolled 20 are sponsored by industry. Other colleges concentrate on the basic business subjects, such as economics, law, accountancy, with some administration, mathematics and statistics.

So far as higher qualifications are concerned, in 1961 a total of 62 colleges granted diplomas in management, while 100 offered courses leading to intermediate level. In that year the numbers of diplomas and certificates granted at colleges were 641 and 812, respectively. Until 1960 there was, however, little enthusiasm in industry for the efforts of technical colleges to train managers. The reasons for this were as follows:

(1) Anyone could enrol to follow a course of management studies, and nearly all students pursued evening courses.

(2) Poor facilities and inadequate teaching combined with unsatisfactory recruitment lead to considerable 'wastage'.

(3) The teachers were unable to carry out research and consultative work owing to the heavy teaching load. (On the average the teachers in this field spend 22 hours a week on contact hours, the corresponding figure in the United States being 10).

(4) Lack of suitable accommodation, unnecessary restrictions and protracted sessions, sometimes of three hours' duration, rendered the teacher's task very difficult.

In general, a tired teacher has been imparting superficial knowledge of many techniques to tired and unsuitable students. However, if the student survived the ordeal, he invariably obtained the College Diploma, for the examination papers were marked by the teachers themselves. The diploma thus lost its value and was largely ignored by the employers. Many firms themselves started managerial training courses, and in certain cases appointed consulting firms to run courses for senior managers. These were residential in character, housed in suitable premises, and the instructors were paid princely salaries in comparison with those of teachers at technical colleges. Oddly enough, there was no diploma at the end of the road, but eventual promotion in industry or commerce. As regards the part played by the management consultants, their long-term objective is education. Member firms of the Management Consultants' Association employ about 1,200 qualified consultants. Some of the firms, as, for example, Associated Industrial Consultants, concentrate on management techniques, others, for example, the Sundridge Park Management, the Production Engineering, Ltd., or the Anne Shaw Organization, hold residential courses for top management; some firms carry out research into new techniques. In the past decade the firms have completed thirty-four assignments to initiate training or personnel development schemes. Consultants have also contributed to education by correspondence courses (H. Whitehead and Partners, Ltd.), lectures, books and articles.

In 1955–56 a Working Party set up by the Ministry of Education investigated this problem, and as a result of their efforts the Ministry published its circular 1/60 in 1960, recommending the establishment of a Diploma in Management Studies. Industry and commerce were expected to sponsor graduate students for sandwich and full-time courses, and the facilities were to be restricted to a limited number of suitable colleges. However, after two years of this experiment the situation shows little improvement. Table 1 shows the comparative figures for 1961–62 and 1962–63.

During 1961–62 nearly 60 per cent of the students attended evening classes only; this has slightly decreased during 1962–63 to about 58 per cent. This proportion is very much greater if one includes the students taking a

Table 1

Full-time sandwich		Block release		Part-time day		Part-time day and evening		Part-time evening	
1961–62	1962–63	1961–62	1962–63	1961–62	1962–63	1961–62	1962–63	1961–62	1962–63
13	31	38	145	155	311	231	525	641	1,348

combined part-time day and evening course. Furthermore, the number of graduates attending the evening courses during 1961–62 was about 150 of a total of 641 students. A large number of students have no academic qualifications; the admission of non-academic students was made possible by the escape clause of the circular according to which students over twenty-seven years of age holding responsible positions are allowed to enrol. The number of colleges offering courses has risen from 32 in 1961–62 to 43 in 1962–63. The proliferation of colleges offering diplomas is probably defeating its main purpose, which is to raise standards by increasing the numbers of full-time courses offered by a few competent institutions recognized to be of high quality as regards teachers, facilities and liaison with industry. According to the director of the British Institute of Management, Mr. J. Marsh (*Technology*, 7 (1), 15; 1963), industry is not yet satisfied that the Diploma is on the right lines.

Thus the old pattern of lack of industrial support and academically qualified students has reasserted itself with some few exceptions, as, for example, in the Lanchester College of Technology, Coventry, where the students are sponsored by industry and the diploma is really a postgraduate qualification. For, as Dr. W. F. Frank, the head of the Department of Management Studies at the Lanchester College, pointed out in a recent letter to *Technology*, management cannot be "taught" as mathematics is; the Diploma course is undergraduate in a sense that the students have no practical managerial experience, and postgraduate in relation to the content of the course and conditions of entry. The important thing for the students is to gain practical experience in industry, which a college cannot provide. At present there are no facilities for technical specialists who are not engaged in management, but who for their own benefit wish to find out something about it. Although large numbers of students attend management courses at various colleges, the prospects for the Diploma are not bright, unless industry can be brought in on a large scale to sponsor students. The urgent task is to persuade the pundits of our economy to depart from the traditional pragmatism and muddle, and in some cases nepotism as well, and to realize that potential managers should go through a specially designed form of higher education, as in the United States and in some Continental countries.

The aim is not just to train young people in techniques and tools of management, such as costing, inventory control, etc., but also in the socio-economic backgrounds of decisions, perception of the total situation, and scientific principles of modern industry. There is an urgent need for a new type of manager with a totally different outlook. This point was emphasized by Mr. J. R. Morrison of McKinsery and Co., management consultants, who pointed out that a radical approach to a complete study of management aspects could bear rich fruit in profitability. Thus one British company with assets of more than £300 million agreed to such a study over a period of four years and the realized saving amounted to tens of millions, though this company had the highest return on its capital in Britain.

At a recent export conference Commander W. E. Whitehead, president of the American subsidiary of Schweppes, told 140 executives that the British industry is failing in the competitive overseas markets owing to the outmoded concepts and faulty attitudes. He emphasized the need for an Institute of International Marketing similar to that at Harvard University. This approach should also apply to personnel management; as the Duke of Edinburgh pointed out in a recent speech to the Institute of Personnel Managers, there is a need for professional training in this field and promoting a careers structure in industry with various levels of qualifications.

The need for competently trained managers has still to be realized by industry. The primary objection sometimes raised against training is the fact that there exists no systematic, conceptual basis of knowledge about management; the theoretical study of managerial techniques, such as accounting, work study of operational research, cannot give a young manager a practical mastery of the art; in other words, management teaching can only render a limited contribution to the normal processes of management, and it is impossible to write down express rules how to run a factory or even a department of a business. This objection is fundamentally true, though to postpone the training until a management science has been created is to ignore the urgency of the problem and the demands of the economy. This is shown by the large sums spent by progressive companies to train their managerial staff, and there is evidence—as the Acton Society's report on *Training Managers* indicates—that the effectiveness of the trainees is increased, since the courses raise their morale, induce self-criticism, influence self-confidence and impart certain skills useful in dealing with difficulties inherent in managerial functions. There is room here for a two-way traffic between industry and educational institutions. Industry could supply a great deal of information to colleges about the internal training methods and organizational problems, and provide constructive criticism about the established courses on the lines already existing in the engineering field. Furthermore, industry ought to provide facilities for academic staff to study managerial problems *in situ* and to 'recharge their batteries', as is already done in various technologies.

Planning for management development is still in its youth; many mistakes will be made and many esoteric proposals will be put forward in this field before maturity is reached. The Acton Society's survey of 1956 (*Management Succession*) showed that of the fifty large British companies investigated, one-third had a comprehensive and systematic management development policy, another third made a feeble attempt at evolving some sort of policy, while the remainder did not evince any interest at all; in most cases comprehensive management planning has only been started after the Second World War. Oddly enough, a survey made by the American Management Association in 1954 (*Survey of Management Development*) disclosed a similar state of affairs, though 2,000 firms were involved. In addition, the concept and *raison d'être* of a manager will have to be elucidated in view of the growing complexity of modern industry.

Perhaps the most comprehensive definition of a manager is that by H. J. Leavitt (*On Training Managers*, 1952), who discards the notion of intellectual supermen and defines the manager as "one whose speciality is skill in the effective integration of many other kinds of specialists . . . a specialist in re-integrating wholes". Leavitt suggests that a manager is in need of skills relating to "an area of creativity and imagination, of insight into relationships among many lists of information". Thus the training of competent managers involves the imparting of knowledge and the development of analytical, synthetic and problem-solving propensities in dealing with men and ideas. Hence

the institutions of higher education have a big part to play in this expanding field, and that involves the universities as well.

According to the Under-Secretary for Further Education, Ministry of Education, in a paper given to the twenty-sixth annual conference of the National Association for Education for Commerce in 1961, large-scale developments in commercial education are likely in the next decade; colleges must be adequately equipped for advanced work and research programmes. In this field of the awakening of public interest the British Institute of Management plays an important part. It has taken a lead in studying the whole subject of management and maintaining a survey of courses in which objectives are analysed and syllabuses and techniques noted. It has also published a conspectus of courses available and is endeavouring to become a recognized centre for the exchange of information and ideas on management education.

Managerial training at the universities. A useful classification of management has been given by A. Cole (*J. Econ. Hist.*, 12, 1946). He envisaged three levels of sophistication; the "empirical", the "rational", and the "cognitive" level. The first is based on experience, and though it has little understanding of the management processes, it has proved to be successful in many cases. Academic training has little to offer at this level.

The "rational" type takes into consideration sophisticated techniques in managing and enterprise; but it is rather indifferent to any changes in the social and economic environment, and the resulting inertia may nullify the gains which come from the use of advanced techniques. At this level most of the training is done by the company training departments consulting firms and technical colleges. Again, the universities were scarcely concerned with training at the "rational" level.

The third or "cognitive" level takes into account the changes in the complex of economic and social events which make up the business environment. It makes use of the practices and ideas developed at the other levels, but at the same time is capable of adaptation to a changing society by engaging in large-scale social research. It has been suggested by Prof. N. C. Hunt of the University of Edinburgh (*BACIE J.*, **15**, 67; 1961) that the role of the academic institutions is to contribute at the "cognitive" level, both in management training and research in subjects such as sociology, economics, psychology and some engineering, while supplementing the work done by consulting firms and technical colleges. Of course, this work can be carried out in addition to the courses leading to commerce degrees—in which the pioneering work was done by the University of Birmingham at the beginning of this century—and to some undergraduate studies in conjunction with social and engineering sciences. For example, Part III of the Cambridge Engineering Tripos includes the study of engineering management; similar courses are run or are being planned at other universities, for example, Edinburgh and Leeds.

The University of Manchester has a School of Management and Administration which organizes courses for practising managers, based on research into the fundamental elements of decision and management. Its aim is to provide managers with information about executive research. On the other hand, a report published in the *Oxford University Gazette* in 1962 considers that the University is not yet equipped for high-grade management studies, while low-grade management studies would not be suitable. The Committee of Social Studies Board, which prepared this report, considers that there is no case for starting in Oxford the kind of diploma course in management or business administration similar to those in some American or other British universities. The report considers, however, that higher management studies, that is, the application of statistical and economic theory to management problems, would be desirable, since they could be developed to a point as a self-contained academic study, as in the United States.

Attempts have been made in universities to develop short, residential courses for established managers, for example, at Oxford, Cambridge, Newcastle, Leeds, Edinburgh and St. Andrews. The Oxford Business Summer School has been running successfully for nine years, and its object is to promote discussions between economics tutors and industrial executives. It is thought at Oxford that courses for business men from three months to a year long are the only sort of business training that could usefully be followed there. The short course at the University of Edinburgh attempts to tackle the problem of increasing specialization in business management and develops a broad understanding of the whole problem of managing an undertaking. In particular, the object of the course is to analyse the impact of the changing social, economic and political environment on the work of a manager; to obtain a clearer view of the interplay of various factors, for example, production, sales, personnel, finance and research; to understand the value of statistical data and their application; and to grasp the human aspect of management. It should again be noted that the participants are industry-sponsored mature individuals, having suitable qualifications and occupying responsible posts in the companies. The course is of three weeks duration and residential, so that the process of learning is continuous and intensive, and emphasis is laid on discussions between the students and interchange of ideas. To broaden the experience of the participants the course is sometimes split into small groups, which discuss selected managerial themes with a member of the staff. The fact that this syndicate work involves managers from a diversity of firms has a salutary effect on those with narrow experience. The course at the University of Bristol consists of two four-week residential periods divided by ten weeks project work in the firm. This sandwich type course implies liaison with industry and a practical aspect.

An interesting development at Edinburgh is the post-graduate course in management studies, which last a whole academic year and is devised for young (25–35 years) managers before they move on to more senior positions, and to graduates in science and technology who are destined for managerial posts. During the year the students return for about four weeks to their firms in order to gather material for their dissertations. The teaching is done by members of the Department of Organization of Industry and Commerce in collaboration with other University Departments, for example, History, Statistics, Political Economy and the Social Science Research Centre.

The problem of co-ordinating academic training with actual works practice is also being tackled by some universities. For example, the new Churchill College sandwich course at Cambridge has been devised in consultation with the Institution of Electrical Engineers in such a way as to relate academic work to industrial practice, and theoretical learning is tested against actual managerial experience. The two-year course at the Manchester College of Science and Technology—the Faculty of Technology at the University of Manchester—is intended for young graduate managers who are working during a part of the period on an industrial problem put forward by their employers; successful candidates are awarded the M.Sc. degree.

Despite these promising advances at the universities, the development of academic training for management has still a long way to go. In a broadcast discussion on the sluggishness in British industry, Mr. F. Zweig expressed the opinion that British management at the upper level lacked imagination, and at lower levels was lacking in leadership. It has been suggested in another broadcast discussion that some of the difficulties were due to the fact that comparatively few able young men had entered

courses in technology in the past hundred years, while professional representation in top management is seldom present. The Chairman of the British Institute of Management, as reported by *The Guardian* on July 1, 1961, stated that administration by men brought up almost entirely in the liberal tradition, who recognized twenty years too late the transcendental power of scientific method, inhibits rapid reaction to change. Hence, compared with American management school graduates, the products of British university and college courses have been termed "magnificiently unprepared" (J. Kitching, *The Observer*, June 4, 1961, p. 24). The two-year course at the Stanford Graduate School of Business leads to a master's degree in business administration, and the curriculum includes both 'pure' and 'applied' subjects. Courses of similar standards are available at some of the Institutes of Technology and the "Ivy League" group, and the subject is viewed there as an academic discipline. In Britain the position is far from satisfactory. It is possible, for example, to obtain a degree at Oxford equipping a student with basic knowledge appropriate to almost any specialized profession but not for business. It has been pointed out by L. F. Urwick (*Manager*, **29**, 608; 1961) that management is a practical art which draws on many disciplines, chiefly economics, engineering, psychology and political science, each of which is an academic subject. However, the integrating character of management runs counter to the universities' tendency towards specialization.

The demands for a business studies school on the Harvard model and similar institutions are gathering momentum in Britain. Various proposals have been put forward to achieve this end, and they are by no means similar. The Foundation for Management Education, which comprises a number of relatively young industrialists, rejected the proposal for one Harvard-type institution for the following reasons: (*a*) lack of suitable teachers; (*b*) uncertainty about the scope and content of the syllabus; (*c*) lack of faith in the viability of such an institution. It has therefore been decided to support a small number of management and business schemes in a restricted number of Universities (Cambridge, Bristol and Sheffield, for example) in the belief that one among them would become similar to Harvard. However, in May 1963 it was announced that the Government and the Foundation were prepared to extend the scheme to nine other universities as well as to one of the colleges of advanced technology and one of the regional colleges of technology. The Foundation, though backed by the Government and the Universities Grants Committee, has been criticized on the grounds that it is encouraging an academic experiment instead of building up a solid course, thus defeating its object of creating an eminent school of business studies.

The idea of a single university becoming like Harvard has been proposed by a small group of people, including Lord Rootes, who wish to see the new University of Warwick as a national centre for advanced business studies. The only criticism levelled so far against this idea is the unavoidable delay involved in establishing the school, while Manchester and others are forging ahead.

Another influential group comprising eminent industrialists and academics holds the view that the schemes proposed by universities or colleges of advanced technology do not match up to industry's need. For example, there is a need for teaching market research and selling, cost analysis, etc., which are not part of academic courses so far. This somewhat critical attitude towards academics and industry is reinforced by some lingering doubts about the facilities at the universities and the motives of the academic staff in establishing management courses.

Despite these divergent views there is an increasing tendency to develop high-grade management courses, and to establish adequate machinery for consultation at national, local and industrial levels between those who provide courses of study and potential users to ensure that the views of both are taken into consideration; hitherto the initiative has come largely from the educationists. Furthermore, the necessity for an eminent school of business studies is supported by the National Economic Development Council in its report *Conditions Favourable to Faster Growth*. It states that "there is a need in this country for at least one very high level new school or institute somewhat on the lines of the Harvard Business School or the School of Industrial Management at the Massachusetts Institute of Technology, either as an independent institution or as a part of some existing university or C.A.T. (College of Advanced Technology)".

A recent survey by Prof. D. Granick (*The European Executive*, 384, Weidenfeld and Nicolson, 1962) examines the managerial problem in Europe at the highest level. He is not impressed by the postgraduate management studies at the British universities, though the Administrative Staff College at Henley has been partly successful, for it attracts first-rate men who are capable of educating themselves. By July 1961, more than 2,500 people, sponsored by a variety of companies, had completed the College course. Their average age was thirty-three, and 40 per cent of the entrants had academic qualifications. The syndicate organization has been developed in order to evolve a wide range of pedagogical devices of great diversity and adaptability; this saves time for experienced and mature students. The significant feature of the course is the relevance and the effective use of prepared resources under pressure of time. This College is a non-profit making venture, and its revenue is derived from fees and subscriptions.

Another point made by Prof. Granick concerns the amateur tradition in British universities and management and the deficiency of enterprise and initiative. This he also ascribes to the lack of able men for management posts, to the tendency to model administration on the Civil Service, and to the relatively low esteem and prestige which business enjoys. Furthermore, scientists and technologists appear to have poor chances of ever reaching the boardroom. The time has come now for universities to play a bigger part than before in management training and research, the latter being essentially a university function. The first object of this research would be—according to W. L. Allen (*Listener*, **66**, 51; 1961)—to convert the blatantly unacademic subject of management into an academic discipline in the form of sociology of industry. Dr. Allen is alarmed at the shape of university management courses; but he feels that the problems set by the complexities of industrial and commercial organizations are such that the intellectual content of management provides a challenge to universities. They must provide the *élite* who ought to be responsible in outlook and professional in conduct. It was suggested at a conference on management training held by the Federation of British Industries in 1961 that the universities should recognize their advantage for a theoretical approach to the subject, while the technical colleges ought to concentrate on the more practical aspects; both types of institutions should recognize that their work and contribution are complementary. The proposals for management studies at some universities and colleges are encouraging, but much remains to be done before we can boast the equivalent of some of the American educational establishments, notably the Harvard Business School. The report of the Robbins Committee, especially the part dealing with higher management education, will therefore be awaited with considerable anxiety and interest. One must also note the strenuous efforts made by the British Institute of Management to reorganize its structure and services and to do some hard thinking about its future policies in the field of management education.

TECHNOLOGY AND THE SIXTH-FORM BOY

By Dr. G. VAN PRAAGH
Senior Science Master, Christ's Hospital

THE Oxford University Department of Education has conducted an enquiry* into recruitment from schools into higher scientific and technological education in England and Wales, and has made an attempt at a comparison with the situation in Sweden, Holland and West Germany.

It is sometimes said that boys tend to read some form of applied science or engineering only when they cannot get a place in faculties of pure science, that is, that the best boys read the pure sciences, and the applied sciences get the rejects. The evidence from the enquiry seems to support this view, and to show that in Great Britain pure science attracts three times as many top-grade boys as does engineering, and that the position is reversed in the case of the lowest grade boys; three times as many read for engineering degrees or Diplomas in Technology as for degrees in a pure science. The question was first considered at a colloquium held in March 1961, the members being drawn from schools, universities, colleges of technology, industry and Government departments. This was followed by a one-day conference of school and university teachers in June 1961. Data were then obtained from three main sources: a study of Advanced Level results, questionnaires completed by sixth-form boys, and interviews in schools. The enquiry covered 121 schools and was concerned with 1,459 boys who had taken the Advanced Level examination. Statistics from these examination results showed that about 65 per cent of boys in the top Advanced Level grading went on to read for degrees in pure science and only about 20 per cent to read engineering. In the lower grades, 2–6, the latter percentage rises through 25 per cent for grade 2 to 60 per cent for grade 6, whereas the percentage reading pure science falls from 60 per cent for grade 2 to 20 per cent for grade 6. These figures fully support the view which the enquiry set out to investigate.

However, some agree that it is best for the future technologist to have a basic education in pure science at the university, followed by a practical training in the particular technology in which he is going to work: "The more intellectually able he is, the later should be the stage at which he moves from the field of pure to applied science". Those who take the opposite view say: "for some of the ablest minds the best approach to applied science is a course where problems of application feature strongly at quite an early stage—this may even be the best approach for some of the best pure scientists".

The latter view assumes that there is adequate practical training provided by industry for pure science graduates. It is doubtful whether this is so, and many industries prefer to take future graduates for a year's training before they go on to university. It would be interesting to know what percentage of these trainees, who had chosen pure science as their university course, change to applied science after this year of industrial experience.

The enquirers were also concerned with the reasons for this state of affairs and attempted to discover these by means of questionnaires, interviews, etc. They sought to find out at what stage boys make the choice between arts and science, and then at what stage they choose a career in pure or applied science. The results showed that the great majority of boys do not seem to make the latter decision until they are in the sixth form. The external factors influencing the choice are listed, and percentages are given of boys who had discussed their careers with parents (94 per cent), subject master (37 per cent), headmaster (34 per cent), careers master (29 per cent), friends (27 per cent), and youth employment officers (17 per cent). Only 12 per cent of the sample said that their choice had been influenced by previous knowledge of the career, that is, through reading, hobbies, vacation work, courses, lectures, etc. Boys' knowledge of technology was vague: on the whole, they think technologists are the products of technical colleges, and confuse them with technicians. Very few knew the abbreviation "C.A.T.".

The conclusion seemed to be that a major factor in a boy's choice is the 'public image' of the two careers: scientists, boys believe, are educated at universities, and go on, if they are fortunate, to 'do research'; research is glamorous, interesting, leads to exciting discoveries, is not really 'work' and may well make you famous. Technologists, on the other hand, are educated at technical colleges, have lower social prestige, are less intelligent and well paid, and their work is 'boring'. The authors have been at pains to find out what a boy means by saying that a job is 'interesting' or 'boring' and came to the conclusion that "in most cases, the terms are simply grunts of approval or disapproval and are probably related to the degree of ignorance of the job concerned".

The Right Hon. W. F. Deedes, Minister without Portfolio, thinks parents are largely to blame for the social attitudes and prejudices which govern the choice of professions and careers in Britain. Speaking at a school prize-giving recently, he said that high status was attached to careers in the Foreign Office, the law, the City and natural science. Lower status attached to being an electrician, a salesman or an engineer. "These prejudices are landing us in difficulties. We are becoming chronically short of able young men in the technical professions. There is much talk of the so-called 'brain drain' from science. We have more cause to worry over the failure of British engineering to attract the best young brains."

One thing is very clear. At this critical stage in a boy's life, the transition from school to higher education, the sixth-former is in very serious need of more information, counsel and guidance. Every school with a sizeable sixth form should have a careers master who is given the facilities to do the job properly, that is, time-off and secretarial assistance. He could probably not teach more than half a timetable, if that. Headmasters, form-masters, house-masters have not the time to make themselves knowledgeable in this field, nor to pass on the necessary information to the boys. Lectures, films, visits, short courses in the holidays, in addition to repeated personal interviews with each boy during the whole of his sixth-form period, are needed.

Britain's need for technologists is well known; are the amateurish and haphazard ways in which boys enter technological training equally well known?

* *Technology and the Sixth Form Boy: a Study of Recruitment to Higher Scientific and Technical Education in England and Wales.* Pp. i+57. (Oxford: Oxford University Department of Education, 1963.)

NEWS and VIEWS

Organic Chemistry at Sheffield : Prof. W. D. Ollis

Dr. W. D. Ollis, at present reader in organic chemistry at the University of Bristol, has been appointed to the chair of organic chemistry at the University of Sheffield in succession to Prof. R. D. Haworth (see *Nature*, **199**, 21; 1963). Dr. Ollis graduated at the University of Bristol and was appointed to the academic staff in 1946. Dr. Ollis's earlier research work, in collaboration with Prof. W. Baker, was concerned with various aspects of organic chemistry, including the investigation and establishment of the novel aromatic structures of a widely varying group of heterocyclics now termed 'meso-ionic' compounds, of which the sydnones are representative, the study of the formation and stereochemistry of macrocyclic compounds of the salicylide, cresotide and thymotide types, and the synthesis of isoflavones. His continued research work on the chemistry of natural products has been widely based, including synthetic, structural and biosynthetic studies on plant products and fungal metabolites. Structural investigations, involving extensive use of modern physical methods, have led to the recognition of several new groups, including the biflavonyls and the dalbergiones, as well as structural variants of the isoflavanoids. The results of his work on the Gymnosperms and members of the Leguminosae family are of considerable phytochemical interest. In 1952 and 1953, Dr. Ollis was a member of the group under Prof. R. B. Woodward, of Harvard University, who achieved the total synthesis of strychnine, and in 1962 he was visiting professor at the University of California, Los Angeles.

Chief Oceanographer, Marine Sciences, Canada: Dr. N. J. Campbell

Dr. Neil J. Campbell, oceanographer-in-charge, Atlantic Oceanographic Group, Fisheries Research Board, has been appointed chief oceanographer, Marine Sciences Branch, Department of Mines and Technical Surveys, Canada. A marine physicist, Dr. Campbell has had several years of experience in oceanography, particularly in the Arctic. Since 1959, he has directed the activities of the Atlantic Oceanographic Group's oceanographers and marine biologists in their investigations of Atlantic and sub-Arctic waters. Born in Los Angeles, he obtained his B.Sc. and M.Sc. from McMaster University in 1950 and 1951, respectively, and his Ph.D. in physics from the University of British Columbia in 1955, attending the Institute of Oceanography there. During this period he participated in a joint U.S.–Canadian Beaufort Sea Expedition in a study of the properties of western sub-Arctic waters off Canada's northern coast. On graduation, Dr. Campbell joined the staff of the Oceanographic Group of Fisheries Research Board in Nanaimo, British Columbia. Later, he was transferred to the Board's Biological Station at St. Andrews, New Brunswick, to take charge of an oceanographic programme of the Canadian Arctic. Under this programme he directed and participated in 1955 and 1956 in two Arctic cruises to Hudson Strait, Foxe Basin and Lancaster Sound. He remained in charge of this programme until 1958, during which time he directed one phase of the International Geophysical Year oceanographic project, 'Deep Water Circulation'. In 1960 and 1961 he acted as scientist-in-charge of joint cruises of the Atlantic Oceanographic Group with Dalhousie and Columbia Universities, which carried out geological investigations of the Earth's crust in the north-west Atlantic Ocean. Dr. Campbell is now a Canadian citizen.

The 1963 Soviet Antarctic Expedition

A British glaciologist, Dr. Charles Swithinbank, is to join the ninth Soviet Antarctic Expedition which is preparing to leave Leningrad in the autumn in the Diesel-electric ship *Ob* and motorship *Co-operatsiya*. This will be the first time a British scientist has participated in a Soviet Antarctic expedition. French scientists will also work with their Soviet colleagues in the Antarctic. They will be flown from Mirny to the *Vostok* station, where they will study the dynamics of the movement of ice. Pavel Haloupka (Czechoslovakia), well known for his work on cosmic rays, will be spending his second winter in Antarctica. There will also be an exchange of scientists between the expeditions of the U.S.S.R. and the United States.

The Building Research Station and the Road Research Laboratory

In reply to questions in the House of Commons on July 30, the Parliamentary Secretary for Science, Mr. D. Freeth, said that the non-industrial staff of the Building Research Station would increase by about 6 per cent in 1964–65 and improved facilities would be available in the new physics laboratories opened earlier in July. The gross expenditure of the Station had risen from £781,000 in 1961–62 to an estimated £970,000 in the next financial year, and he fully agreed that there was an increasing volume of research for the Station. The Department of Scientific and Industrial Research expected to award up to 2,010 postgraduate studentships this year, an increase of 24 per cent on 1962, and the value of the awards was increased by an average of 23 per cent last year. Mr. Freeth also said that the Road Research Laboratory examined the specifications of all motorway and trunk road schemes costing more than £1 million, and monthly liaison meetings were held between the Laboratory and the Ministry of Transport to discuss problems arising from road schemes. When appropriate, visits were paid to schemes in progress. If questions arose on which the advice of geologists and pedologists elsewhere in Government service would be valuable, it could readily be obtained.

The National Centre for Scientific Research, Paris

In the year ended September 30, 1962, the budget of the National Centre for Scientific Research, Paris, exceeded 308,000,000 new francs—an increase of 54 million francs on 1960–61 (Centre National de la Recherche Scientifique, Paris. Rapport d'Activité, Octobre 1961–Octobre 1962. Pp. 440+4 planches. Paris: Centre National de la Recherche Scientifique, 1963.) Of this, some 211 million francs was for functional services, 85 million francs for equipment, and almost 6 million francs for grants for the institution of new activities. The number of research workers increased from 3,409 in 1961 to 3,773, and of these, 3,045 were working in the natural sciences and 762 in the human sciences, compared with 2,712 and 674, respectively, in 1960–61. The report for the year is in three parts. The first reviews briefly the general services given to scientific research, including the provision of equipment, assistance to publications, international exchanges, documentation, etc. The second reviews briefly the activities of the research groups which are not located in the research centres attached to the National Centre itself. These range over the mathematical, terrestrial, and human sciences, physics, chemistry, biology and medicine. The third part summarizes the work proceeding in the several laboratories of the

National Centre itself and in those attached to it such as the Laboratories of Bellevue and of Gif-sur-Yvette, the Centre for Nuclear Research, Strasbourg, and the Centre of Scientific, Industrial and Maritime Research, Marseilles, as well as the activities of the various scientific Commissions. The report displays an impressive range of scientific activities, but seldom gives more than the titles of investigations, and lists of published papers are not included.

Scientific Research in Belgium

THE thirty-fifth annual report of the National Foundation for Scientific Research, Brussels, covers the year ended September 30, 1962, in which grants totalling some 70 million francs were authorized (Pp. 349. Brussels: Fonds National de la Recherche Scientifique, 1963). For 1962–63 grants and subsidies totalling 85 million francs have been budgeted. A special grant of 100,000 francs was made to the Scientific Station of Jungfraujoch, where biological investigations have been resumed. Grants totalling 17·5 million francs were made to the Foundation for Scientific Medical Research: a list of individual recipients of grants and of the subjects of their investigations is included in the report. Grants totalling some 3·7 million francs have also been made for radiobiological research with isotopes and for cancer research at the Universities of Brussels, Ghent, Liège and Louvain, and in all some 24 million francs were awarded during the year for research in the hospitals attached to these universities and in other hospitals and special institutes. The bulk of the report comprises annexes, including an alphabetical list of the 593 recipients of grants, their subjects of investigation and publications during the year, as well as those of 146 foreign recipients of grants, 25 of whom were outside Europe. The report gives the usual details of membership of scientific commissions, etc.

The Nordisk Institut for Teoretisk Atomfysik

AT the end of the academic year 1961–62 Nordita (Nordisk Institut for Teoretisk Atomfysik) completed its fifth year, though it is still continuing on an interim basis (Annual Reports, 1960–61 (pp. 20), and 1961–62 (pp. 32). Copenhagen: Nordita: Nordisk Institut for Teoretisk Atomfysik, 1963). The Board consisted of professors from Denmark, Finland, Iceland, Norway and Sweden, with the late Prof. Niels Bohr as chairman. Mr. L. Neilson of the Danish Ministry of Finance acted as secretary to the Board, and Dr. G. Funke, secretary-general of the Swedish Atomic Energy Research Council, Stockholm, assisted the Board in an advisory capacity. On June 30, 1962, the scientific staff of Nordita consisted of the director, C. Moller, G. E. Brown, B. R. Mottleson, L. Rosenfeld, S. Rozental and A. Winther. Seventeen Research Fellows held appointments during the year. Two visiting professors, R. E. Cutkosky from Pittsburg and E. H. Wichmann from Berkeley, gave lectures and guided the Fellows in their work. The titles of the various courses of lectures, including those given at the Institute for Theoretical Physics, University of Copenhagen, and of the colloquia, together with the papers published and issued as *Nordita Publications* and five volumes of lecture notes, are listed in detail in the annual report, which as usual is printed both in Danish and English. The publications and lecture notes are obtainable free of charge by institutions and physicists interested.

The Organization for European Co-operation and Development

THE Organization for European Co-operation and Development, which on September 30, 1961, succeeded the Organization for European Economic Co-operation, is briefly but fully described in an admirable brochure under that title (Pp. 254. Paris: Organization for Economic Co-operation and Development, 1963). To the three chapters which describe successively the history of the Organization, its aims and tasks, and its structure and functions—the last of which sets out the functions of the Committee of Scientific and Technical Personnel, the Committee for Scientific Research, the Industry Committee, the Energy Committee, and the European Nuclear Energy Agency—there are appended the convention establishing the Organization, the rules of procedure, the statute of the European Nuclear Energy Agency and the text of the European Monetary Agreement and the Codes of Liberalization of Capital Movements and of Current Invisibles.

Radiation Protection Norms

REVISED radiation protection norms were approved by the Council of the Organization for Economic Co-operation and Development on December 18, 1961, in place of those previously adopted on June 12, 1959. The revised norms are given in the booklet *Radiation Protection Norms* (Pp. 44. Paris: European Nuclear Energy Agency, Organization for Economic Co-operation and Development, 1963). They were prepared by the Health and Safety Sub-Committee of the Organization for Economic Co-operation and Development European Nuclear Energy Agency, and take account of recent developments in scientific knowledge and the latest *Recommendations of the International Commission on Radiological Protection*. Some of the maximum permissible dose values and most of the limits of concentration of radionuclides in drinking water and in air inhaled have been altered, but they are still based on the same principles as those previously adopted. The four main sections of the booklet deal with occupational exposure in controlled areas, exposure of special groups of the population, exposure of individual members of the population, and exposure of the population as a whole, and the appendixes list specific data regarding maximum permissible doses and concentrations of radionuclides in drinking-water and in air inhaled. The maximum permissible whole-body dose for persons in controlled areas is governed by the formula $D = 5(N-18)$, where D is the tissue dose in rems and N is the person's age in years. Protective devices should be based on an average weekly dose of 0·1 rem and the maximum dose accumulated during any thirteen consecutive weeks must not exceed 3 rems. There are permitted variations in certain circumstances. The maximum permissible whole-body dose for persons not in controlled areas varies from 0·5 rem per annum according to the particular group, and for all individual members of the general population is 0·5 rem per annum. The maximum permissible genetic dose for the population as a whole is 5 rems *per capita* accumulated to the age of thirty years. The booklet does not indicate where the revised norms differ specifically from those of June 1959.

Co-ordinate Colour Identification for Industry

THERE has long been in industry and science a need for a precise method of classifying and identifying colours. The National Bureau of Standards (NBS) *Technical Note* No. 152 (Pp. ii+9. Washington, D.C.: Government Printing Office, 1962. 15 cents), entitled *Co-ordinated Color Identifications for Industry*, by K. L. Kelly, describes a method of co-ordinating the five levels of fineness of colour designation, which was developed by the Inter-Society Colour Council Subcommittee (ISCC) for Problem 23, "The Expression of Historical Colour Usage". It is based on the ISCC–NBS method of designating colours given in the National Bureau of Standards *Circular* 553 (1955), which also includes a dictionary of colour names, and it lists the methods for changing from one level to another. Examples are given of the use of each level.

Ion–Atom Collisions

THE National Bureau of Standards *Technical Note* No. 185, entitled *Lectures on Ion–Atom Collisions*, by M. R. C. McDowell, at present at Royal Holloway College, London, consists of six lectures delivered at the Joint

Institute for Laboratory Astrophysics (Boulder, Colorado) during September 1962 (Pp. iv+66. Washington, D.C.: Government Printing Office, 1963. 40 cents). The material for the first four lectures on the impact parameter formulation of the theory of atom–atom collisions, the application of the first Born approximation, slow charge transfer, and charge transfer in other cases, respectively, was largely taken from the work of D. R. Bates and was presented for pedagogic purposes only. The other two lectures, dealing with further remarks on charge transfer and the ionization of atomic systems by fast protons, contain original material. The numerical results quoted are taken, in part, from at present unpublished work by M. B. McElroy (Belfast), Rudd and Jorgenson (University of Nebraska) and the author.

Seismology in New Zealand

In addition to the main earthquake observatory at Wellington, the New Zealand Department of Scientific and Industrial Research operates stations at some sixteen other places, together with seismographs at Hallett Station and Scott Base in Antarctica. Records of observations are given in the reports for 1955–57, now available (New Zealand Department of Scientific and Industrial Research: Geophysics Division. Wellington: Government Printer). During 1955, 84 shocks were reported felt by people in New Zealand, and one in the Cheviot region did minor damage. Most of these epicentres were in or near New Zealand, but on February 27 an earthquake in the Kermadec Islands (magnitude about 8) was felt in Tolaga Bay and in Wellington. During 1956, 131 earthquakes were felt in New Zealand, including two which did some damage. The most severe was on December 28 between Opotiki and Tolaga Bay (magnitude 6·4) and the other occurred on March 2 some 10 miles north-west of Tokaanu (magnitude 5·3). During 1957, 88 earthquakes were reported to be felt in New Zealand. The shock of December 31 (magnitude 6·6) had an epicentre about 100 miles west of Milford Sound. Damage to chimneys occurred as far west as Dunedin. Minor damage was done by earthquakes on February 22 in the Ohakune–Raetihi district, on March 13 from the Bay of Plenty to Greymouth, and on September 26 between New Plymouth and Wellington. The earthquake reports for 1958 and 1959 are not yet ready, but during 1960 two earthquakes caused minor damage. That on May 25 had an epicentre at sea some 20 miles north of Milford Sound, and that on February 21 was north of Canterbury. Two earthquakes from foci estimated at 370 miles depth occurred on March 23 under N. Taranaki.

In order to facilitate the study of near earthquakes, G. A. Eiby and M. G. Muir, of the Wellington Seismological Observatory, have compiled some most convenient seismological tables from the Jeffreys–Bullen tables (New Zealand Department of Scientific and Industrial Research: Geophysics Division. *Tables to Facilitate the Study of Near Earthquakes*. Seismological Observatory Bulletin *S*-109. Pp. 31. Wellington: Government Printer, 1961). These are: travel time of P for a given S–P interval; distances corresponding to given times of P, S and S–P; distances corresponding to given travel times of P and S; phases due to crustal structure (Pg, P^*, Sg, S^*); intervals between Pn, Sn and crustal phases. They also give a nomogram for earthquake magnitudes after Richter.

Ultra-structure of Nucleoli and Chromosomes in Normal and X-irradiated Liver Cells

Electron microscope studies on chromosome structure, although fruitful, have never fulfilled the somewhat optimistic hopes which were originally held out for their use in this particular biological field. The difficulties which have been encountered are not simply due to poor fixation, but result from the lack of contrast between chromosome structures and other nuclear materials. The use of 'Araldite' embedding coupled with phosphotungstic acid staining is reported by J. M. G. Davis, of the Department of Radiotherapeutics, University of Cambridge, to overcome partly this problem of nuclear contrast and to make possible studies on changes in chromosome and nucleolar structure during cell development (*Phil. Trans. Roy. Soc., B*, **291**; May 16, 1963. 21*s*. or 3.15 dollars). He describes the changes observed in these structures in rat liver cells stimulated to undergo mitosis by partial hepatectomy, observations being made during the early stages of regeneration and throughout mitosis in both normal and X-irradiated animals. A relation was shown to exist between a change in nucleolar structure from a compact to a diffuse state, due apparently to the loss of pars amorpha, and the formation of new double membraned elements of the endoplasmic reticulum (alpha cytomembranes). The suggested relationship between these two events is reinforced by the observation that shortly after the irradiation of animals with 450 r. of X-rays, both the formation of new alpha cytomembranes and the correlated nucleolar changes are inhibited. This is an important observation, particularly in view of the present interest in the transfer of ribonucleoprotein from the nucleus (nucleolus) to the cytoplasm. It is also of interest to note that this is one of the first reports demonstrating an inhibiting effect of X-rays on a normal sequence of changes in nuclear ultra-structure, and that this inhibition was evident in the first few hours after irradiation. The chromosome studies reported showed that at prophase each chromosome appeared as a strand, 1000 Å in diameter, made up of a dense aggregate of a number of thin 100 Å fibres. In mitotic cells fixed 15–30 min after an X-ray dose of 450 r., no ultra-structural changes could be demonstrated in the chromosome material, but with high doses of 2,000 r. a change in the density of packing of the 100 Å fibres was noted together with some indications of fragmentation of metaphase and anaphase chromosomes.

Nomad Fulani

A monograph, published under the ægis of the Institut d'Ethnologie, Paris, deals with the social structure and economy of the Wodaabe Fulani, who form the bulk of the nomadic Fulani inhabiting the Sahel zone between the Nigerian boundary and the desert and steppes of the Air region of the central Sahara (*Travaux et Mémoires de l'Institut d'Ethnologie*. Nr. 64: *Peuls Nomades: Étude Descriptive des Wodaabe du Sahel Nigérien*. Par Magguerite Dupire. Pp. viii+336+27 planches. Paris: Institut d'Ethnologie, Musée de l'Homme, 1962. 45 francs). The fifty-page introduction, which summarizes the work of other people and which attempts a description of the physical type of the Fulani and of their zebu cattle, is based on quite inadequate biometrical data in the case of the human types and on purely hypothetical migration routes in the case of their beasts. The author is on surer grounds when she comes to her own material, and she divides it into four parts, which deal respectively with the economy, the domestic cycle, Fulani marriage, and with Wodaabe lineage and tribal organization. There are twenty-four plates, fifteen diagrams and sketch maps and one very large map giving details of group migrations and of transhumance routes in the area. Altogether it forms a very valuable addition to Fulani ethnography, the more so since a complementary study of the Wodaabe living south of the Nigerian border has already been made by D. J. Stenning (*Savannah Nomads*. International African Institute, 1959).

The Royal Scottish Museum

With the approval of the Scottish Education Department, a panel of honorary curators has been appointed at the Royal Scottish Museum, Edinburgh. In the report

for the year 1962, the Director states that the function of the panel is purely advisory and it helps to extend greatly the body of specialist knowledge on which the Museum can call. The Director also directs attention to the recruitment of senior officers in museums as a matter which is giving increasing cause for concern. Such posts can be very rewarding to academic individuals especially if they are interested in certain techniques for spreading knowledge and have an original turn of mind. Unfortunately, universities appear to produce fewer and fewer suitable candidates and, for example, it is rare to find an honours graduate in zoology prepared to undertake research in taxonomy. It is pointed out that the Museum has been deprived of the full use of the north-east and north-west wings ever since their structural shortcomings were detected almost twenty years ago. At the best, it will be another three years before they can be reconstructed and equipped.

The Western Australian Museum, Perth

In 1960, the Art Gallery of the Western Australian Museum, Perth, was separated from the Museum premises and a programme of building alterations was immediately commenced (Annual Reports, 1960–61 (pp. 40), and 1961–62 (pp. 43). Perth: Western Australian Museum, 1962). As a result, the staff has at its disposal modern and well-equipped accommodation, a satisfactory library, and good storage facilities for a large part of the collections. As a natural sequence to these improvements, plans were made for the modernization of the exhibition galleries, and these included a rapid-change display case. The report for 1960–61 gives details and plans of this ingenious showcase. In 1961–62 the most important development in the educational services provided by the Museum was the creation of a new Children's Centre. Here specimens could be handled and the pattern followed which is now accepted in many museums schools services. The field work organized by the Museum was extensive and linked both with the Museum collections and the individual research work of members of the staff.

Pugwash Conference in Yugoslavia

Some sixty scientists from two dozen countries will meet in Dubrovnik (Yugoslavia) on September 20 to discuss the crucial problem of how to achieve world-wide disarmament and security. Their meeting, which continues until September 25, is the eleventh in the series of Pugwash Conferences (Conferences on Science and World Affairs) and follows the two held in Cambridge and London in the autumn of last year. The title of the Conference is "Current Problems of Disarmament and World Security", and it will lay particular stress on the part that non-aligned nations can play in disarmament and the possibility of setting up nuclear-free zones in Europe and elsewhere. The main topics to be discussed are: the abolition of delivery systems, inspection and control in the first stage of disarmament, the spread of nuclear weapons, nuclear-free zones, the role of non-aligned nations, and the test ban and problems of detection.

Among the participants will be a dozen scientists from the United States, a dozen from the U.S.S.R. and six from the United Kingdom. Among those coming from the United States are Profs. Paul Doty, Franklin Long and I. I. Rabi; from the U.S.S.R., Academicians V. A. Kirillin, I. E. Tamm and A. N. Tupolev; and from the United Kingdom, Sir John Cockcroft and Prof. P. M. S. Blackett. As with previous Pugwash Conferences, where maximum freedom of discussion is all-important, the Conference will be private. But also, as usual, the findings of the scientists will be sent to Heads of States and other interested bodies. The host for the Conference will be the Council of Yugoslav Academies. Further information can be obtained as follows: until September 13, Prof. J. Rotblat, secretary-general of the Pugwash Continuing Committee, St. Bartholomew's Hospital Medical College, London, E.C.1; from September 13 until 18, Gerald Leach, 110 Regent's Park Road, London, N.W.1.

Announcements

The Minister of Power and the Secretary of State for Scotland have appointed Prof. A. L. L. Baker, professor of concrete structures and technology in the Imperial College of Science and Technology, London, and Mr. R. K. Christy, H.M. Chief Inspector of Factories, to be members of their Nuclear Safety Advisory Committee.

Mr. G. B. Townsend has been appointed technical manager of the Professional Television Department of the Rank Cintel Division of the Rank Organization. Mr. Townsend was formerly with the General Electric Company, having joined its Research Laboratories at Wembley in 1940, where he worked on radar and secret weapons and was afterwards responsible for the research work on television problems. In 1961 he transferred to G.E.C. (Electronics), Ltd., as manager of the new Television Equipment Department. Mr. Townsend is president of the British Amateur Television Club and chairman of the Council of the Television Society.

A meeting of the Biochemical Society will be held in Aberdeen during September 26–27. A colloquium on "The Metabolism of Protozoa" will be held during the meeting. Further information can be obtained from the Meetings Secretary, the Biochemical Society, 20 Park Crescent, London, W.1.

A conference on "The Design and Use of Microwave Valves", arranged by the Electronics Division of the Institute of Electrical Engineers, will be held at the Institute during October 16–18. Further information can be obtained from the Secretary, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

The twenty-fourth national Conference for the Preservation of the Countryside, arranged by the Council for the Preservation of Rural England, will be held in Plymouth during October 10–12. Further information can be obtained from the Secretary, Council for the Preservation of Rural England, 4 Hobart Place, London, S.W.1.

The seventh conference on "Analytical Chemistry in Nuclear Technology", sponsored by the Analytical Chemistry Division of the Oak Ridge National Laboratory, will be held in Gatlinburgh, Tennessee, during October 8–10. Further information can be obtained from C. D. Susano, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee.

An ordinary meeting of the North of England Section of the Society for Analytical Chemistry will be held in Manchester on September 28. A paper on "The Chemistry of Wines and Spirits" will be presented and discussed at the meeting. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

The Basic Science Section of the British Ceramic Society is holding a meeting on "Point Defects in Non-Metallic Solids" at Leamington Spa during October 14–16. Registration is necessary, and further information can be obtained from Dr. J. P. Roberts (honorary section secretary), Houldsworth School of Applied Science, the University, Leeds, 2.

The fourth International Aerosol Congress and Exhibition, organized by the British Aerosol Manufacturers' Association at the invitation of the Federation of European Aerosol Associations, will be held in Brighton during October 7–12. Further information can be obtained from the Conference Secretary, International Aerosol Congress Office, Milbanke Way, Bracknell, Berks.

INDUSTRIAL RESEARCH IN BRITAIN

*RESEARCH for Industry 1962** comprises, as usual, the report of the Industrial Grants Committee, progress reports from research associations the grants of which came up for renewal in 1962 and a directory of the research associations to-day, which this year is much shortened, the lists of publications and an outline of the scope of the present work of each association being omitted. There are also two special articles: one "Bridging the Gap" by H. W. Griffith, general manager of Shirley Developments, Ltd., describes the functioning of Shirley Developments, Ltd., to illustrate the conditions to be met in any such successful venture to bridge the gap between research and the application of research results in industry; the other by Dr. D. F. Galloway, director of the Production Engineering Research Association, on sponsored projects in a research association, describes some of the sponsored projects carried out by that Association. In a third article the Industry Division of the Headquarters of the Department of Scientific and Industrial Research reviews world developments in co-operative research.

This year the work of nine research associations is reviewed. The first of these, the British Boot, Shoe and Allied Trades Research Association, serves an industry which purchases some £100 millions worth of materials and components, expenditure which the Association seeks to minimize by establishing quality targets, grades and specifications. The Association has been conspicuously successful in its contribution to the efficiency of shoe factories and management techniques, and major contributions from physics research have been made to speeding throughput and reducing the volume of work in progress by design of equipment to give appropriate physical treatment to goods in progress. For example, exposure of shoe uppers, while on the last, for a few minutes to moisture and high temperature gives a standard of 'set' superior to that given by many days on the last at room temperature: this has been applied to the design of setting machines. A process-control system developed by the Association is being operated in many factories, and examples of the Association's work are to be found in children's shoes, industrial footwear, including safety boots and shoes, in the Services' footwear and in special footwear for expeditions.

The British Ceramic Research Association refers to the increased exchange of ideas between the main research divisions and the recognition of certain areas of common interest. For example, in the examination of the extrusion of clay, undertaken primarily to establish the cause of lamination in extruded bricks, which has provided new information on the plastic behaviour of clay, and on the role of the different parts of the auger machine in determining the texture of the extruded column and in the study of moisture expansion of fired products which, with the study of fixing methods, has greatly reduced crazing of tiles in service, and has also made it possible to place the moisture expansion of building bricks in its proper perspective as a factor in building costs and maintenance. Studies on basic refractories have contributed substantially to the productivity of the steel industry, and the interest of the Special Ceramics Division has now moved to such compounds as silicon nitride, silicon carbide and boron nitride.

The Coal Tar Research Association has discovered a new method for the industrial separation of the pyridine–picoline fraction of tar bases, but a major share of its attention is now being directed to the development of new and more lucrative outlets for pitch and creosote. Besides special anti-corrosion paints, special plastic enamels for protecting underground pipelines have been developed, improved pitch/asbestos compositions, and pitch/polymer compositions for adhesives and thermoplastic mouldings. Fundamental research has included statistical structural studies on the more complex fractions of pitch and the study of the ozonolysis of polynuclear hydrocarbons; development of a simplified method for the design of continuous distillation columns and of capillary column chromatographic analysis of mixtures of low volatility.

Researches of the British Coal Utilization Research Association on the nature of deposit formation and corrosion from the combustion of pulverized fuel and the causes of corrosion of austenitic steels in the boiler furnace are expected to lead to a better understanding of conditions within a boiler furnace and how these may be controlled, so as to eliminate or alleviate availability problems. Its experience with slagging cyclone combustion led the Ministry of Power to sponsor the investigation and development of a fixed-bed slagging gasification process, eventually to work under pressure. Work on furnace practice has sought to provide specific information on the operation of producer gas-fired furnaces and kilns.

A major technical development in the hatting industry was the introduction, through the work of the British Hat and Allied Feltmakers Research Association, of a fur-felt hardening machine. Also, a system of quality control set up within the industry embodies the standardization of raw materials, and use of process controls and felt quality indexes developed by the Association, which, in the past five years, has extended its activities to hat-finishing processes. Fundamental work has included measurement of the physical properties of felt, including shock-absorbing and frictional properties, and studies of the mechanism of the fur-felting process.

Main achievements of the British Iron and Steel Research Association during the past five years include a device which applies automatic controls to the power input of electric arc furnaces and has greatly improved their efficiency and lowered their running costs; development of a pilot plant for the continuous vacuum treatment of molten steel, giving clean steels with greatly diminished hydrogen content and better fatigue properties; an experimental forging plant embodying better control systems which promotes faster and more accurate working, better utilization of plant and greater saving of labour; and a new annealing cycle and plant which offers substantial reductions in capital expenditure and lower running costs.

The British Paper and Board Industry Research Association, which increased its staff from 70 to 100 during the period and its annual income from £95,000 to £136,000, formulated a new theory for paper structure and properties as a result of a microscopic study of fibre structure and fibre-to-fibre bonding. This theory predicts that many unmeasured and as yet unconsidered properties of the structural components of paper, such as those governing shrinkage, contribute to its mechanical strength. A new method of removing water, devised by the Association, called 'fluidized-bed drying' involves passing the paper web vertically upwards through a bed of sand or glass ballotini which is continually 'fluidized' by air jets, and places have been drawn up for installing five such fluidized beds to operate in series on an experimental machine in a member's mill. A specification for pulp-baling wires, resulting from work in conjunction with the British Iron and Steel Industry Research Association, should considerably reduce the risk of accidents in hand-

* *Research for Industry 1962*: a Report on Work Done by Industrial Research Associations in the Government Scheme. Pp. iv+74+22 plates. (London: H.M.S.O., 1963.) 7*s*. net.

ling these bales by hooking under the wires, since the breakage-resisting qualities of the weaker types of wire will be increased. The Association recently published a book entitled, *A Background Course in Radiological Safety*.

One of the most important research projects of the Timber Research and Development Association has been the development of timber shell roofs, while the development of steel connectors and glues by providing efficient systems of jointing wood has reduced the main restriction to its use for large-scale construction. New systems of house building are being developed to increase the rate of house building by using standardized mass-produced factory components in wood and the Association has co-operated with the London County Council in the design of 700 mobile homes for London's homeless.

A principal technical success of the earlier work of the Water Research Association was research into the mechanism of the chemical process by which turbid waters are clarified in water-treatment plant, and this has laid the basis for a proper understanding of the process. The loss of water by leakage from distribution mains represents a probable cost to the consumer of at least £7 million a year, and an operational research investigation is being made based on such factors as the cost of inspection, the manner in which leakage falls with more frequent inspections, rate of growth of an unrepaired leak, and the increasing damage done by leak with time. Efforts are also being made to improve the technique of detection, and instruments and techniques are also being developed for the more accurate calculation of the yield of water from catchment areas and underground sources.

MANAGEMENT EDUCATION AND TRAINING NEEDS OF INDUSTRY

THE report of the Working Party of the Federation of British Industries on *Management Education and Training Needs of Industry** does not seek to suggest the precise methods by which those existing needs should be met. It seeks rather to clarify the issues, removing the confusion of aims at various levels and defining more precisely the needs of industry itself. By implication it rejects the use of the term 'business' instead of management as suggested at the Home Universities Conference last year, although the report itself restricts the use of the term 'manager' to one who practises management and not to a rank or an appointment. It distinguishes in detail between management and supervision desiderating different qualities for the two functions, and, while critical of the present products of higher education, makes little suggestion as to how this basic education should be obtained. Perhaps the most useful feature of the report is its adoption of the term 'training' rather than education for the measures required to fit the manager for his job, whether at departmental level or in general or higher management.

Postulating that the term 'management' should not be used in the designating of any courses devoted largely to supervisory aspects and that the difference between management and supervision needs to be clearly recognized in the scope of the courses and the terminology used, the Working Party recommends that the curricula in schools and universities should devote much more attention to a knowledge and understanding of the social, economic and political influences of the present time and to the underlying purposes and objections. This, of course, is a matter of the fundamental general education on which any training course for management must be built. However, in its next recommendation the Working Party loses sight of the distinction between education and training and calls for development of a progressive structure of management 'education' for the three clear stages of departmental, general and higher management, no course attempting to cover more than one stage. The first courses of approximately three months or the equivalent are recommended at post-graduate level with opportunities for experience in the practical problems and actual conditions of the chosen environment. The second residential courses of some three months are recommended at not more than one or two centres with the necessary outstanding staff and a specially designed syllabus. The needs of higher management should be met by week-end study conferences or at the most five-day seminars at a few centres where the necessary resources are concentrated. More research is recommended in specific management techniques by those universities and centres which provide management training courses. The courses themselves would benefit and a consultancy service could emerge; but this needs the full support of industry. Training for management must proceed side by side with education, and opportunities must be provided within the organization for progressive instruction and practice in responsibility through staging posts and under senior guidance. In all this there must be the closest link between educational institutions and industry. A critical review of the needs should be the basis of the courses provided and opportunities afforded for the demonstration of principles and observation of practice within industry itself.

* *Management Education and Training Needs of Industry: A Report by an F.B.I. Working Party.* Pp. v+27. (London: Federation of British Industries, 1963.) 6*s*.

VETERINARY SCIENCE AT BRISBANE

THE Colin Wright Tropical Preventive Medicine Laboratory at the Veterinary School Farm, Brisbane, was built with funds from a variety of sources, which included a donation from the Beneficiaries of the Wright Estate, Imperial Chemical Industries of Australia and New Zealand, Ltd., the Australian Cattle and Beef Research Fund, and the Wool Research Fund.

The nieces and nephews of Mr. Colin Wright of 'Waverley', St. Lawrence, intended the Laboratory "to be a memorial of his work, which illustrated that the introduction of Brahman blood into beef cattle herds in the Tropical Coast is a profitable commercial proposition". It is of interest that Waverley Station, which is some 160 miles north of Rockhampton, adjoins the Cattle Station on which Mr. Christian developed the formula of his arsenical dip for cattle ticks in 1895; this formula was used throughout the world with only minor modification for the next fifty years.

Work on the Farm over the past five or six years has shown that the presence of, say, forty engorged ticks on a young bovine will reduce its growth at the rate of approximately 40 lb per annum[1].

The herd of Droughtmaster cattle on the Farm has carried about ten times fewer ticks than British cattle grazed with them, and the Droughtmasters have never had to be dipped or sprayed[2]. Droughtmasters are a

breed similar to the Santa Gertrudis produced by crossing Brahman and British beef cattle. They are approximately half Brahman compared with the three-eighths of the Santa Gertrudis. The importance of this is difficult to overestimate: there are a few British cattle with quite high tick-resistance, but it would obviously take a long time to raise the overall tick-resistance to a high level. On the other hand, one cross with a Brahman bull raises tick-resistance about ten-fold, and we can still select for tick-resistance. These facts were well understood by Dr. R. B. Kelley, who contributed so much in this field, and they have, in fact, been well established, even if their importance has not been well or widely accepted, for at least forty years. Unfortunately, for a long time the subject of 'tick-resistance' seems to have caused considerable confusion in the minds of people regarded as experts on ticks, or at least they have not appreciated its importance. However, a few people have had a clear understanding, and a paper published early in this century described how pastures grazed by tick-resistant cattle were steadily 'cleansed' of ticks. The Droughtmasters and Brahman-hybrid cattle are also considerably more resistant to babesiosis[3] than European cattle.

The Farm, of some 700 acres, is only seven miles from the new Veterinary School at St. Lucia which was opened in August 1961 by the Hon. C. F. Adermann, Minister for Primary Industry. There are now almost 100 students in each of the first two years of this school, and 36 post-graduate students.

JOHN FRANCIS

[1] Little, D. A., *Austral. Vet. J.*, **39**, 6 (1963).
[2] Francis, J., and Little, D. A. (submitted to *Austral. Vet. J.*).
[3] Francis, J. (submitted to *Austral. Vet. J.*).

AUSTRALIAN DEFENCE STANDARDS LABORATORIES

THE main sections of the illustrated annual report of the Department of Supply of the Australian Defence Standards Laboratories for the year ended June 30, 1962, deal with the work carried out in the fields of physics, metallurgy and chemistry.

The Physics Division consists of the metrology, electronics, electrical measurements, chemical physics and optics, and xerography groups and branches in New South Wales and in South Australia. The two items of special interest in the Division's activities during the year are the production of calibration tables for platinum resistance thermometers by means of an IBM 1620 digital computer and the acquisition of one of the highest precision Mueller resistance thermometer bridges yet built.

The bridge, which incorporates all the latest developments and modifications from the United States National Bureau of Standards, has nine dials covering the range 0–422 ohms in increments of microhms. The Division's 'Varian' wide-line nuclear magnetic resonance spectrometer was used in the study of a number of chemical and physical problems, including the determination of the separation between hydrogen atoms in the water of crystallization in calcium sulphate hemihydrate and the observation of some nitrogen, zinc and chromium resonances. The resonance of the tin-119 isotope, which is of low natural abundance and of relatively poor sensitivity, was recorded in a series of liquid tributyl tin compounds of particular interest in the development of plastics resistant to corrosion and to penetration by fungi. The shift of the tin resonance in each molecule relative to the resonance of the stannous ion was measured and explained on the basis of simplified models.

Mention was made in the 1961 annual report of investigations on the vaporization processes occurring on the surfaces of metal electrodes when subjected to low-current long-duration pulsed discharges. These have been continued. In the field of solid-state physics small-scale experimental work was started on hot carrier conduction in silicon. In the metrology group a method was devised for the use of the 'Talyrond' roundness measuring instrument for the accurate measurement of standard ring gauges, and an experimental mercury manometer of 5-cm bore was constructed for the measurement of pressures of 0–60 millibars to an accuracy of 10^{-2} millibar.

The Metallurgy Division was mainly concerned with the production of pure chromium and with the study of the properties of chromium and chromium alloys. In collaboration with Dr. P. A. Jacquet a new technique was developed for the preparation of chromium for microscopic examination with either the optical or electron microscope. Internal friction studies established that the extent of magneto-mechanical damping and associated hysteresis in chromium is markedly dependent on grain size and on impurity content. Measurements of the dynamic shear modulus of chromium at 1 c/s over the temperature-range $-100°$ C to $100°$ C showed that the modulus has a maximum value at the antiferromagnetic transition temperature (Néel temperature). The metallographic study during 1961 of abraded germanium surfaces led to the surprising result that the damaged layer consisted of cracks and not of large densities of dislocations. This work was extended during 1962 to the examination by microscopy and by taper-sectioning techniques of the extent to which the damage is removed by etching with '*CP*-4' reagent. It was found that the reagent penetrated the cracks and opened them into broad crevices, but that at the same time the surface was smoothed out with the production of a characteristic cellular pattern. The damage in electric spark-machined surfaces was also studied.

Some interesting results were obtained from the structural studies and stereochemistry investigations in the Chemistry Division of the aliphatic cyanides and isocyanides which, despite identical molecular formulæ, differ considerably in chemical and physiological activity. The contribution of the isocyanide bond to the molecular refraction is much greater than that of the cyanide bond, and the effects of hydrogen-bonding solvents on the infrared spectra of the two families of organic compounds and of carbonyl compounds differ markedly. Other activities in the Chemistry Division include the determination of gases in metals, in particular oxygen in chromium by the vacuum fusion and the Adcock chemical methods, and phosphorus in irons and steels using quinoline molybdate instead of the more usual ammonium molybdate, and X-ray fluorescence analysis of the ternary alloys Fe-Cr-Ni, Cu-Ni-Zn and Cu-Zn-Sn.

The annual report lists the various personnel of the Laboratories and their status, and gives details of the publications by members of the staff during the year under review. The staff numbered 724 on June 30, 1962—an increase of 37 during the year, reflecting the somewhat easier position in the recruitment to sub-professional positions. Nine members of the staff spent all or part of the year in the United Kingdom. Visitors to the Laboratories included the Australian Minister for Supply; Prof. K. H. Hunt of Monash University; Prof. S. Kikuchi of Tokyo University; Sir Leslie Martin, chairman of the Australian Defence Research and Development Policy Committee; Dr. P. Cheosakal of the National Research Council of Thailand; Dr. W. J. Youden of the United States National Bureau of Standards; and a party of sixty officers from the Australian Army Staff College.

S. WEINTROUB

NORWEGIAN GLACIERS

GLACIOLOGICAL research at the present time consists of two main tasks: first, the recording of the state and behaviour of glaciers; and secondly, the study of the various factors controlling their behaviour. The latter normally progresses by intensive studies in limited areas or of specific problems. The general recording of glaciers and their behaviour requires widespread survey, mapping and a continued interest in glaciers and the surrounding country spread over many years. Adolf Hoel and the late Werner Werenskiold have had both broad and specific interests in the glaciers of Norway throughout their lives. In the *Glaciological Bibliography of Norway* * entries under Adolf Hoel are dated from 1906–56, and under Werner Werenskiold from 1913–61. Their lifetime interest and knowledge of Norwegian glaciers makes their general review of *Glaciers and Snowfields in Norway* † a work of authority and value. It is written in English, and will thus be useful to students in many countries.

The first section of *Glaciers and Snowfields in Norway* deals with the history of glaciological research in Norway, then lists the glaciers of Norway with details of their area. Dates of surveys are given, since it has not been possible to survey all glaciers contemporaneously. Data for southern Norway are mostly up to date (about 960 glaciers), but in northern Norway many surveys used were made around the beginning of this century, since when a marked recession may have decreased the area of glaciers by perhaps 30 per cent. However, dates are given and changes to the present day can be determined by field parties visiting these areas. The present senior glaciologist of the Norsk Polarinstitutt, Olav Liestol, has always been a ready guide and helper to those seeking advice on where mapping of the glaciers will be most useful, and this volume may stimulate further work.

Former glacier fluctuations are discussed in the light of indirect evidence, such as the preservation of arrowheads and ropes by ice, which indicates that snow and ice cover must have increased from at least A.D. 1500 until A.D. 1750, when a general recession commenced. Although some advance occurred in the period 1850–55, it appears less marked than that in the European Alps. Spitsbergen glaciers are also discussed, where, apart from a general decrease in size in recent decades, some catastrophic advances, such as that which added 600 km² to the area of Bråsvelbreen, are noted.

The section discussing benefits derived from glaciers starts naturally with their relations to hydroelectric power. Glaciers also provide useful lines of communication of use between settlements as well as by tourists. Commercial exploitation of glacier ice is also covered. Collection by fishing vessels for refrigeration purposes was important for at least thirty years up to 1949 near Oksfordjokulln, but other forms of export of ice, although pursued, were apparently less successful in this area.

The remaining 170 pages of the book presents a wealth of data on glaciers studied by Hoel, Werenskiold and their associated workers. Work in the Jotunheim area over the period 1927–48 covers surveys, accumulation, ablation, velocity, stream discharge and other measurements. Studies have concentrated especially on Hellstugubreen and Tverråbreen. Maps of both glaciers and a series of maps of Tverråbreen during 1927–38 are presented in a separate folder. Similar investigations on glaciers in northern Norway are presented.

The *Glaciological Bibliography of Norway* contains some 1,500 references to work on Norwegian glaciers presented both as a regional and as a chronological (author) catalogue. Its usefulness is increased by symbols denoting that the papers contain maps or photographs of special use for future comparisons. References are cited up to 1958 with some additions to 1961.

All those interested in the study of Norwegian glaciers will benefit greatly by these publications, which not only draw together a considerable amount of scattered work, but also contain many sections which would interest anyone visiting these areas. G. DE Q. ROBIN

* Norsk Polarinstitutt. (Det Kongelige Departement for Industri og Handverk.) Skrifter Nr. 126: *Glaciological Bibliography of Norway*. By Adolf Hoel and Johannes Norvik. Pp. 242. (Oslo: Norsk Polarinstitutt. Distributed by Oslo University Press, 1962.) 30 Kr.

† Norsk Polarinstitutt. (Det Kongelige Departement for Industri og Handverk.) Skrifter Nr. 114: *Glaciers and Snowfields in Norway*. By Adolf Hoel and Werner Werenskiold. Pp. 291+8 maps. (Oslo: Norsk Polarinstitutt. Distributed by Oslo University Press, 1962.) 40 Kr.

FORAGING AND FEEDING BEHAVIOUR

A SYMPOSIUM of the Society for the Study of Animal Behaviour held at the London Zoological Gardens during July 9–11 was devoted to the topic of "Foraging and Feeding Behaviour". Most of the papers read on the first day of the meeting dealt with insects and had an ecological rather than ethological flavour. F. Raw (Rothamsted Experimental Station) was more concerned with the aspects and effects of foliage disposal by earthworms than with the behavioural mechanisms operative in this remarkable affair, interest in which goes back to Darwin. "As fallen foliage in orchards harbours stages in the life-cycle of fruit tree pests its speedy disposal is an important factor in the health of the fruit trees." Leaf burial by earthworms (*Lumbricus terrestris*) is, therefore, of great potential ecological importance.

Barbara A. Hopkins (Ascot Field Station of the Imperial College of Science and Technology) reported on the "Probing Response of *Stomoxys calcitrans* (the Stable Fly) to Odours". Ammonia chiefly and a series of other components were offered to individual flies after and during a 24-h period following a blood or sugar meal, and their proboscal probing was observed. Probing does not seem to be associated with need for water and shows no obvious 24 h or longer period rhythmicity. Its threshold also is unaffected by larval environment or crowding. This gradually fell during the 24 h after feeding. There is some evidence that receptors responsible for the probing sequence are located on the tarsi. *N*-propyl amine and several lower fatty acids approach ammonia in stimulating effect. A. J. Cockbain (Rothamsted) also dealt with probing in his paper on "The Probing and Feeding Behaviour of Alate Aphids in Relation to the Transmission of Some Plant Viruses". He showed that infectivity with non-persistent viruses such as pea and sugar beet mosaic virus is associated with short duration probing and not with prolonged feeding on infected plants. Many *Myzus persicae* individuals but few *Aphis fabae* probe for a short time (15–60 sec) before flying from infected plants on which they have been feeding. This may explain the difference in infectivity of the two species. *A. fabae* also seem to fail to insert their stylets into the plant tissues when probing.

"The Feeding and Excretion Behaviour of Aphids" was analysed quantitatively by C. J. Banks (Rothamsted). He demonstrated that most aphids feed on the phloem tissues of the host plant. That they feed on the sieve tube elements has only rarely been convincingly demonstrated. It may take them as long as 14–16 h to reach the maximum rate of feeding. In this aphids do not seem to rely entirely on the sap pressures within the plant and there is some evidence for active sucking and relation of intake to need during post-reproductive ageing when excretion has stopped.

J. Sudd (Department of Zoology, Hull) analysed the behaviour involved in the transport of prey by ants and finds two chief mechanisms: namely, dragging and pulling. Successful dragging presupposes visual orientation as it involves reversal of the ant's alignment with respect to the homing direction. Pulling is compatible with preponderantly olfactory and tactile tracking. In *Formica lugubris* disordered movement by a number of collaborating ants is followed by a pause of several minutes before rapid and orderly concerted movement towards the nest occurs. The middle pause of no movement seems to be a state of equilibrium.

J. B. Free (Rothamsted) returned to the orchard with a study on "The Foraging of Honeybees in Fruit Orchards" and showed that nectar gatherers, especially those that stand on the petals, do not touch the stigma and thus fail to pollinate although they may receive pollen from flowers with spreading stamens such as apricot, peach, pear and plum. On some apple varieties with stiff, upright stamens no pollen is transferred to the nectar gatherer. Feeding with sugar syrup switches a greater proportion of foragers to pollen collecting and thus increases the chances of successful pollination. Bees keep to a row of trees and change to trees nearest to those initially collected from. This is of importance for maximum fruit set in trees needing cross-pollination and points to an optimal pattern of arrangement of varieties in apple orchards. As bees tend to forage near their homes, especially in unfavourable weather, colonies should be sited in small groups each near the centre of the area to be pollinated. Colonies should not be taken to the orchard until flowering has begun, as bees will not readily change to the fruit blossoms after having collected from other plants.

The rest of the first day was devoted to lower vertebrates. L. de Ruiter (Zoology Laboratory, Groningen) gave a paper on "The Feeding and Foraging in the Stickleback: Causes and Consequences" in which he demonstrated the factors involving visual feeding under quantitatively controlled conditions, and J. S. Kenny (Department of Zoology, Birkbeck College, London) gave an account of "A Typical Use of the Ciliary Feeding Mechanism in Some Anuran Larvæ" in various ecological niches. The primary feeding mechanism involves the buccal rasp, filters and mucus string aggregation and transport of particles by ciliary action. Mid-water and surface-feeders may show modification in the primary mechanism such as increase in filtering area and loss of the buccal rasp. Secondary adaptations of tail and lips were also described.

The second day of the symposium, devoted entirely to bird behaviour, opened with a paper by P. M. Driver (Department of Extra-Mural Studies, Bristol) on "The Development of Feeding Mechanisms in *Mergini*", followed by an account by Janet Kear (The Wild Fowl Trust, Slimbridge) on the "Parental Feeding Behaviour in the *Anatidae*". True ducks scarcely ever feed their young, but in *Anseranas*, *Dendrocygna* and *Cygnus* parental feeding is well established. The development of parental feeding may start with the young feeding on surplus food falling from the bill of the parent. This is supported by innate pecking upwards of newly hatched anatids towards the tip of pointed objects, including the bills of siblings and parents. In the magpie goose, begging and the brightly coloured head of the goslings act as releasers for parental feeding. "The Feeding Behaviour of the Wood-pigeon" was analysed by R. K. Murton (Field Research Laboratory, Worplesdon, Surrey). When food supplies are limited *Columba palumbus* feeds gregariously and 95 per cent of the food is made up of clover leaves. The birds feed almost continuously and selectively. The importance of a 'specific search image' was demonstrated. The speed of searching is correlated with food density. The prime function of flocking is to maintain a maximum population size where random distribution of birds without competition leads to excessive mortality.

D. J. McFerland (Institute of Experimental Psychology, Oxford) reported on "Some Interactions of Feeding and Drinking Behaviour in the Barbary Dove". These birds eat less when thirsty and drink less when hungry. This was held to be the consequence of an antagonism between the hypothalamic feeding and drinking systems. In a Skinner box thirsty birds go on working for food but do not eat it (displacement feeding ?). It appears that the food deficiency incurred in thirsty doves is capable of activating the relevant appetitive behaviour despite the fact that the birds are unable to eat the food which they obtain in this way.

In the afternoon those attending the symposium were given an opportunity to visit the Zoo and the Granada Television Film Unit and to see there a preview of unedited film material, including a fascinating close-up colour film of cobbler ants at work building their nests from leaves sewn together with the silk produced from the silk glands of their larvæ.

In a paper not originally included in the programme of the third (day which was devoted to mammalian behaviour) J. A. Chmurzyński (Nencki Institute of Experimental Biology, Warsaw) described "The Stages in the Spatial Orientation of the Digger Wasp, *Bembex rostrata* L.". There are three stages of spatial orientation: distant orientation outside and within the life-range (about ½ km from the nest) with random and chiefly visual orientation respectively; proximal orientation (about 4 m from the nest depending on frequency and distinctiveness of natural landmarks—here visual and olfactory senses guide the orientation and recognition; "immediate orientation" (not more than 6 cm from the nest) when the insect finds the nest by making use chiefly of tactile clues. Three papers on various aspects of feeding in ruminants by A. Brownlee (Agricultural Research Council Institute for Research on Animal Diseases, Compton, Newbury), C. C. Balch and R. C. Campling (National Institute for Research in Dairying, Shinfield, Reading) and D. T. Chambers (Grassland Research Institute, Hurley) dealt with rumen development as related to food regimen, with the rate of eating and relative length of periods of ruminating in relation to different kinds of food, and with selective grazing habits of cattle and the associated problem of wastage respectively. A. J. B. Rudge (Infestation Control Laboratory, Guildford) reported on a preliminary investigation into baiting of wild rabbits. Whole carrots were shown to be a most suitable form of unpoisoned bait. P. R. Wiepkema (Zoological Laboratory, Groningen) spoke on the physiological significance of feeding-patterns in mice. Hyperphagia, aphagia and finickiness are considered in their relation to lesions in the ventro-median nucleus of the hypothalmus. Perception of food quality activates a hunger system which in turn stimulates a motoric eating centre. After food uptake three negative feed-back channels can be distinguished, namely, a stomach factor, a blood glucose factor and a body-fat factor. They sum up into a satiety system which inhibits the hunger system. The probable effects of ventro-median lesions on these feed-back systems were discussed.

"The Ethology of Food Hoarding in Mammals" was discussed by M. Lyall-Watson (Zoological Society of London). He gave a comprehensive survey of the different methods utilized by mammals in accumulating stores of food. Larder hoarding and scatter hoarding can be distinguished and related to seasonal requirements and

types of food. The scatter hoarding in South American green acouchi, *Myoprocta pratti*, was subjected to a closer ethological analysis.

The symposium closed with an analysis of "The Aetiology of Food Reward in Monkeys" by L. Weiskrantz (Psychological Laboratory, Cambridge) and a paper on "Chimpanzee Behaviour in Relation to the Predominant Food Sources" in which V. Reynolds (London) described the overall pattern of chimpanzee activity as an alternation between convergence on areas with plentiful food and scattering when food is scarcer. This lead to optimal exploitation. The loose social structure of chimpanzees who live in bands of ever-changing size and composition may be considered to be an adaptation to the type of food distribution.

The topics discussed at this symposium clearly belonged to the borderland between ecology, horticulture, husbandry, physiology and ethology, and, although behavioural aspects were frequently not in the foreground, the meeting served the useful purpose of cross-fertilization during lively and informative discussions.

O. Lowenstein

IONOSPHERIC EFFECTS DUE TO THE EARTH'S ORBITAL POSITION

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In the article by Sir Edward Appleton on "Ionospheric Consequences of the Earth's Orbital Eccentricity"[1] it was shown how the peak electrons number present in 1 cm³ of the E ionospheric layer ($N_m(E)$) changes during the year according to the variation of the distance of the Earth from the Sun. In Fig. 1 of Appleton's article (Fig. 1(a) of this paper) is shown a graph obtained by working on the results of ionospheric soundings performed during 1949–59 by several stations and leaving out all effects due to the variation of the solar activity (R) and to the variation of the zenithal distance (χ) in the places of observations; on the whole this result must be considered valid for R = constant and $\chi = 0$.

Going from the perihelion to the aphelion, that is from the beginning of January towards the beginning of July, the distance between Earth and Sun (r) changes of 3·4 per cent; this means a variation of the solar flux of 6·5 per cent during this interval. Such value is in good agreement with the experimental one shown in Fig. 1 of Appleton's article if we assume the electronic density ($N_m(E)$) proportional to the flux of the ionizing radiation (q) (electrons loss described by an attachment law). There is still a difference of 1 per cent between the variation of q and that of $N_m(E)$ to be explained: according to Appleton this is due to an unknown cause.

In this article I would like to show how the discrepancy between theoretical values and results is not only due to this difference—even in the hypothesis of an attachment law for the electrons—and to explain its cause.

In the curve of Appleton's Fig. 1 the width of the concavity is too small compared with the curve showing the behaviour of $1/r^2$: in fact, if 'a' is the length of the major semi-axis of the Earth orbit, it can be easily seen

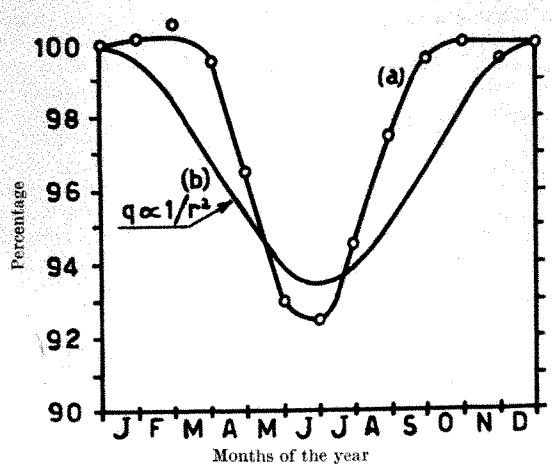


Fig. 1. Comparison between $Nm(E)$ (curve a) and the solar flux supposed proportional to $1/r^2$ (curve b)

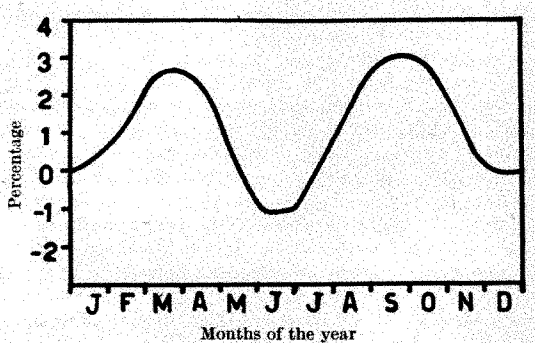


Fig. 2. Difference between the curves (a) and (b) of Fig. 1; that is, annual variation of the E layer electron concentration due to that part of the ionizing flux which is not proportional to r^{-2}

that the average between the upper and the lower limits of the function $1/r^2$ is larger than $1/a^2$. Besides, the arc of the ellipse made by the Earth from the perihelion to the instant in which $r = a$ (and naturally $1/r^2 = 1/a^2$) is equal—for well-known properties of the ellipse—to a

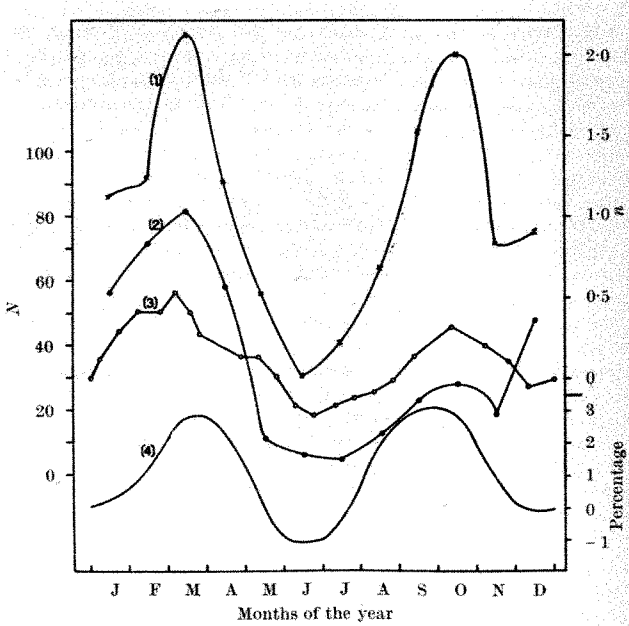


Fig. 3. (1) Annual variation of the number of nights with auroræ in Denmark (ref. 2) during 1897–1937 (scale on the right top). (2) Annual variation of the number of nights with zodiacal light (ref. 3) during 1847–1875, according to Heis (scale on the left). (3) Annual variation of the number of magnetic perturbations in Paris (ref. 4) during 1883–1923 (scale on the left). (4) Annual variation of the 'residual' peak electron concentration in the E layer (that is, the curve of Fig. 2) (scale on the right, below)

quarter of the ellipse total length. Let us consider now the time needed by the Earth to make that part of the ellipse are going from the point where $r = a$ to the symmetric point passing through the aphelion. For the second Kepler's law this time is more than that needed to make the complementary arc; it is now clear that the width at half depth (that is to a level higher than $1/a^2$) of the concavity of Appleton's Fig. 1 should be larger than six months if such curve is to behave according to $1/r^2$. This width is instead much smaller.

However, the curve showing the behaviour of $N_m(E)$ has another important characteristic, besides being too deep, which cannot be simply explained by the variation of the distance Earth–Sun. This can be seen in Fig. 1 of this article where the curve (a) is compared with the graph of $1/r^2$ (b); in Fig. 2 the difference (a) – (b) is made evident.

Particularly interesting is the graph of Fig. 2 which can be considered as showing the temporary behaviour of that electrons number fraction (as a percentage of the maximum value) which is not explained by the simple model $N_m(E) \propto q \propto 1/r^2$. Obviously this fraction contains an undefined additive constant and therefore the position of the zero in Fig. 2 is arbitrary.

It can now be seen that the phenomenon showed by such a curve is much more complicated than being a simple excess in the reduction of the electronic concentration in June. Nevertheless from this new characteristic I am now able to suggest the origin of this effect. In fact some other phenomena show seasonal variations of this type, with maxima around the equinoxes and minima around the solstices: between such phenomena there are: aurora polaris[2], zodiacal light[3] and geomagnetic perturbations[4] (Fig. 3). (Note that the increase of electron density in the E layer during magnetic storms is proved.) Their behaviour seems due[5] to the fact that near solstices (that is, at the beginning of June and December) the Earth passes through the plane of the solar equator, and therefore it faces a solar region of low activity. Vice versa in March and September the radius Earth–Sun is nearest to the region of medium latitude—north or south—where the sunspots are present. From these regions a corpuscular radiation, responsible for phenomena of this type, should be emitted. In other words, the Wolf relative number R has a different weight when the Earth faces a solar region in which there are spots or when the Earth faces a less-active equatorial region.

It is to be noticed that the remarked greater depth of the curve in June compared with December is a characteristic common to all mentioned phenomena (Fig. 3).

Furthermore I must emphasize that a curve similar to that of Fig 2 is also obtained if one supposes the electron loss in the E region described by a recombination law ($N_m(E) \propto q^{1/2}$) or by an intermediate law ($N_m(E) \propto q^n$; $0{\cdot}5 < n < 1$).

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[2] Egedal, J., *Publik. fra Det Danske Meteor. Inst. Tillaeg, Copenhagen* (1937).
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RED CELL ACID PHOSPHATASE VARIANTS: A NEW HUMAN POLYMORPHISM

By Dr. D. A. HOPKINSON, Dr. N. SPENCER and Prof. H. HARRIS

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WHEN human red cell hæmolysates are examined by starch-gel electrophoresis, more than one zone of acid phosphatase activity is observed. Furthermore, the pattern of these zones may differ from person to person. So far we have been able to distinguish five distinct red cell acid phosphatase patterns and they appear to be characteristic for the individual and to be genetically determined.

These electrophoretic patterns may be identified by the following procedure. Hæmolysates are prepared by the addition of 2 volumes of distilled water to 1 volume of washed red cells. Horizontal starch-gel electrophoresis is then carried out at *p*H 6·0 (gel buffer: 0·005 M succinic acid and 0·0092 M *tris*, bridge buffer: 0·41 M citric acid/sodium hydroxide *p*H 6·0) using very thick filter paper inserts (Whatman No. 17). Electrophoresis is performed for 17 h with a potential gradient of 6 V/cm. in a cold room at +5° C. After electrophoresis the gels are sliced horizontally and placed in 'Perspex' boxes which have lids. The cut surfaces are then covered with a solution of 0·005 M phenolphthalein diphosphate sodium salt in 0·05 M citrate buffer *p*H 6·0 and incubated for 3 h at 37° C. After this the reagent is poured off, and a few drops of ammonia solution (s.g. 0·88) are added to the box which is then re-covered. The ammoniacal atmosphere makes the surface of the gel alkaline, and the areas where phenolphthalein has been liberated from the phenolphthalein phosphate by acid phosphatase appear as red zones. The phenolphthalein slowly diffuses so that sharp definition of the various zones only persists for about half an hour. The zones of activity may also be observed using *p*-nitrophenyl phosphate as substrate instead of phenolphthalein phosphate. However, the *p*-nitrophenol formed diffuses very much more rapidly than does the phenolphthalein and the zones are therefore much less clearly defined. We have been unable to detect these zones using α- or β-naphthyl phosphate as substrate with an appropriate diazo reagent to couple with any α- or β-naphthol liberated. This is presumably a consequence of the peculiar pattern of substrate specificity of the red cell acid phosphatase, because certain other human acid phosphatases can be readily detected with these substrates under the foregoing conditions.

Fig. 1 shows typical examples of the five different patterns of activity so far distinguished in different individuals. These types will be referred to as *A*, *BA*, *B*, *CA* and *CB*. They can be approximately described, as indicated in Table 1, in terms of the relative activity of three zones, 'fast', 'intermediate', and 'slow', which migrate towards the anode in that order. In type *A* the 'fast' zone is generally clearly defined and well separated from the 'intermediate' zone. In type *BA*, however, the 'fast' zone is less sharply defined and appears as a smear running forward from the 'intermediate' zone, which is in this type very much more intense than in type *A*. A pattern very similar to that observed in type *BA* can be obtained by the electrophoresis of a mixture of hæmolysates of type *A* and type *B* in roughly equal amounts. Types *CA* and *CB* are characterized by the presence of a very intense 'slow' zone. In type *CA* this is associated with both 'fast' and 'intermediate' zones similar to those observed in type *A* and very much less intense than the

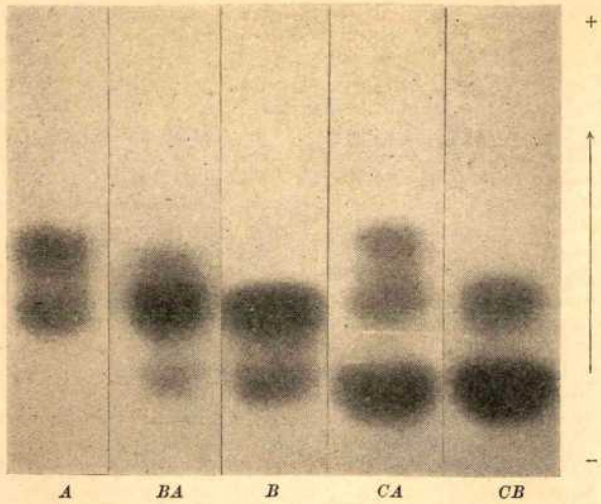


Fig. 1. Five red cell acid phosphatase patterns which may be seen after starch gel electrophoresis of hæmolysates from different individuals. The lower edge of the photograph is the start line. The hæmoglobin moves towards the cathode and is not shown

'slow' zone. In type *CB* it is associated with an 'intermediate' zone similar to that observed in type *B* and significantly less intense than the 'slow' zone. It must be noted that a distinct 'slow' zone is also observed in type *B*, but this is relatively less intense than the 'intermediate' zone in this type, and is also much less intense than the 'slow' zones observed in types *CA* and *CB*. A very weak 'slow' zone can also be regularly observed in type *BA*. In some experiments it appeared as if a faint trace of such a 'slow' zone was also present in some samples of type *A*, but this has not been observed consistently.

Despite the complexity of the patterns there is in practice little difficulty in classifying individual hæmolysates, particularly when these are fresh. Storage at +4° C and especially at −10° C results in a progressive fall in activity, and also often in some modification of the pattern, with the frequent appearance of one or more zones moving even more rapidly than the 'fast' zone referred to previously. The activity of stored samples is maintained somewhat better if 2-mercaptoethanol (1 µl. per ml. hæmolysate) is added before storage. Using this we can generally get satisfactory electrophoretic patterns up to about ten days after the hæmolysate was originally prepared.

Table 1. Approximate Description of the Five Red Cell Acid Phosphatase Electrophoretic Patterns

Zone of enzymatic activity	Red cell acid phosphatase type				
	A	*BA*	*B*	*CA*	*CB*
'Fast'	+	+	−	+	−
'Intermediate'	+	++	+++	+	++
'Slow'	−	trace	++	++++	++++

As a routine both in the frequency studies and in the family studies described later, both surfaces of the sliced gels have been classified independently by two observers. In many instances the hæmolysate has also been subjected to electrophoresis on more than one occasion. Furthermore, in a number of cases more than one sample obtained on different occasions from given individuals has been examined. In general, the classifications have been unambiguous and in agreement.

It has been shown that red cell acid phosphatase activity can be inhibited by formalin[1] but is relatively unaffected by D(+)-tartrate[2], and these characteristics have been used to distinguish this kind of acid phosphatase activity from that found in certain other tissues (for example, prostate). We have observed that if formalin is added to the reaction mixture, described, to a final concentration of 0·5 per cent, no zones of activity in any of the five types are visible after the usual 3 h incubation. On the other hand D(+)-tartrate (0·03 M in the final reaction mixture) produces no obvious change in the patterns observed. Previous evidence indicating heterogeneity of red cell acid phosphatase has been obtained from the study of *p*H activity curves[3], and from elution patterns obtained by chromatography on DEAE cellulose[4]. It is not at present clear in what way these findings are related to the variations described here.

Table 2. Incidence of the Red Cell Acid Phosphatase Types in Randomly Selected English Males and Females

Type	Males	Females	Total	Frequency
A	10	4	14	0·101
BA	50	14	64	0·460
B	33	15	48	0·345
CA	2	3	5	0·036
CB	6	2	8	0·058
Total	101	38	139	1·000

Red cell hæmolysates from 139 unrelated English people of both sexes have been examined, and the classifications obtained are given in Table 2. Since no gross sex differences in the frequencies of the various types were observed, the data for the two sexes have been combined to obtain estimates of population frequencies.

Studies have been carried out on 42 families, in each of which blood samples were obtained from both parents and one or more of their children. The results are summarized in Table 3. The families do not represent an entirely random sample, because in the early stages of the investigation we were especially concerned to study families in which the less-common types *A*, *CA* and *CB* occurred. It is apparent that the distribution of the types among the children of particular sorts of mating is non-random. Thus it may be noted that all the 16 children of the matings between parents both of whom were of type *B*, were also type *B*. Also all the children of types *CA* and *CB* observed were derived from matings in which at least one parent was also type *CA* or *CB*. In the single family of mating type *A* × *B*, all 4 children were type *BA*.

Although the family data are still rather scanty, it is perhaps useful at this stage to consider how they might be accounted for in genetical terms. One hypothesis which is consistent with the present data is the following. We suppose that the variations observed are determined by three allelic autosomal genes P^A, P^B and P^C, and that the various possible genotypes correspond to the phenotypes observed in the manner shown in the first two columns of Table 4. It will be noted that this hypothesis predicts the occurrence of a sixth phenotype, *C*, which has not as yet been observed. This phenotype, as will be shown later, would be expected to be much less frequent than any of the other phenotypes.

With six genotypes, twenty-one parental combinations have to be considered, assuming that reciprocal matings produce the same results. Many of these combinations would, of course, be relatively uncommon and in the 42 families so far studied only eleven of them have been observed. The particular phenotypes expected among the children of these matings can be readily determined, and it will be seen from Table 3 that, among the 86 children so far investigated, only the phenotypes predicted by the hypothesis actually occurred. The data are also consistent with the expected Mendelian ratios after allowance has

Table 3. Distribution of Red Cell Acid Phosphatase Phenotypes Among the Children of 11 Different Parental Combinations

Type of mating	No. of families	Children				
		A	*BA*	*B*	*CA*	*CB*
A × *BA*	5	6	5	—	—	—
BA × *BA*	10	4	12	2	—	—
A × *B*	1	—	4	—	—	—
BA × *B*	10	—	10	7	—	—
B × *B*	6	—	—	16	—	—
A × *CA*	1	—	—	—	2	—
A × *CB*	2	—	2	—	4	—
BA × *CA*	3	1	—	—	—	4
BA × *CB*	1	—	—	—	1	1
B × *CA*	2	—	1	—	—	2
B × *CB*	1	—	—	1	—	1
Totals	42	11	34	26	7	8

been made for the way in which the different families were selected, but larger numbers will clearly be required to test this aspect of the hypothesis adequately.

If the hypothesis is correct then estimates of the gene frequencies p, q and r of the three alleles P^A, P^B and P^C can be obtained from the frequency of the various phenotypes in the general population, in the following manner: p = [frequency of type A] + ½[frequency of type BA] + ½[frequency of type CA]; q = [frequency of type B] + ½[frequency of type BA] + ½[frequency of type CB]; r = [frequency of predicted type C] + ½[frequency of type CA] + ½[frequency of type CB]. Using the results given in Table 2, which are effectively random, the values for the gene frequencies are: $p = 0{\cdot}349$; $q = 0{\cdot}604$; and $r = 0{\cdot}047$.

From these estimates one can determine the expected frequencies of the various phenotypes from the expansion $(p + q + r)^2$ assuming random mating and the absence of appreciable selection. These can be compared with the observed frequencies. The appropriate calculations are given in Table 4. There is good agreement between the observed numbers of individuals of the different phenotypes and the numbers expected. This provides further evidence in favour of the hypothesis.

The calculations given in Table 4 suggest that the predicted but not yet observed phenotype C may be expected to occur in about 1 in 400 randomly selected individuals in an English population. One would imagine that such individuals would show a red cell acid phosphatase pattern in which there was no 'fast' zone, a very weak or perhaps absent 'intermediate' zone, and a very prominent 'slow' zone which would account for nearly all the activity present.

A number of other observations about these variations may be briefly mentioned. (1) Studies on the red cells from cord bloods have revealed patterns not obviously different from those seen in adults. Out of 12 cord bloods examined 2 were of type A, 6 of type BA and 4 of type B. (2) Inspection of the gels suggests that the overall acid phosphatase activity varies from type to type. Preliminary studies in which the enzyme activity has been assayed by standard procedures using p-nitrophenyl-phosphate as the substrate, suggest that the relative activities are $A < BA < B \sim CA < CB$. (3) In much of the family and population data reported here typing for blood group antigens, haptoglobins and transferrins, has been carried out. No obvious associations between any of these markers and the red cell acid phosphatase variants have been noted. Detailed linkage analyses have not yet been performed.

Table 4. Observed and Expected Numbers of Different Phenotypes Assuming Random Mating

Genotype	Phenotype	Expected frequency		Expected numbers in sample in Table 2	Observed numbers in sample in Table 2
P^AP^A	A	p^2	0·1218	16·9	14
P^AP^B	BA	$2pq$	0·4215	58·6	64
P^BP^B	B	q^2	0·3648	50·7	48
P^AP^C	CA	$2pr$	0·0328	4·6	5
P^BP^C	CB	$2qr$	0·0568	7·9	8
P^CP^C	[C]	r^2	0·0022	0·3	0
Total			0·9999	139·0	139

Further collection of family and population data of the sort described here should show whether the simple genetical hypothesis which we have put forward is correct, or whether some more complex explanation is required. It should also indicate whether the predicted phenotype C actually exists.

We thank Dr. M. Glen-Bott for her help with the family studies, Dr. T. E. Cleghorn for determining the blood groups, and Dr. E. B. Robson for determining the haptoglobins and transferrins.

Note added in proof. Improved separations are obtained by halving the concentration of the gel buffer. The zones of type A are then retarded so that the faster zone is ahead of and the slower zone is slightly behind the main zone of type B.

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[2] Abul-Fadl, M. A. M., and King, E. J., *Biochem. J.*, **42**, xxvii (1948).
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EFFECT OF INSTANTANEOUS FLASHES ON ADAPTATION OF THE EYE

Dark Adaptation after Exposing the Eye to an Instantaneous Flash

FLASH bleaching. Rhodopsin consists of the protein opsin combined with the 11-*cis* form of retinene. The primary effect of light is to isomerize retinene, and since the all-*trans* combination is unstable at room (or body) temperature it becomes dissociated from the opsin and results in a change in spectral absorption called 'bleaching'. The dissociation is irreversible, thus long-continued light exposure of rhodopsin in solution will eventually bleach all the rhodopsin. The situation is different if thermal dissociation is arrested by low temperature[1,2]. In this condition, however great the light exposure, only about half the rhodopsin is found to have been bleached when measured after the subsequent thermal dissociation in the dark, and the half that remains has not quite the same absorption spectrum as the original rhodopsin.

In the living eye the thermal reaction cannot be delayed by low temperature but the same result may be obtained by bleaching with a powerful flash lasting 1 msec or so during which very little thermal change can occur. Hagins[3] in this way showed in the eye of decerebrate albino rabbits that only about half the rhodopsin content of the exposed retina could be bleached by a single flash no matter how bright this was.

The elegant analysis of Hubbard and Kropf[4] in solution at low temperature explained these phenomena. Before the irreversible thermal dissociation has occurred, the all-*trans* isomer still attached to opsin (metarhodopsin) is capable of reisomerization by light to form 11-*cis* which is rhodopsin, or 9-*cis* (isorhodopsin). Excessive light can do no more than produce the photo-equilibrium mixture of rhodopsin, isorhodopsin and metarhodopsin. The proportions depend somewhat on the colour of the light, but roughly half is metarhodopsin, and one-quarter each of the thermo-stable ingredients. This accounts for the fact that only half is bleached by the subsequent dark reaction and also for the final product being somewhat different from 11-*cis* rhodopsin, for it is a mixture of 11-*cis* and 9-*cis*.

Dark adaptation. It has long been held that the insensitiveness of the eye after exposure to strong light is closely linked to the bleaching and subsequent regeneration of the visual pigments. With regard to rod vision and rhodopsin this has now been placed on a fairly satisfactory footing by two rather different types of experiment. Dowling and Wald[5] exposed albino rats to bright lights and after various subsequent periods in the dark the threshold for the electroretinogram was taken; alternatively, the animal was killed and the rhodopsin content of the retina measured. A linear relation was found between rhodopsin content and log threshold.

This same linear result was found by Rushton[6] in man between the visual threshold for rods and their rhodopsin content as measured by reflexion densitometry in the living eye. As is well known, the dark-adaptation curve of the normal human eye consists of two branches, the first due to the cones which recover faster, the second to the rods which eventually become more sensitive. A difficulty in relating rod threshold with rhodopsin regeneration in man is that the rod threshold is normally hidden by the lower cone threshold for the first 15 min or so after a full bleaching exposure, and after 15 min rhodopsin is about 90 per cent regenerated, so there is little further regeneration occurring to relate with the 100-fold change seen in the rod threshold.

The most striking evidence was in fact obtained from a 'rod monochromat' who had normal rods and rhodopsin but very few cones; in that case the rod threshold could be followed well over a million-fold range of rod sensitivity.

Dark adaptation and flash bleaching. Now in the first section it appeared that a maximum brief flash only bleaches about half the rhodopsin to the unstable meta-rhodopsin and leaves half the pigment photosensitive in the form of rhodopsin and isorhodopsin. If isorhodopsin acts like rhodopsin in vision we might therefore expect from section 2 that dark adaptation, following a full flash bleach, will give the same result as that following a prolonged bleaching light that removed only half the rhodopsin.

Westheimer and I[7], working on rod dark-adaptation curves following flash bleachings, were surprised to find that the curve following a maximal flash (7 msec duration) was the same as that following a sustained exposure of 30 sec duration, also maximal in the sense that half that intensity lasting 30 sec gave the same dark-adaptation curve. The curves would be expected not to coincide but to run one about 4 min earlier than the other, which was certainly not the case. We had no densitometer at Berkeley and I have taken up the matter again at Cambridge with Dr. P. E. Hallett (Oxford) and Dr. H. D. Baker (Florida) as subjects.

Experiment. The results of bleaching with a bright flash (4 msec) or a 30-sec exposure were measured in a single experiment by bleaching in quick succession two retinal regions 5° apart, both 15° from the fovea. Just before and 15 sec after the bleach the change in rhodopsin density was measured at each retinal region. In confirmation of Hagins it was found that the 30-sec exposure produced about twice as much bleaching as did the flash. Dark-adaptation curves were then taken with a 2° test flash exposed for 0·25 sec each sec. The 2° test circle was half yellow and half blue of such relative intensities that they matched by rod vision and appeared a uniform grey circle. But by cone vision near threshold it appeared a yellow semicircle. It was thus easy to tell when the rod branch of the dark adaptation curve was reached.

Fig. 1 shows the rod branch of a dark-adaptation curve typical of many that have been obtained both from Dr. Hallett and from Dr. Baker. The black circles correspond to the results of flash bleaching, the white circles to a bleaching exposure of 30 sec. Since the latter light was extinguished 30 sec after the flash, the white circles in Fig. 1 have been displaced half a minute to the left so that both sets of points represent correctly the time that has elapsed from the end of the bleaching exposure. The coincidence is perfect and speaks for the skill of my two observers.

Discussion. Judged by reflexion densitometry a maximum flash only bleaches about half as much rhodopsin as is bleached by 30 sec of a maximum exposure; nevertheless the resulting rod dark-adaptation curves are exactly the same in the two cases. This is unexpected and some of our views about the course of pigment regeneration or its relation to dark adaptation will need enlargement.

One relation seems a simple starting point. Both the flash and the 30-sec exposure were maximal in the sense

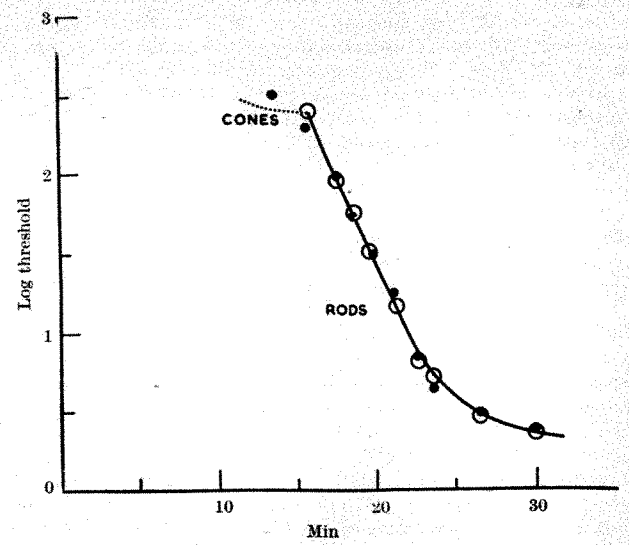


Fig. 1. Dark adaptation curve of rods (○) following a 30-sec exposure which appears to bleach away all the rhodopsin; (●) a 4 msec flash which appears to bleach only half. Cones are more sensitive than rods for the first 16 min. (Subject P. E. H.)

that doubling the intensity produced no further rise in rod threshold. Now this upper limit of threshold was in fact the same in the two cases so perhaps each light was able to push to the limit whatever change it is that causes the rod threshold to rise after bleaching. Unfortunately, however, it is not easy to reconcile this idea neatly with the known relation between threshold and the regeneration of rhodopsin.

A difficulty with rod adaptation in man is that following a maximal bleaching light we have to wait 15 min before rods are sensitive enough for their thresholds to fall below the cones and become measurable. If following the bleaching flash, rhodopsin regenerated more slowly than following the 30-sec exposure, perhaps 15 min later both retinal regions would be at about the same state of regeneration and thereafter march together.

This did not seem to me likely, but I had no knowledge of the isomers of retinene and their effect on regeneration, so I asked the experts, Hubbard and Dowling, when I visited Harvard recently. Their answer has been a series of excellent experiments on the rat which are described in the following communication. If in man the regeneration of rhodopsin follows the pattern they have shown so clearly in the rat it would account for our results. But with human rods I think that this is not the case, and with human cones I know that this is not the case.

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Effects of Brilliant Flashes on Light and Dark Adaptation

Several years ago, Hagins[1] reported that a flash of light, no matter how intense, could not bleach more than about half the rhodopsin in a dark-adapted rabbit's eye, provided that it lasted no longer than 1 msec. This observation, though difficult to explain at the time, is a

direct consequence of the way in which light acts on rhodopsin[2,3].

Rhodopsin contains 11-*cis* retinal (formerly retinene)[4] attached as chromophore to a colourless protein, opsin. Light isomerizes the chromophore, predominantly to the all-*trans* configuration, and thereby produces a series of unstable intermediates: pre-lumirhodopsin, lumirhodopsin, metarhodopsin I and metarhodopsin II. These differ widely in their absorption properties, but all contain all-*trans* retinal still attached at the chromophoric site on opsin[5–7]. Finally, metarhodopsin II hydrolyses to all-*trans* retinal and opsin, and *in vivo* this is followed by the reduction of retinal to retinol (formerly vitamin A)[4].

The crucial point for Hagins's observation is that light isomerizes the chromophores of any of the all-*trans* chromoproteins—that is, the intermediates in which all-*trans* retinal is still at the chromophoric site—back in part to the 11-*cis* configuration, thus regenerating rhodopsin, and in part also to the 9-*cis* configuration, forming the rhodopsin analogue, isorhodopsin. In other words, any light which is bright enough to deliver large numbers of quanta within the relatively brief life-times of the unstable intermediates, isomerizes a fraction of them to rhodospin and isorhodopsin, and this fraction remains as a thermally stable but photosensitive residue, when the all-*trans* fraction has been hydrolysed to retinal and opsin (cf. refs. 8 and 9). No more photopigment (rhodopsin + isorhodopsin) can be bleached by increasing the intensity of a flash. What is now needed is time for the thermolabile all-*trans* chromoprotein to hydrolyse. As hydrolysis proceeds, fewer and fewer chromophores are available to absorb the light and be isomerized by it, until eventually all the rhodopsin and isorhodopsin have been converted to retinal and opsin. How much time this requires depends on the light intensity and the temperature. The former determines the rates of the photo-isomerizations (initially from 11-*cis* to all-*trans*, but shortly also from all-*trans* to 11-*cis* and 9-*cis*); the latter, the rate of hydrolysis.

In the preceding communication Rushton has outlined the paradox which arises from these reactions. He finds that following an intense, short flash, the course of human rod dark adaptation is essentially the same as after a longer exposure to bright light, which bleaches about twice as much photopigment. This is surprising, for ordinarily bleaching more photopigment produces a higher state of light adaptation, and therefore prolongs dark adaptation. Many years ago, Wald and Clark observed that following a photoflash of 20–40 msec duration, human dark adaptation was very slow, as though from a high state of light adaptation[10]. (A photoflash leaves a residue of about 35 per cent photoregenerated rhodopsin + isorhodopsin.) The inconsistency between these results and Hagins's discovery of the low efficacy of flashes of light to bleach rhodopsin, however, went unnoticed until Rushton juxtaposed the two types of observation in one experiment.

After Rushton told us about his observations (personal communication), we decided to examine this phenomenon in the albino rat, which has the advantage over most other vertebrates that its electroretinogram (ERG) behaves as though it had an all-rod retina, though its retina is known from anatomical studies to contain a few cones. In the albino rat one can therefore observe the entire course of rod dark adaptation as well as of rhodopsin formation[11,12]. We have light adapted our animals with a xenon flash gun ('Ultrablitz Meteor *SP*') held at a distance of about 4 in. from the eye. The flash has an intensity rating of about 80 W sec delivered in 1 msec; but we measure a peak in intensity at about 0·7 msec. after which it decays with a half-time of about 2·5 msec. Visual thresholds are determined by means of the ERG; photopigment concentrations by direct extraction[11,12].

A single flash delivered by our flash gun leaves the eye of the albino rat with about 35 per cent photopigment, with λ_{max} 496 mμ. The λ_{max} of rat rhodopsin is at 498 mμ, and that of isorhodopsin is shifted about 12 mμ to shorter wave-lengths. The photoregenerated pigment therefore contains roughly a 5 : 1 mixture of rhodopsin and isorhodopsin.

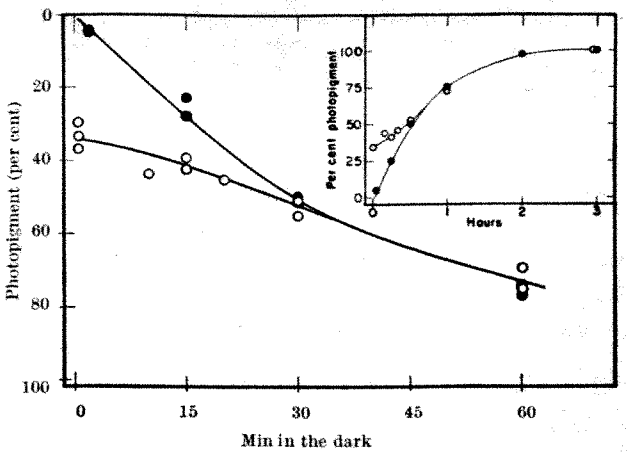


Fig. 1. The course of rhodopsin formation in the albino rat following light adaptation by a xenon flash gun (open circles) or by 30 min exposure to bright white light (filled circles). Prior to light adaptation with the flash gun, each animal was lightly anæsthetized with ether. The two eyes were light adapted successively, and each experimental point represents the results obtained with the two eyes of one animal. Unless the eyes were to be enucleated immediately on light adaptation, the animals were allowed to recover in the dark, and were anæsthetized with ether and nembutal just before removing the eyes. For the prolonged light adaptation, untreated animals were kept in a white enamel pan, and observed periodically to prevent them from shielding their eyes; they were then anæsthetized deeply just prior to enucleation of the eyes. Rhodopsin was prepared as described in ref. 12, and its concentration expressed as the percentage of photopigment found in the completely dark-adapted eyes (see inset). Experimental points in the inset are averages; in the main part of the figure, they are results obtained with individual animals. As can be seen, these are reasonably reproducible among different animals

Fig. 1 illustrates the way in which rhodopsin accumulates in the dark in animals light adapted by a single flash (1 msec) (open circles). The concentration of photopigment increases slowly during the first 20–30 min, so that after about 30 min in the dark, the flash-adapted eye (open circles) contains no more photopigment than an eye in which essentially all the rhodopsin had been bleached by prolonged adaptation to bright light (30 min) (filled circles). Thereafter, rhodopsin accumulates at about the same rate following both types of adaptation. (Both rhodopsin and isorhodopsin are formed in the light; but only rhodopsin is regenerated in the dark.) In other words, following a flash, the course of rhodopsin synthesis is S-shaped, and joins the dark-adaptation curve from a much higher state of prolonged light adaptation after about 30 min in the dark (see inset to Fig. 1). It therefore takes a flash-adapted eye as long to recover completely as one in which much more rhodopsin had been bleached away by prolonged light adaptation.

The ERG threshold recovers in a similar way. As shown in Fig. 2, a single flash of light (1 msec) raises the threshold about 3·75 log units above the dark-adapted level (open circles). (The threshold is measured 1 min after the flash, after the bulk of the initial rapid changes of threshold —presumably due to the neural component of dark adaptation—are completed[12]). This rise of threshold corresponds roughly to a rhodopsin concentration of about 35 per cent. Then, during the first 20–30 min in the dark, the threshold falls very slowly, so that after about 30 min in the dark the dark-adaptation curve of the flash-adapted eye joins that of an eye which has been light adapted to a much higher threshold by prolonged exposure to bright light (5 or 10 min) (filled circles).

Also shown in Fig. 2 is the dark-adaptation curve measured after prolonged adaptation to a dimmer light, which raises the threshold only about 3 log units above the dark-adapted level (half-filled circles). In this case, the threshold recovers in much the same way as after prolonged

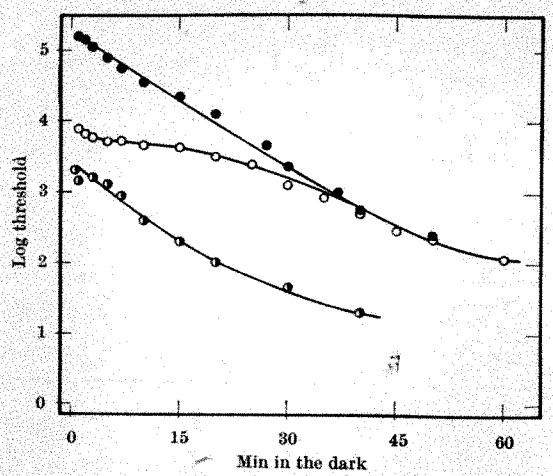


Fig. 2. Recovery of visual sensitivity following light adaptation with a xenon flash gun (open circles) or to bright white light for 5–10 min (filled circles). The course of dark adaptation after 5 min adaptation to white light of 1/10 the intensity is shown by half-filled circles. The animals were light adapted as described in Fig. 1, except that all animals were anæsthetized with nembutal (5 mg/100 g body-weight) during the entire course of light and dark adaptation, and allowed to recover at the end of the experiment. The ERG thresholds were determined as described in ref. 12

adaptation to the ten times brighter light, along an entirely different course from that observed after a flash.

In summary, a short, brilliant flash, which photo-regenerates about 35 per cent photopigment, raises the threshold to a level which corresponds roughly to the presence of that amount of visual pigment. Following the flash, rhodopsin accumulates very slowly during the first 20–30 min in the dark, and there is a correspondingly slow fall of threshold. After about 30 min in the dark, both functions join the recovery curves from a much higher state of prolonged light adaptation.

These observations agree with Rushton's, and serve to resolve them. In man, the initial stages of rod dark adaptation are obscured by the lower thresholds of the cones, so that one sees only the final coincidence of rod dark adaptation following a flash and following prolonged adaptation to bright light.

One reason why the flash causes an initial delay in rhodopsin formation is clear from what has already been said. For the very fact that the flash causes photo-regeneration implies that the quanta are delivered within the life-times of the all-*trans* chromoproteins, that is, before retinal has left the chromophoric site. Immediately after the flash, that fraction of opsin molecules which does not already possess an 11-*cis* or 9-*cis* chromophore, therefore cannot combine with 11-*cis* retinal until the all-*trans* isomer hydrolyses off the chromophoric site. It is easy to demonstrate a competition between the hydrolysis of metarhodopsin and the formation of rhodopsin in solution, that is, the competition between all-*trans* retinal on the site and 11-*cis* retinal seeking to occupy it. The presence of an excess of 11-*cis* retinal accelerates the disappearance of metarhodopsin I; and conversely, rhodopsin is regenerated more slowly if 11-*cis* retinal is added immediately after it has been bleached, than if it is added 2 h later, when all the all-*trans* chromoprotein has been hydrolysed in the dark.

It has sometimes been suggested that the retina may possess enzymes which isomerize one or another of the labile intermediates back to rhodopsin, while the all-*trans* chromophore is still attached to the chromophoric site[13]. This type of isomerization reaction almost surely takes place in lobsters, squid and other cephalopods, in which retinal apparently never leaves the chromophoric site *in vivo*[14–16]. The experiments described here show that this reaction does not occur in rats or men during dark adaptation. Nor does it seem to occur in frogs, where Zewi many years ago observed a delay in rhodopsin formation, precisely under conditions comparable with those delay it in the present experiments: namely, follov brief exposures to bright light, particularly at low temp ture[17]. It is therefore likely that all vertebrates regene rhodopsin in the dark only from free retinal and ops

This introduces a second reason for the initial dela rhodopsin synthesis. For if rhodopsin must be for from 11-*cis* retinal and opsin, the availability of 11 retinal is crucial to the rate of regeneration. The all rat, when dark adapted, contains little free ret or retinol[18]. The hydrolysis of the all-*trans* intermedi therefore not only clears the chromophoric site on op but is the main source of retinal or retinol for isomer tion to the 11-*cis* configuration and rhodopsin synth This factor does not have the same force in frogs a albino rats, since frogs store considerable retinol in t eyes, and some in the 11-*cis* configuration[19].

The length of the initial delay is necessarily also affec by the rate at which opsin combines with 11-*cis* reti This depends on the affinity of opsin for retinal, as as on their respective availabilities. The affinity is kn to differ in rod and cone opsins from the same anim and also among rod opsins from different animals[21]. I therefore impossible beforehand to predict the extent the delay in a specific animal or experimental situati

In general, one would expect it to be more pronoun in the albino rat than in most other warm-bloo vertebrates, since rhodopsin formation in the rat is v slow; much slower, for example, than in man[11,18,22]. the albino rat, we observe an initial slowing of d adaptation even after adaptations to bright lights last up to 1 min, though the lag under these circumstan lasts only 5 or 10 min (cf. Fig. 4, ref. 13). No delay usually seen after light adaptations of more than 5 m even if these are produced by repeated flashes, rather t by a steady bright light. As seen in Fig. 3, it takes li plus time in the dark to abolish the delay, just as both required to bleach all the photopigment. The two effe go hand in hand, since both are caused by the persiste of all-*trans* retinal on the chromophoric site.

The open circles in Fig. 3 summarize experiments which the albino rat has been exposed to successive flas delivered 2–5 min apart, thresholds being measured min after each flash. The first flash raises the thresh

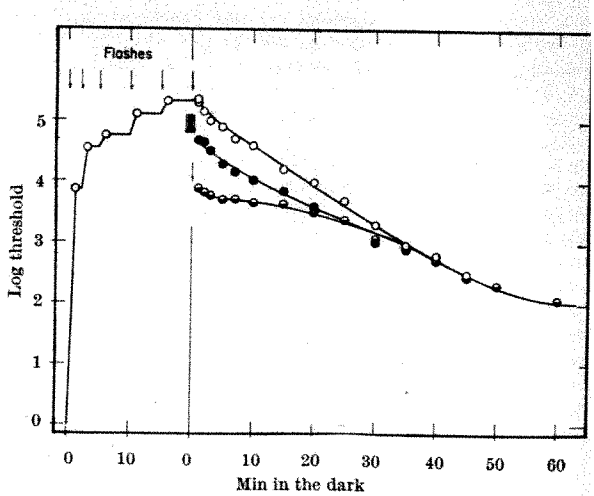


Fig. 3. Effectiveness of short, brilliant flashes to light adapt the eye, and the subsequent course of dark adaptation. The eyes of anæsthetized albino rats were exposed to flashes from a xenon flash gun, and the ERG threshold measured as described in ref. 12. Open circles are averages from three experiments, in which the eye was exposed to six flashes of light delivered in the course of 20 min at the times shown by the arrows, the threshold always being determined 1 min after the flash. Filled circles are averages from five experiments, in which the eye was exposed to the same number of flashes, all delivered within 1 min. The half-filled circles, averages from six experiments, show the results of exposing the eye to a single flash (cf. Fig. 2). The course of dark adaptation in each case is shown only for the first hour, by which time all three curves coincide. But dark adaptation is not complete until the animals have been in the dark about 3 h (cf. Fig. 1)

by about 3·8 log units (cf. Fig. 2), the second about 0·7 log unit further. Succeeding flashes raise it less and less, until it is raised about 5·3 log units, corresponding to a rhodopsin content of 1–2 per cent[12], and cannot be raised further. This takes about 6 flashes delivered over a period of 10–15 min.

If 6 flashes are delivered within 1 min (Fig. 3, filled circles), the threshold is raised only about 4·7 log units, about the same level as after only 2 flashes spaced 1–2 min apart. In other words, 6 flashes bleach no more rhodopsin than 2 flashes, unless they are spaced sufficiently widely apart to let some of the all-*trans* chromophore be hydrolysed from opsin. In both cases, recovery follows an intermediate course between that observed after a single flash (Fig. 3, half-filled circles), and that seen after complete bleaching by 6 or 7 flashes spread over 15 min; and all three curves coincide roughly after about 35 min in the dark.

We have measured the rise in visual threshold also during adaptation to continuous bright light. In the albino rat, the brightest lights we have tested cannot raise the threshold more than about 4·5 log units during the first min, the same as 2 or 6 flashes spaced over 1 min. This probably represents the limit imposed by the rates of the relevant thermal reactions at body temperature. As might be expected, all these lights produce a similar initial lag in dark adaptation. The time required to dark adapt completely is again the same as in rats the rhodopsin of which has been bleached completely by prolonged light adaptation.

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CRYSTALLIZATION OF ω-DICARBOXYLIC ACIDS IN POLYMER FILMS

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IT is well known[1] that the environmental conditions under which crystal growth occurs can have a marked influence on the external visible shape of certain crystals. This effect is manifested in two ways. In single crystals the crystal habit may vary either because the growth develops on different crystal planes or because the relative development of the same set of faces differs. Alternatively, when the entities actually observed are not single crystals, but are aggregates (for example, spherulites) consisting of crystalline sub-units such as crystallites or fibrils, the visible shape of the aggregates will be determined by the size and orientation of these sub-units. This applies especially to polymeric materials.

A number of unusual crystalline modifications, resulting from particular conditions of growth, have been reported from time to time. Thus, there are many references to changes of habit in single crystals of simple inorganic salts[2–7]. Rarer forms such as curled platelets in paraffin wax have been observed by Edwards[8], and helical structures have been seen in micro-crystals of certain soaps[9] and in sublimed zinc sulphide[10].

Irregularly branched dendritic crystalline aggregates occur in many systems[2]; usually under conditions where a high degree of super-cooling or super-saturation can be achieved. Spherulitic crystalline aggregates, first observed in certain minerals, are now frequently found in melt crystallized polymers[11] (though polymer single crystals[12] can also be grown from very dilute solutions). However, many simpler organic substances, for example, sucrose[13], resorcinol[14], malonamide[15,16], and hydroquinone[15], and the many inorganic salts studied by Morse and Donnay[17], all of which are normally grown from solution in the form of single crystals, can also be made to crystallize in spherulitic form if the experimental conditions are suitably chosen.

The purpose of the present communication is to report microscope observations of a wide range of birefringent crystalline forms, and notably spherulitic forms, which appear in solution-cast polymer films containing ω-dicarboxylic acids. The work stems from the adventitious inclusion of oxalic acid in an aqueous dispersion of pectic acid which when dried out exhibited spherulitic and dendritic structural features. Similar forms were subsequently observed in films of poly (vinyl alcohol) and poly (vinyl chloride) containing ω-dicarboxylic acids and these observations therefore provide a further example of the effect of environment on crystal growth.

The pectic acid was extracted from Bright or Connecticut Broad Leaf tobacco stems by the methods of Masters and Richards[18]. Poly (vinyl alcohol) was supplied by L. Light and Co., and poly (vinyl chloride) as compound '*RA*124' from British Geon, Ltd. Adipic and sebacic acids used were laboratory grade reagents recrystallized from aqueous solution and oxalic acid was Analar grade.

In the earlier experiments pectic acid, partially neutralized with sodium hydroxide and containing about 5–10 per cent oxalic acid and 30–50 per cent glycerol, was used. The films were cast from aqueous solution at 20° ± 1° C and 65 ± 2 per cent relative humidity. Birefringent structures began to form in the films during the later stages of removal of water and the structures often continued to grow for several days. In subsequent experiments both casting temperature and concentration of oxalic acid were systematically varied and the glycerol was omitted. The films gave a crystalline X-ray diffraction pattern,

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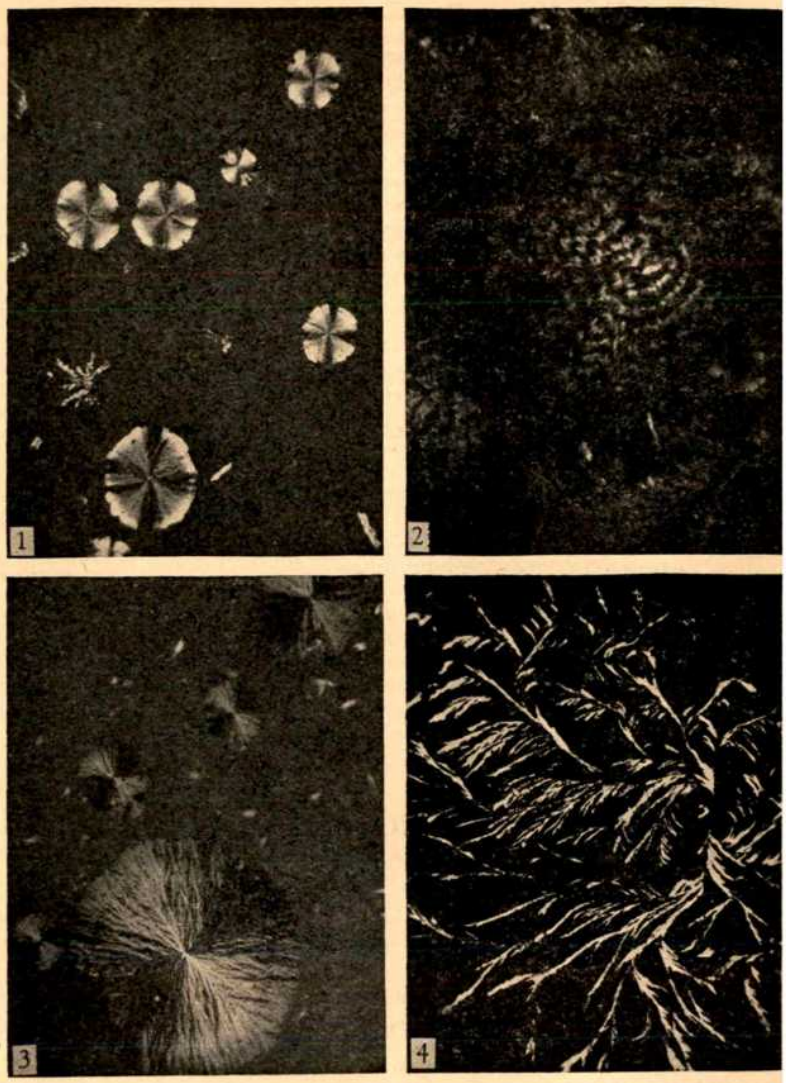
dependent on the extent of neutralization (cf. ref. 18). Crystalline diffraction was also observed in the absence of oxalic acid, although such films were completely non-birefringent.

Several different types of birefringent entities, namely, spherulites, spheruloids and dendritic formations were distinguished in the different films and certain trends are apparent in the type of structure formed. For example, as the casting temperature was increased entities became less complex and more numerous and at 75° C extensive nucleation occurred producing a very grainy birefringent appearance throughout the film. A similar effect was noted with increasing neutralization of the pectic acid. No systematic influence of glycerol content on the structures could be detected. Particular forms could not be identified with films of particular oxalic acid content when the latter was varied from 5–20 per cent, and it is evident that local concentration fluctuations and conditions are more important than the overall ratio of pectic to oxalic acid. Although birefringent structures formed during the later stages of the evaporation of solvent, controlled evaporation in enclosures of constant but different relative humidity did not appear to lead to the production of specific forms. Very rapid removal of water in high vacuum, however, generally caused less dense, highly branched dendritic growths. On standing for 2–3 days at 20° C, 65 per cent relative humidity, dendritic structures in thin films cast from 0·5 per cent solution coalesced to form well-defined single crystals of oxalic acid.

The phenomenon is not specific to the pectic acid/oxalic acid/water system. Thus in films cast under ambient conditions from 10–20 per cent (w/w) poly (vinyl alcohol)/water solutions containing oxalic acid a similar wide range of dendritic and spherulitic structures was produced. Adipic acid and sebacic acid were also shown to be effective and spherulites of the latter could be grown within the 20 per cent poly (vinyl alcohol) solution. These entities when subjected to small shearing stress yielded radically cleaved fragments. Sodium oxalate usually produced only single crystals in poly (vinyl alcohol) films, but these sometimes appeared in the form of dendritic or spherical agglomerates.

Solution cast films of pure poly (vinyl alcohol) are partially crystalline but are not birefringent. However, poly (vinyl chloride) is neither crystalline nor birefringent and this polymer was also found to provide a medium in which ω-dicarboxylic acids would crystallize in birefringent and spherulitic forms, in this case from *cyclo*hexanone solution. A similar observation has been made recently by Schuur[19].

Examples of typical structures in the systems studied are shown in Figs. 1–4.



		Casting solution	Film thickness (mm)	Scale (field diameter, mm)
Fig. 1.	Spherulites	16 per cent w/w poly (vinyl alcohol)/water. Oxalic acid 30 per cent	0·05	1·0
Fig. 2.	Ringed spherulites	0·4 per cent w/w pectic acid/water; *p*H = 3·6. Glycerol 40 per cent, oxalic acid about 10 per cent	0·05	1·0
Fig. 3.	Spheruloids	20 per cent w/w poly (vinyl alcohol)/water. Oxalic acid 20 per cent	0·05	1·8
Fig. 4.	Dendritic structure	0·3 per cent w/w pectic acid/water; *p*H = 3·6. Glycerol 50 per cent, oxalic acid about 10 per cent	0·01	2·4

Solution *p*H adjusted using 5 N sodium hydroxide solution. Plasticizer and dicarboxylic acid content expressed as percentage dry weight of polymer. All samples were viewed between crossed polaroids, the axes of which were parallel to the edges of the photographs

Numerous, but unsuccessful, attempts have been made to induce the low-molecular-weight dicarboxylic acids to crystallize in other than the normal crystal form without added polymer. Dendritic oxalic acid crystals have been grown when thin layers of a dilute solution were allowed to evaporate, but the dendrites appear to be agglomerates of single crystals. Single crystals were also obtained from organic solvents such as acetone, ethanol, or glycerol, without any obvious change in the habit of the acid crystals. One notable exception however was the production of long needles (10–20 mm × about 1 mm diameter) of adipic acid from a saturated solution in xylene.

Since a similar wide range of crystalline forms is observed in films of polymers which are different chemically and which are either crystalline or non-crystalline, with the ω-dicarboxylic acids the only common factor, it seems reasonably certain that these are structures of the ω-dicarboxylic acids themselves.

From saturated aqueous solution, however, the lower dicarboxylic acids appear in the form of single

crystals, usually short monoclinic needles, so that the polymers present in the system studied here evidently exert a controlling influence over the crystallization process.

It is generally accepted that crystallization proceeds via consecutive nucleation and growth steps and in so far as single crystal growth is concerned the mechanism is quite well understood. Now it would not be unreasonable to suppose that the observed structures stem from single crystal nuclei. We must, therefore, conclude that the number of effective nuclei is limited and that the subsequent development is also modified by the polymer.

There are many instances of the supression of homogeneous nucleation in media of high viscosity. Glasses are often formed for just this reason. Again, certain polymeric materials, which normally exist in a crystalline form, may exist indefinitely in a non-crystalline state if quenched from the melt to temperatures where the high viscosity hinders the formation of crystal nuclei. Concentrated aqueous solutions of sucrose[13] may be maintained for long periods at high degrees of super-saturation without crystallization taking place, and it has been stated[16] that nucleation is also greatly diminished in melts of low molecular weight materials, for example, malonamide and resorcinol, to which certain colloids have been added to increase the viscosity.

It is now suggested therefore that, in the solutions and films under consideration here, the viscosity is so increased by the presence of the polymer as to reduce greatly the rate of formation of effective crystal nuclei of the dicarboxylic acids.

If limitation of nuclei were the only effect of the polymers on crystallization of the dicarboxylic acids, however, the result would be to prevent their crystallization or to produce a few large 'normal' crystals. We must therefore seek factors which favour spherulitic and dendritic forms of growth.

The ability to form long needle-shaped crystals or fibrils is an important property in this respect and very probably a necessary requirement for the production of dendritic and spherulitic structures. The lower ω-dicarboxylic acids adopt an acicular habit when grown from aqueous solution and this inherent tendency is almost certainly encouraged under the present conditions. For example, the preferential growth of 'thin stemmed' crystals has long been known to be favoured in a medium of high viscosity[20]. Furthermore, similar though apparently specific effects on crystal habit have been noticed in the presence of relatively small concentrations of added colloidal substances or polymers. Among the latter, and of particular significance, is the effect of pectic acid[6] or poly (vinyl alcohol)[7] in promoting the formation of fibrous or needle-shaped crystals from aqueous solutions of inorganic salts.

The ultimate formation of dendritic or spherulitic structures from a needle-shaped nucleus or a bundle of needles must, however, depend on a twinning or branching process. This would lead to intermediate sheaf-like forms composed either of fibrillar single crystals or fibrillar aggregates of crystallites. Spherulite precursors of just this shape have indeed been observed in the present work. Subsequent growth would then develop to give the large-scale aggregates with more or less spherical symmetry exhibiting a radially oriented fibrous fine structure. With one exception[19] this type of mechanism forms the basis for the hypotheses proposed to account for spherulite growth and in general terms at least has proved adequate. Keith and Padden[21] have recently presented a unified theory of spherulite growth which is dependent on the presence of more than one component on high viscosity and on slow crystal growth. All these factors are present in our systems, and, furthermore, dendritic and spherulitic crystalline aggregates appear in the same film. It is likely, therefore, that the growth mechanisms of the two forms should not be too different and that both could arise from branching or twinning of needle-shaped crystals as outlined above.

Dendrite formation occurs more frequently where crystallization is able to occur rapidly, as in the very thin films or when solvent is removed rapidly under high vacuum. Open, irregularly, branched structures are apparently then a consequence of a high degree of supersaturation and rapid growth. This is consistent with the generalization of Buckley[2] on factors favouring dendritic growth.

The spherulites, on the other hand, have grown slowly. They are more condensed structures and evidently consist of a more symmetrical arrangement of crystalline sub-units, as is revealed by the extinction cross (Fig. 1) or rings (Fig. 2) produced when these entities are viewed between crossed polaroids. Their resemblance to spherulites often seen in melt crystallized polymers is striking and suggests the following explanation for the wide variety of crystalline forms observed here.

The acicular crystals of the lower ω-dicarboxylic acids consist of arrays of linear chains of molecules linked together head to tail through hydrogen bonding between carboxyl groups[22]. In addition, it is frequently found that the molecular chain axis lies perpendicular to the long axis of the crystals, a fact which is consistent with a growth mechanism involving lateral accretion of chains or chain segments. It is possible, therefore, that molecular associations of this type to form linear chains precedes crystallization in the systems investigated here. This would then lead to the analogy with melt crystallized polymers, the various crystalline forms possibly arising from a range of chain lengths which determine the mobility of the crystallizing species. Observations of dendritic (herring-bone) spherulites in low molecular weight polypropylene[19] and in a low molecular weight fraction of polyethylene[23], support the proposal that chain length may be an important factor in determining spherulite fine structure.

It is not suggested that linear chains of molecules are always necessary before crystallization can occur in spherulitic form. Indeed, the work of Morse and Donnay[17] describing ringed spherulites in a wide range of inorganic substances of different chemical types shows that such a generalization is unwarranted. The results described above, however, indicate that the unusual crystalline habits of these dicarboxylic acids are probably due to both linear intermolecular association and increased viscosity due to the base polymer, these two effects being interrelated.

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POSSIBLE TRANSITION IN THE INITIATING MECHANISM LEADING TO ELECTRICAL BREAKDOWN IN VACUUM

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MANY experiments have been carried out to determine the initiating event leading to electrical breakdown between metallic electrodes separated by high vacuum[1,2]. While the mechanism causing breakdown in certain cases, such as when using sharply pointed cathodes in extremely clean conditions, is now understood, the more practical case of plane-parallel electrodes with mechanically polished and oxidized surfaces is still under active investigation[3].

Of the mechanisms thought to cause breakdown, those that have received most attention are:

(*a*) those in which discrete beams of electrons, from small areas or asperities on the cathode, bombard areas on the anode. Resistive heating effects at the cathode or bombardment effects at the anode cause localized rises in temperature and the release of gases or vapours in which low pressure gas breakdown can occur;

(*b*) those in which the transport across the gap of aggregates of material, or clumps, and their resultant impact on the opposite electrode gives rise to heating and hence to the release of vapour.

The Electron Beam Mechanism. The mechanism of electron beams causing anode heating was first suggested by Semenov[4] and has been supported by the comprehensive experimental and theoretical work of Maitland[5,6] and by others. Maitland showed that craters were produced on the anode during the breakdown process and he postulated that these were due to the existence of a multiple system of electron beams in the gap initiating breakdown. That copious electron emission occurs from metallic electrodes, having mechanically polished and oxidized surfaces, at the stresses noted by the foregoing investigators (in the region of 5×10^5 V/cm) has recently been confirmed[7].

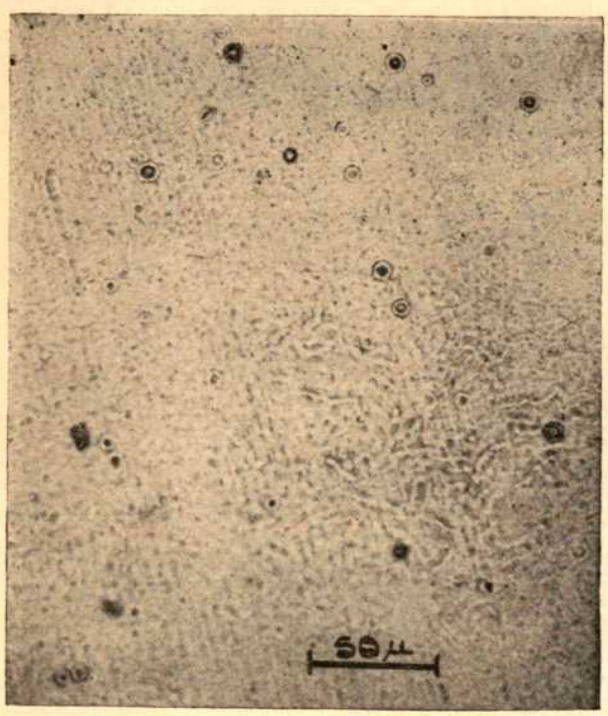


Fig. 1. Pre-breakdown markings on anode of commercial copper. Gap = 1 mm

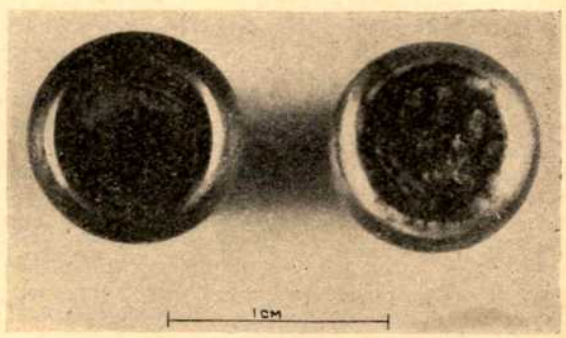


Fig. 2. Markings after ten breakdowns between copper electrodes in a 1-mm gap. Breakdown voltages = 30–40 kV

Hawley and Walley[8] attempted to see if evidence of the presence of clumps could be obtained, in gaps up to 0·75 mm, by observing pre-breakdown surface effects at copper anodes through a microscope. Though embedded inclusions were torn from the anode they did not initiate breakdown on crossing the gap. In addition, 'spots' sometimes appeared on the anode prior to breakdown, at stresses of about $4–6 \times 10^5$ V/cm. More recent analysis of these spots, using an interference microscope, has shown they are craters (Fig. 1) similar to those reported by Maitland[6], consisting of a pip in a central depression surrounded by a raised rim; and so it can be assumed they are caused by electron beam heating.

The maximum gap studied by those investigators postulating an electron beam mechanism is 2 mm. The bigger the gap the larger the area bombarded at the anode by the divergent electron beam, so the greater the input power at the surface required for breakdown. However, Trump and Van de Graaff[9], using gaps of 0·25 mm to 70 mm, showed that as the gap is increased the cathode gradient (where gradient is taken as voltage divided by gap and no account taken of intensification factors due to the surface condition) required for breakdown decreases rapidly so that the electron emission must also decrease (also see ref. 10). This is the opposite to what would be expected if the electron beam mechanism were applicable to all gaps.

The Clump Mechanism. In small gaps a clump may possess insufficient energy to cause breakdown due to it being accelerated by a low voltage. In longer gaps, because of the higher voltage required to produce breakdown, the clump energy would be correspondingly increased and a clump mechanism may be possible. Thus as the gap is increased this mechanism may take preference over an electron beam mechanism.

One objection to a clump mechanism is that as the electrical stress required for breakdown falls with increasing gap, so also must the electrostatic forces available to remove a clump from the surface of the electrodes. However, studies of electrode surfaces after breakdown in 20 mm gaps[11] have shown that clumps can exist even at

stresses as low as 4×10^4 V/cm though it was not possible to show that clumps were responsible for the actual initiation of the breakdown. This has long been an experimental difficulty. Since the existence of clumps is proved, one approach would be to study the characteristics of the breakdown mechanism, in gaps of increasing size, to see if evidence of a transition in the initiating event can be found. This can be done by examining the markings on the electrodes after breakdown has taken place and also by noting the shape of the breakdown voltage-gap curve.

Evidence of a Transition. Studies of the breakdown markings in small gaps (up to 2 mm) have shown that the electron beam mechanism is characterized by craters on the anode surface[6,12]. With similar conditions and using uniform field electrodes, 1 cm and 2·5 cm in diameter and gaps up to 5 mm, the markings occurring after ten breakdowns were studied. Direct voltages were used, the voltage level being gradually increased until breakdown occurred. After ten such voltage applications the electrodes were removed, examined, repolished to a 1μ diamond paste finish and replaced in the cell ready for the next test. Fig. 2 illustrates the gross markings, on commercial copper electrodes, that were typical for gaps of 0–1 mm. Each gross mark on the anode contained craters (Fig. 3). In 2-mm gaps the number of craters and the severity of the gross markings were reduced while in 3-mm and larger gaps the character of the markings had changed completely. At the anode no gross markings or craters were found. This difference in the markings could therefore be taken as evidence of a change in the breakdown mechanism.

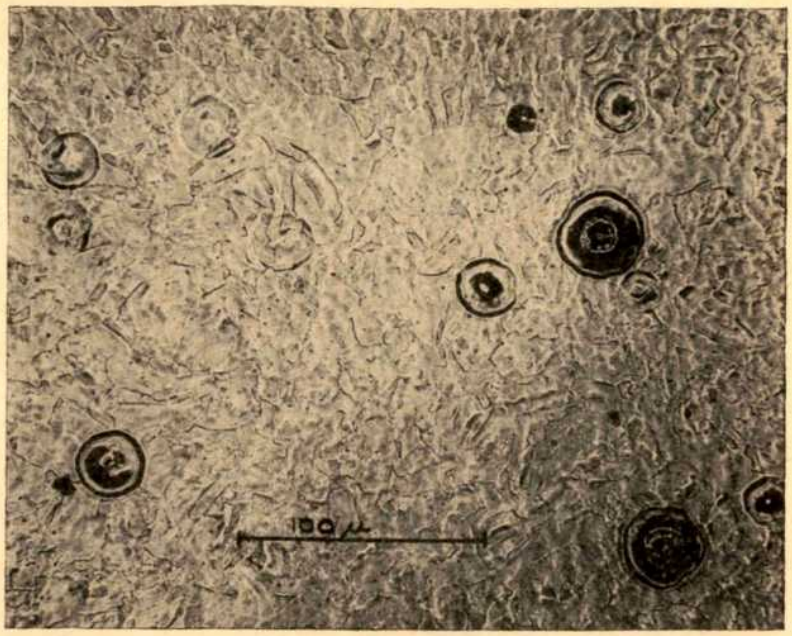


Fig. 3. Melting and cratering effects on anode of commercial copper. Gap = 1 mm. Breakdown voltages = 30–40 kV

An empirical relationship between the electrode spacing and the breakdown voltage is:

$$V = kd^{\alpha} \qquad (1)$$

where V = breakdown voltage
d = gap
k and α = constants, depending on experimental conditions

The Maitland hypothesis[6], based on electron beams, predicts $\alpha = 0{\cdot}8$, while the Cranberg[13] and Slivkov[14] hypotheses, based on clumps, predict $\alpha = 0{\cdot}5$ and 0·625 respectively. Therefore, another way to detect any change in the breakdown mechanism is to obtain the breakdown voltage-gap curve and see if a change occurs in the value of α with increase in gap.

Once a breakdown has taken place in a vacuum gap it is possible that the arc, by causing melting, etc., could give rise to the formation of clumps at the electrode surface. Also the value of α has been shown by Maitland to depend on the previous number of breakdowns in the gap[15]. Therefore to avoid variation of α due to experimental procedure and also to avoid the possible formation of clumps by the arc, values of the first breakdown voltages have been obtained between freshly polished uniform field electrodes[2]. For gaps below 5 mm, 2·5-cm diameter electrodes and direct voltages were used, for 5 mm and greater gaps, 7 cm diameter electrodes and negative 1/50 wave impulse voltage were used. In all cases the vacuum was of the order of 10^{-5} torr, and after each breakdown the electrodes were removed and repolished to a 1μ diamond paste finish.

The magnitudes of the first breakdown voltages obtained in this manner for stainless steel and for copper electrodes are given in Fig. 4, the slope of the curves being α. There is an apparent change in α at 17 mm for stainless steel electrodes and at 7 mm for copper electrodes (Table 1). Slivkov[14] measured the breakdown voltages

Table 1. VALUES OF α

Electrode material	Values of α	
	Small gaps	Large gaps
Stainless steel	0·58	0·19
Copper	0·53	0·29

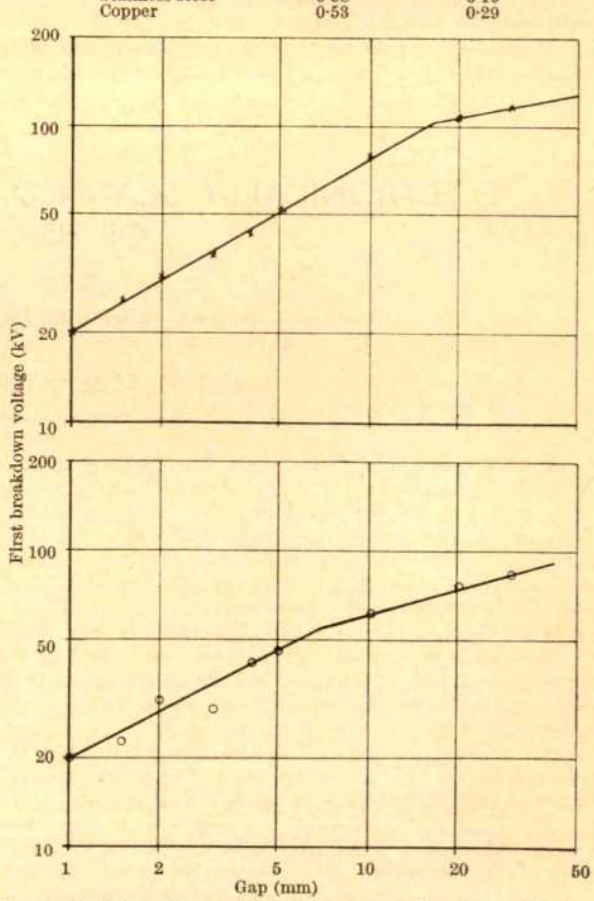


Fig. 4. First breakdown voltages between smooth uniform field electrodes in vacuum. Above, Steel electrodes; below, copper electrodes

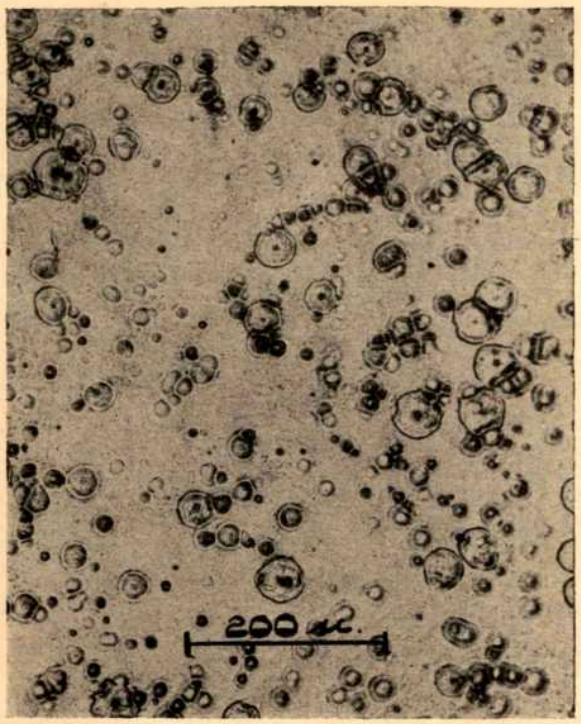


Fig. 5. Markings on anode of stainless steel after impulse voltage breakdown. Gap = 20 mm

between steel electrodes in gaps up to 8 mm and adjusted his theoretically obtained equations to suit the values found by experiment. Under the present experimental conditions the critical gap for stainless steel is about 17 mm so that it is possible that Slivkov was not observing a clump-type breakdown. If this was so then his value of 0·63 for α is in good agreement with the present value of 0·58 obtained for stainless steel for the smaller gaps.

The foregoing results are highly tentative and need to be confirmed using a consistent type of voltage before any definite conclusions are drawn. Also there is conflicting evidence as to the gap regions in which the different mechanisms apply. For example, in several tests using 20 mm gaps (that is, gaps greater than the apparent critical gap) and stainless steel electrodes, markings were found on the anode (Fig. 5) identical to those reported by Maitland[16] which he attributed to an electron beam mechanism. In addition, Rozanova[17] has recently shown, by deliberately introducing clumps on to clean electrodes, that clumps can initiate breakdown in gaps as small as 0·4–0·8 mm.

Obviously the critical gap will depend strongly on the experimental conditions, for example, the state of the electrode surfaces, the electrode material, etc., but the above results, although obtained using different types of voltages, provide some evidence of a change in the breakdown mechanism in vacuum with increase in gap.

I thank the Directors of C. A. Parsons and Co., Ltd., for permission to publish this article and Dr. R. L. Craik, Mr. E. Ridley and Mr. B. Ambrose for assistance.

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ELECTRONICALLY SCANNED SONAR IN THE INVESTIGATION OF FISH BEHAVIOUR

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AND
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RECENT preliminary tests with a new high-resolution sector scanning sonar have shown that equipment of this type has an important application to research on fish behaviour. The sonar set used was one designed at the request of the Marine Laboratory, Aberdeen, under a grant from the Development Commission and built in the Electrical Engineering Department of the University of Birmingham, in the course of a programme of research in underwater acoustics. Theoretical investigations of the problems involved and also a fuller description of the design and construction of the set are published elsewhere[1–6].

A carrier wave at 500 kc/s is modulated with 100 μsec pulses, corresponding to a range resolution of about 15 cm. The receiver has a nominal 3 db directional beam-width of $\pm \frac{1}{2}°$ and this is scanned over a sector of $\pm 15°$ within each pulse duration (that is, the scanning rate is 10,000/s). The receiving transducer array contains 30 lead–zirconate plates and the electronic scanning system has 30 signal channels, each associated with one element of the transducer array. The transmit array is arranged to radiate a peak within-pulse power of about 80 W into the water over the $\pm 15°$ scanned sector. Provision is made for the use of multiplicative signal processing which approximately doubles the angular resolution of the set, at the expense of some reduction in its effective maximum range. The output is presented on a plan position indicator (P.P.I.) display in which horizontal deflexion represents target range and vertical deflexion represents target bearing relative to the axis of the transducer array. With multiplicative processing, the set is capable of resolving objects at the same range when their angular separation is one degree.

The set was tested in a concrete tank holding sea-water and measuring 16 m × 13 m, the depth of water being about 1·6 m. The transducer was placed about 4 m from one end facing across the tank. The tank contained about 40 herring (25–30 cm long), five cod (63–69 cm), four skate (34–54 cm) and three dogfish (60–63 cm) and a number of small roundfish and flatfish. The fish had been

established in the tank as part of a project to test the reactions of commercial species to fishing gear, and especially to assess the herding effect of moving panels of netting at different light intensities. One of the problems had been to observe the extent of herding in low light intensities. Up to this stage, flash photography had been used; but it was found possible to observe individual fish so well with the scanner that a series of herding experiments was performed at dusk using a panel of white nylon netting with a mesh 22·5 cm², the behaviour of the fish being observed on the scanner.

Fig. 1 shows a typical frame from a ciné film of the P.P.I. display during such a herding experiment. Individual fish may be seen between the warps pulling the net. When any sonar is used in a confined space, such as a tank, spurious traces tend to be caused by acoustic reflexions from the boundaries of the medium, and also, as a result of multiple transmission path effects, the amplitudes of the wanted target echoes fluctuate rapidly with time. Even under these conditions, when the display was viewed directly, persistence of vision caused the wanted targets (in this case fish and the net) to appear as continuously moving spots on the screen which could easily be distinguished from unwanted 'noise', whereas much less information was contained in a single photograph. For this reason sketches have been used here (Fig. 2) to illustrate the observed results in more detail.

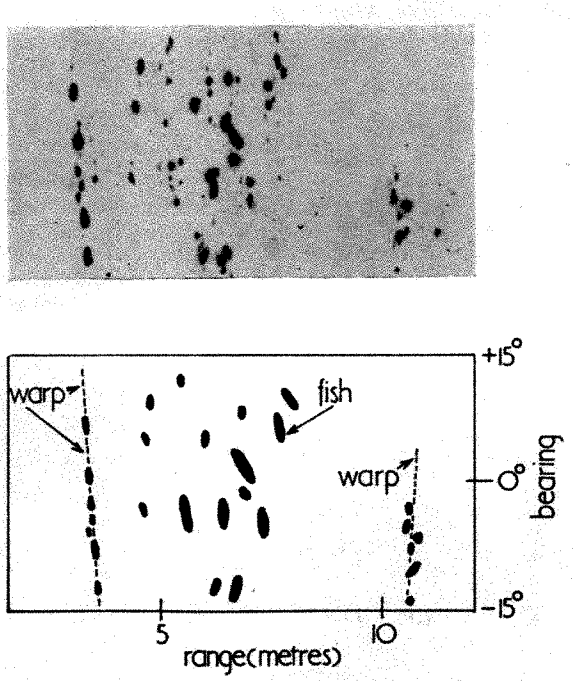


Fig. 1. Single frame from ciné film of sonar P.P.I. display, shown in negative form (with explanatory sketch below), taken during experiments on fish in a sea-water tank

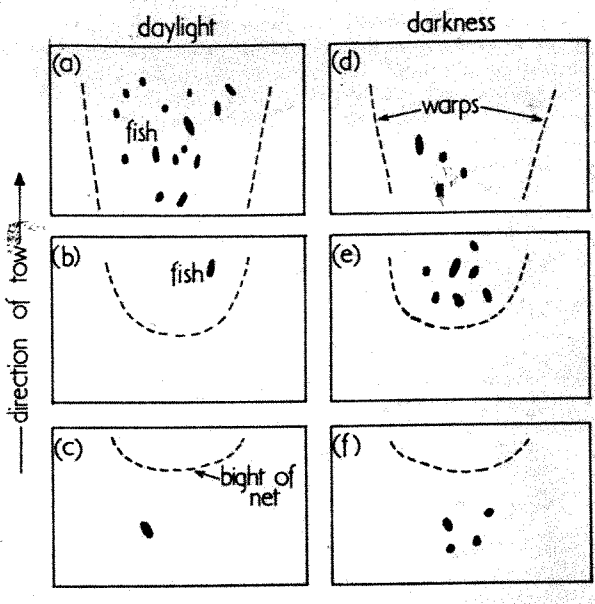


Fig. 2. Sketches of P.P.I. display showing typical results of herding experiments on fish in daylight and darkness

In Fig. 2, sketches (*a*), (*b*), (*c*) refer to daylight and (*d*), (*e*), (*f*) to darkness. The pattern of herding was found to alter as the light intensity dropped. Fig. 2 (*a*) shows a fairly large number of fish well in front of the net in daylight, being herded partly by the warps of the net. Fig. 2 (*b*) shows the bight of the net, with only one fish visible and Fig. 2 (*c*) shows a later stage in the haul, with one fish left behind (presumably having passed through the meshes of the net). At night (Fig. 2 (*d*)–(*f*)) the total number of fish herded became much reduced, the fish being much nearer the net and more passing through it.

Numerous experiments of this sort, using both the sonar and flash photography, showed that the pattern of herding changed at about 0·5 to 0·05 photopic lux. Clearly this is of importance in relation to the use of visual herding devices on trawls; these devices would become less effective at low light intensities.

The potential value of a high-resolution within-pulse scanning sonar for observing fish concentrations and movements in darkness or in turbid water can scarcely be over-emphasized, and the apparatus may well prove to have value for other types of observation in the dark.

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MICRO-CULTURE OF HELA CELLS AND HUMAN EMBRYO LIVER ON POYTHENE DISKS

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CULTURE of organs from chick embryos has been accomplished by squashing fragments of the organs to form thin films and sticking them to polythene surfaces with clotted mouse plasma. The technique was considered inapplicable to adapted cell lines and to embryo liver[1]. In certain experiments, the surface on which organ fragments and cell lines were cultivated consisted of reconstituted rat tail collagen[2,6]. This article describes a micropolythene technique for the cultivation of adapted cell lines and human embryonic tissues.

Polythene disks, 0·003 cm thick, and with a diameter of 1·5 cm were cut out with a metal punch and sterilized by immersion in ether[1]. 6–12 disks were then placed in a sterile Petri dish and on each disk was centred a sterile glass ring with an internal diameter of 0·7 cm, external diameter of 0·9 cm, and height of 0·65 cm (Fig. 1).

HeLa cells were propagated in Roux bottles in a growth medium consisting of 80 per cent 'Eagle basal special' medium and 20 per cent bovine serum. The 'Eagle basal special', prepared by the Poliomyelitis Research Foundation, Johannesburg, was an 'Eagle basic medium' in Hanks's base[2] containing 0·5 per cent (w/v) lactalbumin hydrolysate to which was added 1·9 mM *l*-arginine (thus bringing the *l*-arginine content up to 2 mM), 0·1 mM glycine and 0·1 mM *l*-serine; to this medium was added the crystalline sodium salt of penicillin *G* (200 u/ml.) and streptomycin sulphate (100 μg/ml.).

The cells were stripped with 0·25 per cent Difco trypsin in Dulbecco buffer[4], lightly spun down and re-suspended in the growth medium to a concentration of approximately $1·2–1·5 \times 10^4$ per ml. Of the suspension, 0·1 ml. was placed in each ring. Very little, or no leakage occurred at the junction of the polythene disk and glass ring. The Petri dish covered with lid was placed in a 6-in. vacuum desiccator and 90 per cent of the air was replaced with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. The final gaseous mixture, therefore, contained 1–2 per cent oxygen. The jar was incubated at 35° C. Every 2–3 days the medium was pipetted off and replaced with a maintenance medium consisting of 95 per cent 'Eagle basal special' and 5 per cent bovine serum. The cells were examined by placing the polythene disks with the cells uppermost on a small drop of glycerol on a slide. A further drop of glycerol was placed on top of the disk, covered with a coverslip, sealed with two coats of nail varnish and examined under phase contrast illumination. Cultures of HeLa cells were successfully maintained in this manner for 10–14 days (Fig. 2).

The cultivation of human embryo liver was accomplished on polythene disks coated with a film of reconstituted rat tail collagen. Fresh rat tails were obtained from the Johannesburg City Health Department and stored at —20° C. The collagen was prepared in a manner similar to that described by Watson *et al.*[5], and Ehrmann and Gey[6]. After being soaked in 95 per cent alcohol for 10 min, the tail was successively fractured. As each 0·5 in. fragment was drawn away from the tail, the long, silvery tendon became stretched for 3–4 in. before the fragment came away, leaving stretched tendon still attached to the tail. The tendon was cut with sterile scissors and dropped into 10 ml. sterile, distilled water. All the tendon strands from one tail were teased apart and then transferred to a sterile 250-ml. centrifuge bottle containing 150 ml. of 1 : 1,000 acetic acid solution. This bottle was sealed and stored at 0°–4° C for 48 h during which time, as a result of further swelling of the tendon strands, the contents became transformed into 'a translucent jelly-like mass'. Following centrifugation at 4° C for 2 h at approximately 1,200*g*, 40 ml. of clear viscid fluid were transferred to McCartney bottles.

The acetic acid collagen solution was then dialysed against distilled water in an autoclaved 'Cellophane' bag at 0°–4° C. After 3 days the viscosity of the partially dialysed solution was markedly increased; it was then transferred to McCartney bottles and stored at 0°–4° C. Sterilized polythene disks with glass rings were placed in a standard Petri dish. Approximately 0·03 ml. of the viscous collagen solution was dropped into the centre of each ring without actually coming into contact with the sides of the ring. The Petri dish was left uncovered under an ultra-violet strip lamp while the collagen gelled. After 2 h, the collagen formed an invisible transparent film on the polythene disk and sterility was ensured at the same time. Approximately 0·1 ml. of the culture medium was then placed in each ring. Equilibration was allowed to occur; first for 1 h at room temperature and then overnight at 0°–4° C. The following morning, the medium was removed with a Pasteur pipette, and the collagen surface on the polythene disk was now ready for implantation. The Petri dishes thus prepared were sealed with parafilm and storable at 0°–4° C for at least 4–6 months.

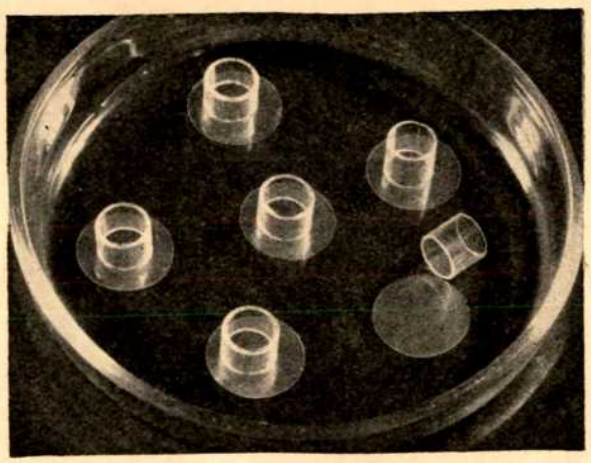


Fig. 1. Petri dish with polythene disks and glass rings in position

Liver tissue was obtained from 6–15 cm human fœtuses, partly from spontaneous abortions and partly from therapeutic terminations of pregnancy. Often 12 h elapsed before the material was received. The liver was minced moderately with fine-pointed scissors in a Petri dish containing 3–4 ml. Dulbecco buffer[4], washed 2–3 times with 5 ml. fresh Dulbecco buffer[4] to remove as much blood as possible, and then re-suspended in the culture medium. The tissue was then re-minced in order to obtain particles of less than 1 mm[3]. Approximately 0·1 ml. of the suspension was placed in each ring. The gaseous environment was the same as for the HeLa cells. Liver cells were



Fig. 2. HeLa cell culture, day 9, on untreated polythene disk; phase contrast illumination (×600)

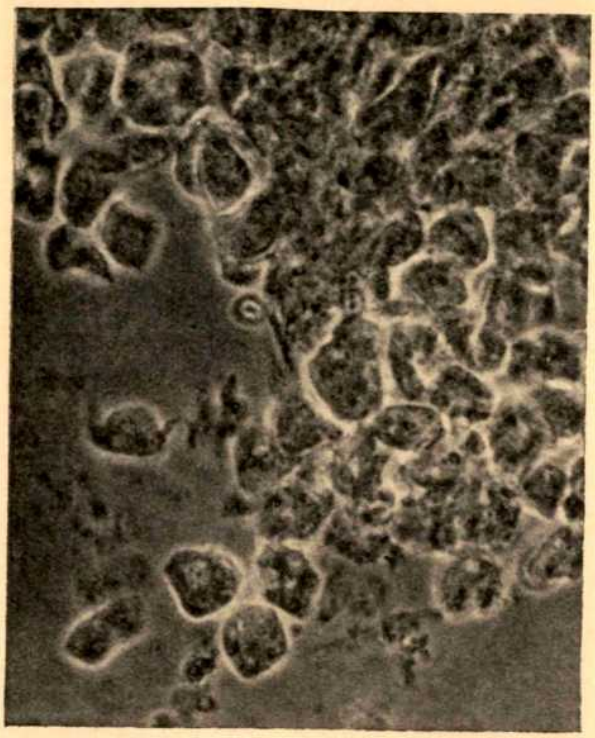


Fig. 3. Human embryonic liver explant day 2 on collagen coated polythene disk; culture medium: 80 per cent 'Eagle basal special', 20 per cent bovine serum, changed at weekly intervals; phase contrast illumination (×600)

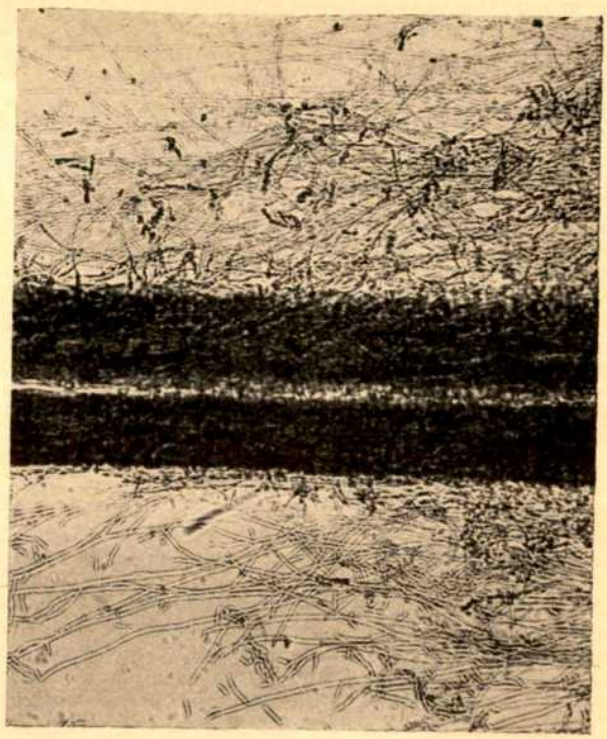


Fig. 4. Hair infected with dermatophyte; day 4 on untreated polythene disk showing mycelial outgrowth; phase contrast illumination (×120)

cultured in this way for at least 39 days (Fig. 3). Subculture of the cells has not as yet been attempted.

The microtechnique may also be successfully adapted to the growth of fungi. In this modification the polythene disk is floated on the surface of sterile saline contained in a screw-capped jar, 3 cm in diameter. Glass rings are not used; 0·05 ml. of a suspension of the infected material in an appropriate fungus medium is seeded directly on to the untreated polythene surface. Several such jars can be prepared from each specimen and examined at regular intervals. An example of such a culture is illustrated in Fig. 4, in which a dermatophyte may be seen growing out of an infected hair after 4 days incubation at room temperature.

I thank Dr. V. Bokkenheuser for criticism, Miss M. A. S. Birrell for assistance, Mr. M. Ulrich for the photographs, the director of this Institute, Dr. J. H. S. Gear, and Dr. P. D. Swanepoel, superintendent of the South Rand Hospital, Johannesburg, for facilities granted.

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OXYGEN IN THE GAS VACUOLE OF THE RHIZOPOD PROTOZOAN, *ARCELLA*

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THE rhizopod protozoan *Arcella* is perhaps unique in repeatedly forming large gas-filled vacuoles in its cytoplasm. These gas vacuoles, first observed by Perty[1], attracted the attention of Engelmann[2,3], and were investigated by Bles[4]. F. Gowland Hopkins and the late D. Keilin, in their preface to Bles's posthumously published paper, state: "we believe . . . that it was Dr. [J. S.] Haldane's firmly expressed belief in the great biological importance of gas production as observed in Arcella which, more than anything else, urged Bles to his experimental study of the phenomenon".

Bles's many ingenious experiments with vital dyes led him to believe that the gas in the vacuole was oxygen produced by the chemical action of 'oxidases'. It is perhaps difficult to-day to reconstruct the full impact of these experiments; but both J. S. Haldane and D. Keilin, who followed Bles's work closely, felt that he had convincingly shown that the gas was oxygen (Keilin, D., private communication). However, certain identification of the gas ultimately must rest on direct analysis, and Bles's monograph[4], edited after his death and thirteen years after the work was done, presents only a single experiment on identification of the gas by chemical means. He crushed *Arcella* containing gas vacuoles in a solution of potassium hydroxide and concluded: "The experiments with caustic potash solution show not only that the gas in

the vacuole is not CO_2 but that it must be one of the gases forming the bulk of air because saturation of the solution with air increased the time taken to dissolve the gas from the vacuole. This sequence of experiments points again to the gas being oxygen . . . ".

We believe that Bles failed to appreciate the speed with which gases dissolved in the potassium hydroxide solution invade a very small bubble and replace the gases initially present. In fact, Krogh[5] had found that because of this difficulty the practical lower limit for the size of the bubble that could be analysed when freely suspended was 0·01 μl., 100 times larger than the bubble from *Arcella*. We have observed that the bubbles, formed from the vacuole contents when *Arcella* are crushed in alkaline pyrogallol, often enlarge abruptly before diminishing gradually. The abrupt enlargement points to another difficulty to be circumvented if a meaningful analysis is sought[6]. Two solutions of different concentration, each saturated with air, become greatly supersaturated when mixed and will give off gas through any available surface. In this case, mixing of adhering culture medium or protoplasmic water with the strong potassium hydroxide may double the local gas tension. For analysis of the vacuole contents the gas bubble must be protected from invasion by gases in the environment.

For a brief period cultures of *Arcella vulgaris* which had been established some two years earlier for the purpose of investigating gas vacuole formation had many individuals containing gas-filled vacuoles, Fig. 1. The cultures were maintained with alternating periods of light and darkness; by chance the light period fell during the night. From 2100 to 0400 h about one-third of the animals contained a gas vacuole at any one time, and since the life-time of a vacuole is short and new vacuoles were continually forming in quiescent animals, most individuals must have formed vacuoles many times during the night. The animals were remarkably uniform in size, the test was 170μ in diameter; the protoplasm, not completely filling the test, averaged 145μ diameter. The gas vacuoles ranged from 30μ to 60μ diameter, their volume being 2 to 8×10^{-5} μl., 5–10 per cent of the volume of protoplasm.

To analyse the gases of the vacuole, individuals were transferred to a glass slide together with a minute droplet of culture medium, and the diameter of the vacuoles estimated by comparison with a grid mounted in the microscope ocular. A drop of potassium hydroxide solution (50 g in 100 g water) which had been boiled and evacuated to remove gases or a drop of potassium hydroxide–pyrogallol solution (10 g pyrogallol dissolved in 100 ml. of the degassed potassium hydroxide solution and once again degassed *in vacuo*) was placed over alternate animals, and a cover-slip was dropped on the pool of reagent so that the violent currents resulting swept the residual culture medium from the animal. It was essential that the culture medium be completely removed, and accordingly the success of this manœuvre was verified in separate experiments with droplets of indian ink. As soon as the animal could be found in the microscope field (usually after 20 sec), the vacuole diameter was once again noted and observed until it disappeared. On contact with the potassium hydroxide solution the vacuole lost its complex shape and became spherical, apparently with some shrinkage whether pyrogallol was present or not.

The rate of loss of gas from the vacuole will be governed by the rate of outward diffusion across the now dead but still intact protoplasm, and this in turn will depend on the partial pressure difference for each gas between the bubble and the potassium hydroxide solution immediately adjacent to the animal. Because the solution is viscous and unstirred, and because the solubility of gases in the concentrated potassium hydroxide is very limited, gases diffusing from the animal will diffuse only very slowly into the bulk of the solution, but will tend to remain in a thin

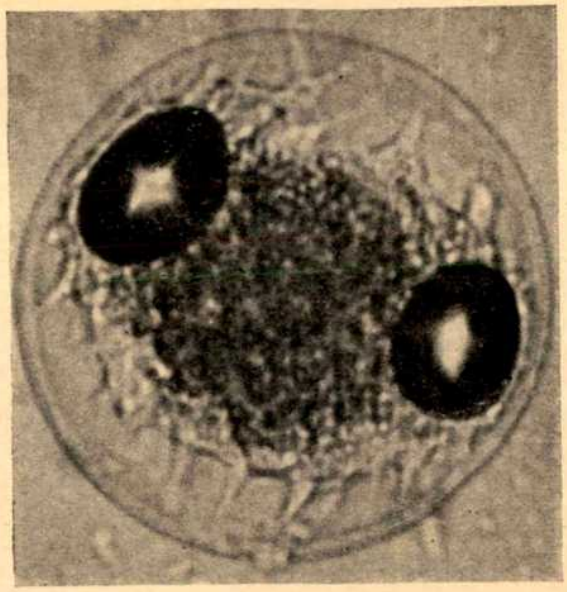


Fig. 1. Photograph of an *Arcella* containing two gas vacuoles

shell of fluid surrounding the animal. The gases accumulating in this very thin layer of potassium hydroxide solution, because of their limited solubility in that solution, exert substantial partial pressures which oppose further loss of gas from the vacuole. If the potassium hydroxide solution contains pyrogallol, oxygen leaving the animal is instantaneously consumed, the oxygen tension adjacent to the animal remains zero, and the rate of oxygen loss from the bubble should be greater than for other gases.

The rates of shrinkage of the gas bubbles in: (*a*) animals treated with potassium hydroxide solution; (*b*) animals treated with potassium hydroxide–pyrogallol solution are compared in Fig. 2. The initial bubble volume was taken as the volume 1 min after adding the reagent, since earlier readings were difficult and the vacuole in the living animal has a complex shape. The final volume was arbitrarily taken 5 min after adding the reagent. (The results are not different if the final volume is taken as the volume at 3 min). Fig. 2 is a frequency diagram relating number of animals to fractions of the initial gas volume absorbed during this interval. Clearly the bubble tended to persist in animals flooded with potassium hydroxide solution and tended to disappear in animals flooded with potassium hydroxide–pyrogallol solution. Application of the median test[7] confirms that the two groups, *a* and *b*, are drawn from populations having different medians (at the 5 per cent confidence level).

Oxygen, we conclude, makes up the largest part of the gases present in the gas vacuole of *Arcella*.

The volume of large gas vacuoles may be as much as 10–15 per cent of the protoplasmic volume. Since the amount of oxygen dissolved in protoplasm is only 0·6 volume per cent, the oxygen filling the bubble must be brought from outside the animal. To understand this transport one turns to the observations of Bles and, in his words (*loc. cit.*, p. 556): "An arcella which had formed five large gas-vacuoles . . . was crushed in the usual way, and it was found that the five bubbles pressed out did not coalesce to form a single bubble: in fact, two of them were caught inside the broken test; of the three which escaped, two in the course of the first minute after crushing were absorbed by protoplasm adhering to the coverglass. The third, instead of disappearing, began rapidly to expand. It was measured by the eye-piece micrometer one minute after crushing; four minutes after crushing it had grown $1\frac{2}{3}$ times its diameter when first measured. It then steadily increased in size for 1 hr. 42 min., during

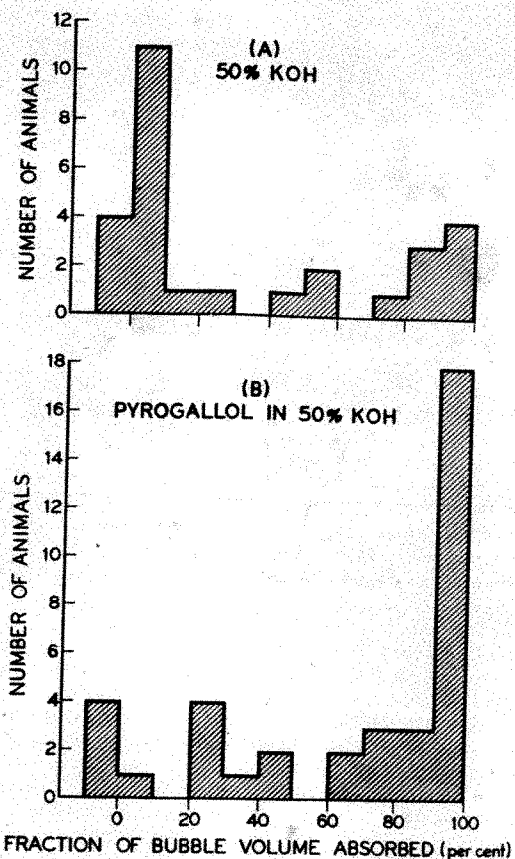


Fig. 2. Frequency diagram relating the numbers of animals to the fraction of the bubble volume (measured 1 min after adding the reagent) which is absorbed during the ensuing 4 min

which it was measured every few minutes, and found every time to be growing in diameter. At the end of this period it had grown to 5½ times its original diameter, its volume having increased, assuming that temperature and pressure had remained constant, to [155] times its original volume". Also: "A further crushing experiment was made in order to test the action of peroxide of hydrogen on a fragment of protoplasm containing a minute remnant of a gas vacuole. After crushing, a piece of the protoplasm was found containing a gas vacuole which was watched and seen to be shrinking. Eight minutes after crushing a weak solution of peroxide of hydrogen (about 1 per cent) was run in and within thirty seconds the size of this gas vacuole was measured. One minute later the diameter was six times greater and the vacuole continued to grow. The vacuole had increased in diameter about ninety times in forty-three minutes. . . . The gas in both experiments may be the product of catalase action upon a peroxide formed by the oxidase activity of the cell. In the peroxide experiment there can be little or no doubt that the gas in the vacuole is due to the reduction of the peroxide of hydrogen, and the growth of the gas-vacuole in the peroxide experiment corresponds exactly with the spontaneous growth of the gas vacuole in the crushing experiment described above. The difference between the two cases is that the peroxide of hydrogen was supplied in one of them, and in the other the peroxide was present in the protoplasm".

The work recorded here establishes by direct analysis that the gas brought into the vacuole of *Arcella* is predominantly oxygen. Presumably oxygen from the medium is reduced to hydrogen peroxide, diffused in this form and, after transport, is decomposed to oxygen, probably by catalase. The morphology of the specialized cytoplasm adjacent to the bubble is being studied in collaboration with D. E. Copeland and A. Fitzjarrell of Tulane University. The technique for culturing *Arcella* will be described elsewhere.

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INDUCTION OF BUDS ON OLDER LEAF PRIMORDIA IN FERNS

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SEVERAL recent investigations of morphogenesis in ferns have yielded information about the developmental potentialities of leaf primordia. For example, in *Dryopteris dilatata* (Hoffm.) A. Gray (*D. aristata* (Vill.) Druce) it has been shown that leaf sites and very young leaf primordia are initially capable of development either as leaves or as buds, since they can be induced to develop as buds if isolated from the shoot apex[1–3]. After a certain stage of development, coinciding with the formation of the lenticular leaf apical cell, the primordium can no longer be induced to develop as a bud but even when isolated from the shoot apex will develop as a normal, dorsiventral leaf primordium. Occasionally, small buds are formed in association with such isolated older leaf primordia, some distance up from the base of the petiole[4].

In *Osmunda cinnamomea* L., leaf primordia excised from the subtending shoot after a certain stage of development are capable of developing into small but morphologically normal fronds when grown on a comparatively simple nutrient medium[5], but some of the youngest primordia, similarly treated, give rise to shoots and later complete plants[6]. The nine youngest leaf primordia of *O. cinnamomea* are considered to be capable of development as shoots[7], whereas in *Dryopteris* only the three youngest visible primordia have this potentiality[3]. In recent experiments[8], it has been shown that the halves of leaf primordia of *O. cinnamomea* which were bisected and grown in culture could regenerate either as leaves or shoots, according to the age of the primordia and other conditions. The occurrence and development of buds in

ferns have been surveyed by several of the older morphologists, notably Bower[9]. In *Dryopteris* buds are typically formed in interfoliar positions; where the shoot apex is intact, buds are not usually formed in the apical or immediately sub-apical regions.

With this background of information, it was considered that experiments involving various incisions of older leaf primordia of *Dryopteris dilatata* (those known to be determined as leaves) might induce interesting new morphogenetic developments, both foliar and cauline, and at the same time yield information about the distribution of meristematic, or potentially meristematic, tissues within the leaf and their control during growth and development. In one series of experiments, leaf primordia of *D. dilatata* ranging in age from the third youngest, P_3, to P_{20}, the majority being from P_5 to P_{15}, were either radially bisected with a microscalpel or punctured with a tungsten needle; some of the primordia in each group were also isolated from the shoot apex. Usually only the apical cell of the leaf primordium was punctured, but the resulting necrosis often spread to neighbouring cells; damage from the bisecting incision also spread a little. The regions of leaf tissue on either side of the damage resulting from the puncture or bisection (half-primordia) can regenerate either leaf primordia or buds. In all the experimental treatments of older leaf primordia, no evidence was obtained of bud formation as a result of a transformation of the leaf apical meristem. The results of these experiments, in terms of the fate of the half-primordia, are shown in Table 1, from which a few anomalous results, to be described in a later paper, have been omitted. Both in Table 1 and afterwards, all foliar developments are termed 'leaf'; we realize, however, that some of these developments may be of pinna nature. In Table 1 the category 'leaf + bud' comprises regenerated leaves which bore buds on the petiole; these were obtained both from bisected (Fig. 1) and from punctured primordia (Fig. 2).

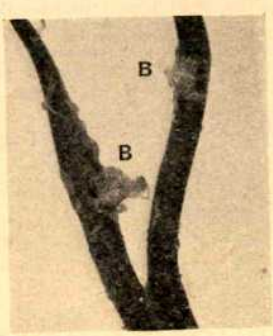


Fig. 1. Part of the petioles of the two leaves obtained from P_{20}, which was bisected and isolated from the shoot apex. Buds (*B*) are borne on both petioles. Eleven weeks after treatment. (All photographs by Mr. G. Grange.) (Same scale)

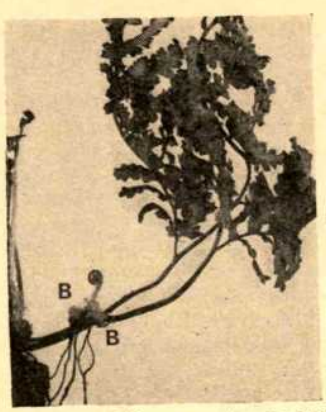


Fig. 2. A leaf primordium which was punctured and isolated. Fifteen weeks later, two expanded leaves had been formed, and two buds with roots were present on the petiole. (× 0·5)

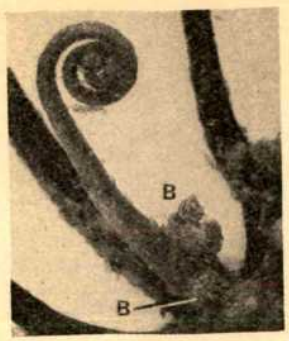


Fig. 3. P_{12}, isolated from the shoot apex by an adaxial incision, developed as a normal, dorsiventral leaf primordium, with a bud (*B*) on one flank and a smaller bud (arrowed) near the base of the other flank. Eleven weeks after isolation. The formation of two buds was unusual. (× 2)

Growth in general, and especially bud growth, was greatly stimulated by isolation from the shoot apex (Table 1). Where buds occurred on primordia which were not isolated, their appearance was belated, occurring 2 months or more after the experimental treatment, by which time the primordia were at some distance from the shoot apex.

Seventeen leaf primordia ranging in age from P_5 to P_{19} were isolated from the shoot apex without further treatment, their apices remaining intact. Of these, all developed as dorsiventral leaf primordia of approximately normal size, but 10 bore buds laterally or adaxially on the petiole (Fig. 3). These results were in accordance with previous findings[4].

Table 1. Effects of Puncturing or Bisecting Leaf Primordia, With or Without Isolation

Treatment	No organized development	Result: Leaf	Leaf + bud	Bud	Total
Punctured					
Not isolated	17	5	2	1	25
Isolated	25	14	12	24	75
Bisected					
Not isolated	26	5	6	—	37
Isolated	15	7	16	22	60

In another series of experiments, either one or two small, approximately horizontal incisions were made laterally into the subdistal, that is, presumptive petiole, region of leaf primordia ranging in age from P_9 to P_{22}. Such incisions partially isolated portions of the marginal meristem from the influence of the leaf apex. Some of the primordia were also isolated from the shoot apex. The results are summarized in Table 2. In this case, the category 'leaf and bud' implies that both organs were formed, separately but in proximity, in relation to the incisions (Fig. 4).

In most instances the outgrowths were formed below the cuts, often between them if there were two cuts, and sometimes rather adaxially. In a few specimens, not included in Table 2, meristematic outgrowths were induced on the opposite flank of the petiole to the one which had in fact been incised. In another experiment, incisions similar

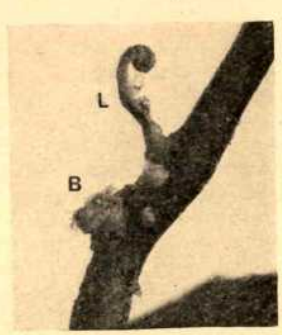


Fig. 4. The petiole of P_{15}, given two cuts across the marginal meristem and isolated from the shoot apex, now shows a bud (*B*) and a leaf primordium or pinna (*L*) formed in relation to the cuts. Thirteen weeks after treatment. (× 2)

Table 2. Effects of Lateral Incisions on Leaf Primordia, With or Without Isolation

Treatment	No organized development	Result Leaf	Leaf and bud	Bud	Root	Total
Lateral incisions						
Not isolated	31	—	1	4	1	37
Isolated	13	—	1	10	—	24

to these were made into the abaxial side of the primordia; such growths as were obtained occurred adaxial to these cuts, not between or directly below them.

Meristematic activity was induced not only in primordia isolated from the shoot apex, but also in those only given lateral incisions. Occasionally two outgrowths were formed below the cut, an upper leaf primordium (or pinna) and a bud below this. Whereas the cuts were originally made fairly close to the tip of the primordium, the ultimate position of the outgrowths was fairly low down on the petiole (Figs. 5 and 6). It appears, therefore, that the incisions were made in and the meristems grew out from that part of the fern petiole which does not normally undergo much increase in height[10].

In 11 primordia, ranging in age from P_{10} to P_{19}, vertical incisions were made abaxially in the base of the petiole, with the intention of disrupting the incipient vascular

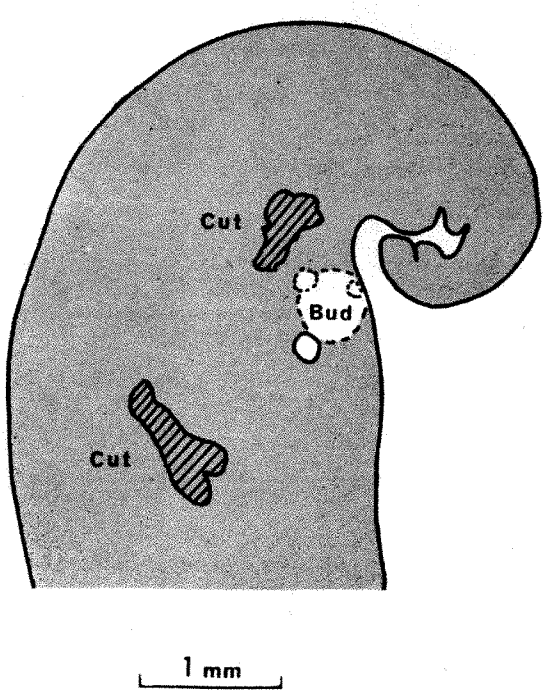


Fig. 5. A bud has formed between and slightly adaxial to two cuts (now opened out and visible as necrosed tissue, cross-hatched) across the marginal meristem of P_{12}, which was also isolated from the shoot apex. Seven weeks after treatment. (see Fig. 6)



Fig. 6. P_{12} with the bud shown in Fig. 5, four weeks later. The leaf tip has grown on considerably. (× 2)

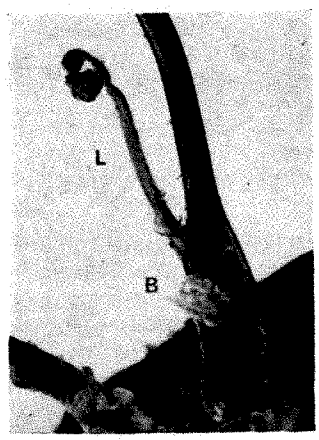


Fig. 7. P_{11}, split abaxially near the base of the petiole and isolated from the shoot apex, bears a leaf primordium or pinna (L) on one flank of the petiole, and a bud adaxially, above the archway formed by the split. Thirteen weeks after treatment. (× 2)

tissue therein. Seven of these primordia were also isolated from the shoot apex. Of the 4 primordia which were not isolated, one had a double tip, although there was no evidence of apical damage; the other 3 showed no response to the slit. Of the 7 isolated primordia, 4 gave rise to buds, 2 of which were formed more or less directly above the archway resulting from the split; one gave rise to a leaf primordium or pinna on one flank, with a bud above the archway (Fig. 7), and 2 showed no response.

In all these experiments, presumptive petiolar regions were induced to grow out as organized meristems of various kinds, indicating that lateral and adaxial regions of leaf primordia of *Dryopteris* are, under certain circumstances, capable of organogenic developments which they do not normally undergo. This was usually effected by isolating them from the leaf apex, or by destroying this. Possible direct or indirect effects of wound hormones may to some extent be involved. Isolation of older leaf primordia from the shoot apex, that is, in addition to the other treatments, typically resulted in the induction of a higher proportion of organized outgrowths.

Collectively, these results suggest that the apex of the leaf primordium of *Dryopteris* exercises a controlling effect on the tissues below it akin to that of the shoot apex on its lateral meristematic tissues. Growth centres in the marginal meristems grow out as pinnæ, just as leaf primordia originate from growth centres in the shoot apical meristem: but other regions of the leaf do not grow out in the normal development, although these experiments indicate that they can be induced to do so, not only as foliar organs but as buds. Thus the organized leaf apical meristem, of which the lenticular apical cell is an integral component, seems to determine the form and structure of the leaf. Notwithstanding the excellent classical studies of leaf apical and marginal meristems in ferns, a fuller investigation of the distribution of these meristematic or potentially meristematic tissues is now seen to be required. Discussion of the various problems which these new discoveries evidently raise, and also of the cognate findings of contemporary workers, will be held over until the anatomical studies of our experimental materials, now in progress, are completed.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Bolometric-Correction and Effective-Temperature Scale

In recent years the application of high-speed digital computers to problems in theoretical astrophysics has combined with fundamental research in nuclear physics and solar convection to achieve considerable understanding of the physics of not only stellar interiors but also stellar evolution. The connecting link between this increasingly elaborate theory and the corresponding ensemble of observations is the scale of bolometric-corrections (*B.C.*) and effective-temperatures (*Te*).

Much of the early theoretical work dealt with luminosities and effective-temperatures in a range where the uncertainties in these scales have been generally felt to be less severe than uncertainties stemming from the assumptions inherent in the stellar models. The sources of uncertainty in the *B.C.* and *Te* scales have been reviewed by Popper[1], whose arguments will not be reproduced here. The scales are in all ranges in a position to benefit from modern methods of radiometry, applied both from ground observatories and spacecraft, and from a vastly improved knowledge of the transmission of the atmosphere.

In particular, however, a stellar model of 15·6 solar masses has now been constructed by Hayashi and Cameron[2]. The initial and final portions of the evolutionary track for this supergiant can only be related to observation by way of an educated guess. The early supergiant phase requires the *B.C.* and *Te* from model atmospheres, the empirical scales being precluded by the atmospheric ozone cut-off. The *M* supergiant phase requires that an already poorly determined empirical scale for late type stars, which definitely suggests a dispersion due to luminosity class that cannot be adequately accounted for because of a paucity of data, must be extrapolated.

Work is at present being carried out to alleviate this situation, but which, unfortunately, will not be available for some time. In the meantime this communication is an attempt to compile the most reliable set of *B.C.*'s and *Te*'s from the literature; which scales will be used in a forthcoming investigation of the region surrounding *h* and χ Persei.

A number of recently proposed *B.C.* and *Te* scales have been drawn in Figs. 1 and 2 together with mean relations extracted by me from pure data-point plots also recently published. I shall now briefly review the source of each in chronological order.

(1) Arp[3] has conducted the most recent review and compilation which extends over the entire observational range of temperature. Earlier than *B*2, the *B.C.*'s have been obtained from the prediction of *B.C.* and *Sp* from the model atmospheres of Underhill and McDonald. Later than *B*5 the *B.C.*'s are from the early comparison of the radiometric magnitudes of Pettit and Nicholson with the corresponding photovisual magnitudes. For the *Te* scale model atmospheres are again used early through *B*2. For later stars the scale is based on a colour-temperature-interpolation by six-colour photometry between the real *Te*'s of the Sun, β Aurigæ (eclipsing binary), and those based on interferometric diameters of bright giants and supergiants (Pease). The real *Te*'s in each case involve the angular diameter (either directly measured or inferred from other measurements) and the *B.C.*'s of Pettit and Nicholson.

(2) Popper[1] has revised the *B.C.*'s chiefly by changing the argument of the scale from *Sp* to $(B-V)_0$ (photoelectric), and returning to the original radiometric data of Pettit and Nicholson and subtracting photoelectric *V* magnitudes. The radiometric data of Emberson with an adjusted zero-point was also used. For the effective-temperatures, beyond the improvement afforded by the revised *B.C.*'s, an additional fundamental datum is

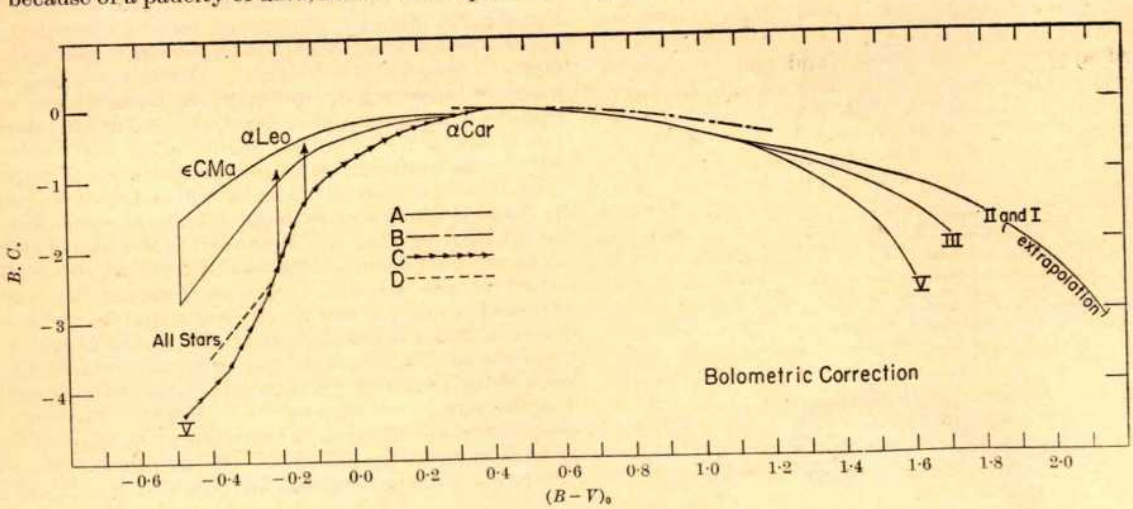


Fig. 1. Bolometric-correction Scales proposed by Various Authors

The solid curve (*A*) is the mean relation for Popper's analysis. The centre-lined curve (*B*) is Oke's. The arrow-head curve (*C*) is due to Arp. The dashed curve (*D*) is Schmalberger's. The recommended relation is given by the following table

$(B-V)_0$	Class I	Class II	Class III	Class IV	Class V
−0·48 to −0·40	*C* + 0·40	*C* + 0·40	*C* + 0·40	*C* + 0·40	*C* + 0·40
−0·40 to −0·22	*D*	*D*	*D*	*D*	*D*
−0·22 to 0·30	*C*	*C*	*C*	*C*	*C*
0·30 to 0·40	*B*	(*B* + *C*)/2	*C*	*C*	*C*
0·40 to 1·20	*B*	(*B* + *A*)/2	*A*	*A*	*A*
1·20 to —	*A* + 0·23	*A* + 0·23	*A*	*A*	*A*

The curves in the figure are labelled according to luminosity class.

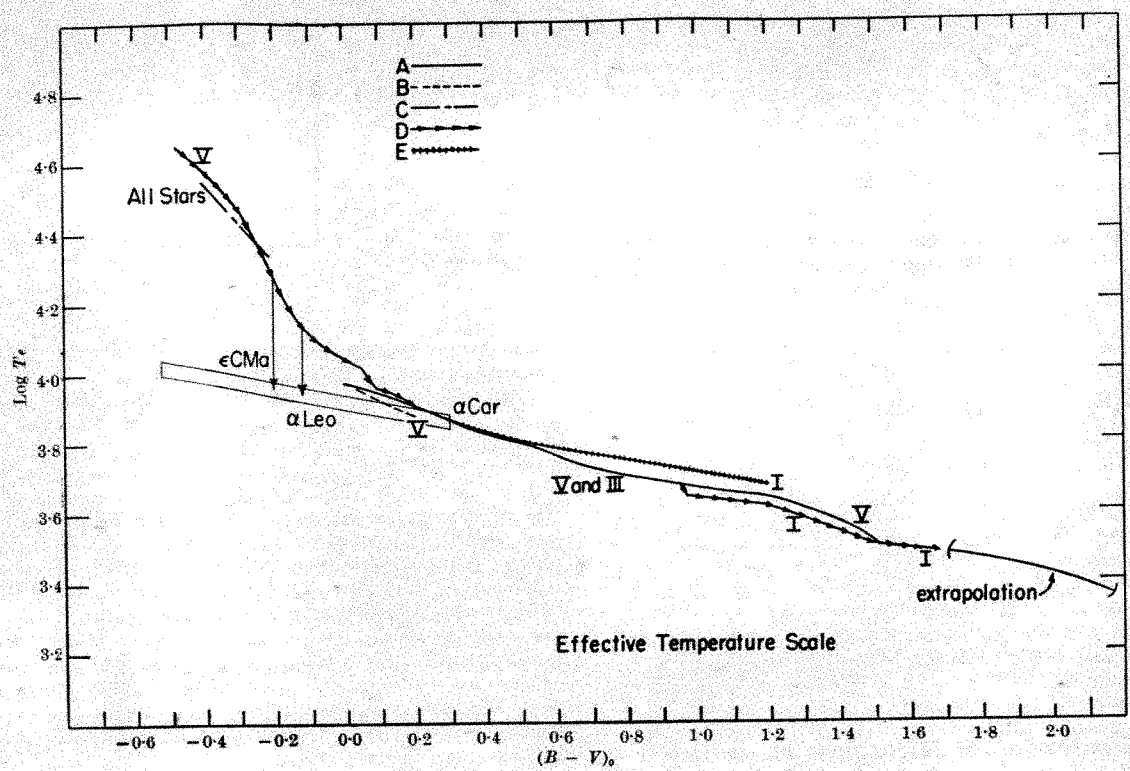


Fig. 2. EFFECTIVE-TEMPERATURE SCALES PROPOSED BY VARIOUS AUTHORS

Solid curve (*A*), Popper; dashed curve (*B*), Bless; centre-lined curve (*C*), Schmalberger; arrow-head curve (*D*), Arp; and lazy-*T* curve (*E*), Oke. The recommended relation is given by the following table

$(B-V)_0$	Class I	Class II	Class III	Class IV	Class V
−0·48 to −0·40	$D-0{\cdot}036$	$D-0{\cdot}036$	$D-0{\cdot}036$	$D-0{\cdot}036$	$D-0{\cdot}036$
−0·40 to −0·22	C	C	C	C	C
−0·22 to 0·00	D	D	D	D	D
0·00 to 0·20	$(B+A)/2$	$(B+A)/2$	$(B+A)/2$	$(B+A)/2$	$(B+A)/2$
0·20 to 0·30	A	A	A	A	A
0·30 to 1·20	E	$(E+A)/2$	A	A	A
1·20 to 1·30	$D+0{\cdot}061$	$(D+A)/2+0{\cdot}030$	A	A	A
1·30 to —	$D+0{\cdot}061$	$(D_I+D_V)/2+0{\cdot}030$	D	D	D

The curves in the figure are labelled according to luminosity class.

admitted due to the measurement of the diameter of Sirius by Hanbury Brown and Twiss. Popper suggests further that empirical *B.C.*'s be used no earlier than *FO* because of atmospheric extinction and relies on model atmosphere predictions in the earlier range (Hunger, Osawa, Saito, and Underhill and McDonald).

(3) Schmalberger[4] gives *B.C.*'s and *Te*'s for *O* and *B* stars from the model atmosphere computations of *Sp*, *B.C.*, and *Te* from Pecker, Underhill, McDonald, and Traving.

(4) Bless[5] has made photoelectric spectrum scans of *A V* stars and obtained the *Te* on the basis of several predicted (model atmospheres) and measured criteria. The criteria chosen were the Balmer discontinuity and the monochromatic magnitude differences: $m(\lambda 4{,}600)-m(\lambda 4{,}190)$; $m(\lambda 5{,}600)-m(\lambda 4{,}400)$, and $m(\lambda 6{,}000)-m(\lambda 5{,}000)$.

(5) Oke[6] has obtained photoelectric spectrum scans for various phases throughout the light cycle of δ Cephei. With concurrent high-dispersion spectrograms he has measured the line-blanketing at various wave-lengths and corrected the scans to obtain the true continuum as a function of phase. He has fitted model atmospheres to each continuum and thereby obtained the *Te* and *B.C.* as a function of phase hence $(B-V)_0$. These have then been recorrected for the blanketing-effect, thus providing *Te* and *B.C.* versus $(B-V)_0$ for true stars rather than their continua.

The reader is referred to the foregoing five authors for reference to authors referred to by them.

In the present compilations the argument has been converted from *Sp* to $(B-V)_0$ whenever necessary. The *Sp* versus $(B-V)_0$ relation used was that given by Arp[3] except for *O* stars and class I stars where the relation was derived from results in *h* and χ Persei to be published later.

I have indicated in the figure captions what I feel to be as reliable a choice of scales as is possible at the present time.

Ultra-violet observations already published[7] show such disparity with model atmospheres that their effect on the *B.C.* and log *Te* scales can scarcely be evaluated until the ultra-violet spectrophotometry is successfully carried out on *O* stars and in addition pushed farther toward the X-ray region. Nevertheless, a crude indication has been obtained of the correction to be applied to the selected curves of Figs. 1 and 2. The fluxes of ε *CMa*[7] and α Leo[7] have been extrapolated by eye to zero wave-length under the assumption that the second derivative of the energy distribution curve is no larger in this region than in the observed portion of the ultra-violet. Both this energy distribution and that of the comparison model-atmosphere[7] were planimetered for each star. Their ratio provided a Δ *B.C.* $=4$ Δ log *Te*. These are shown in Figs. 1 and 2 as vectors. Schematic indications of what one might expect as the *B.C.* and log *Te* scales of the future are shown by the blocked-in areas in the early parts of the Figs. Agreement is produced between these prognostications and present scales at $(B-V)_0=0{\cdot}30$, since Stecher and Milligan's[7] ultra-violet observations of α Car are in agreement with theory.

In summary, I have presented *B.C.* and *Te* scales which I feel reflect the most recent theory and data. However, it must be recognized that crucial observations have not

yet been carried out using modern techniques. When improvements afforded by: (1) the use of detectors of increased accuracy and sensitivity extending farther into the ultra-violet to X-ray region and into the 2–8μ infra-red; (2) satellite-based observing stations; (3) reliable absolute calibrations of detector responsivities; (4) interference filters which enable one to make Earth-bound observations only through atmospheric windows, the extinction law of which is properly evaluated, are incorporated in the final analysis, we must be prepared to accept revisions in *B.C.* which *may* reach tens of per cent for intermediate spectral types to nearly orders of magnitude for early *O* and late *M* stars.

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Concentration of Nightglow H_α-emission to the Ecliptic and the Radial Velocities of this Line

During the winter of 1962–63 I examined the distribution of H_α-intensity over the night sky. A Fabry-Pérot étalon was used with a spacing of 0·3 mm and reflexion coefficient 0·91 at H_α. An interference filter of 30 Å half-width was used as premonochromator. A contact image intensifier was used for registering the fringes. The whole system was installed on a small equatorial mounted in a tube in which temperature was controlled. The observations were made at the high-altitude station of the Sternberg Astronomical Institute ($\varphi = 44°$, $h = 3{,}000$ m) near Alma-Ata. One-hour exposure was sufficient to record H and OH fringes at any point of the sky, including the zenith.

The distribution of the night-glow H_α-emission shows a considerable concentration to the ecliptic and to the Sun. If the H_α intensity in the antisolar point is taken as 1, the intensity on the ecliptic at elongations 70°–90° from the Sun reaches 4–5 (Fig. 1). The distribution is markedly asymmetrical, the morning intensities being greater. The H_α intensity distribution across the ecliptic at the elongation of 90° (morning side) is shown in Fig. 2. The intensity of H_α in other point of the night sky at the same zenith distance, but far from the ecliptic, shows no enhancement. During the observations the regions close to the Sun were situated far from the Milky Way; its position is marked on Fig. 1. The H_α-intensity at 70°

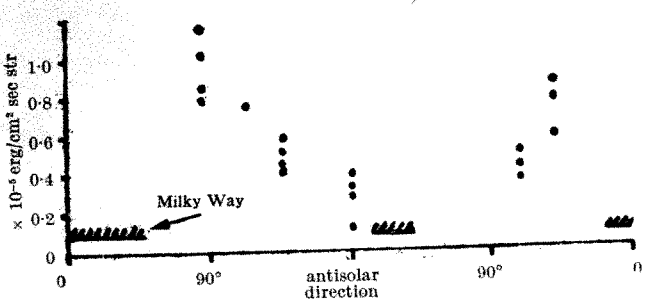


Fig. 1

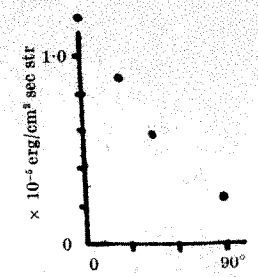


Fig. 2

elongation is greater than in a region of Milky Way without emission nebulæ.

An attempt was made to measure the radial velocities of H_α at 90° elongations. The dispersion of the monochromator used was 2 Å/mm for the fringe measured; the width of the H_α-line in the night-glow does not exceed 0·3 Å. A collimating lens of 2-m focal length sends into the apparatus the image of a white cross centred on the axis and illuminated by an H discharge tube. On the plate, points from the calibration cross were superimposed on the night sky fringes. The width of the line being 0·3 Å, a shift of 0·1 Å, corresponding to a radial velocity of 4·5 km/sec, could be easily detected. The measurements gave no certain evidence of relative shift of the two lines and the night-glow H_α radial velocity at 90° elongations must be less than the aforementioned value. In the antisolar direction a positive radial velocity was observed but not very certainly.

The small radial velocity and narrowness of the night-glow H_α line are strong arguments for its atmospheric origin. The observation can be fitted with the following model: neutral hydrogen forms a disk in the ecliptic plane 5,000 km thick and extended up to 3,000 km in the evening and to 10,000 km in the morning. During the night the hydrogen exosphere becomes denser as shown in a recent paper of Donahue (private communication). At sunrise intense dissipation begins, but the density remains high enough during several hours. It is a plausible explanation of the east–west asymmetry of the H_α night-glow. Such measurements can be used for determining the density of the exosphere, which depends strongly on the temperature of the thermopause. An interesting observational problem is the interaction of the Doppler-shifted Fraunhofer line in the zodiacal light and the H_α night-glow when observed with finite spectral resolving power. We have perhaps observed a darkening of the emission line at elongation near 90° due to such interaction.

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RADIOPHYSICS

21-cm Absorption of the Radiation from 3C273

The radio source 3*C*273 has been identified[1,2] with a star-like object of about thirteenth magnitude having a faint jet. However, the optical spectrum of the star contains a number of broad emission features which can be explained as hydrogen, magnesium and oxygen lines with a red-shift $\Delta\lambda/\lambda_0$ of 0·158 (refs. 2 and 3). Schmidt suggests that the stellar object is either a nearby ultra-dense star or, more probably, the abnormally luminous nuclear region of a galaxy at a distance of about 500 megaparsecs. The second possibility would make 3*C*273 the most luminous object known.

One method of deciding whether 3*C*273 is outside our Galaxy is to check whether its radio spectrum shows

absorption by the layer of neutral hydrogen in the galactic plane. Unfortunately 3*C*273 is at galactic latitude $b_{II} = 64°$, so that the opacity of the hydrogen layer is small. Over a large area around 3*C*273 the neutral hydrogen emission profile observed with a 14′ beam has a peak brightness temperature of 4° K and a half-width of 20 km/s (ref. 4). Assuming that the gas is uniformly distributed and has a spin temperature of 100° K, we could expect an opacity not greater than 4 per cent. With the 210-ft. reflector at Parkes the antenna temperature of 3*C*273 is 24° K, so that there would be an absorption dip of about 1° K if the source is extragalactic. A direct measurement of the absorption profile for this source with high accuracy presents considerable experimental difficulties.

Instead of measuring the whole absorption profile of 3*C*273, a shorter experiment has been performed to compare the opacity in front of this source and the source 3*C*270. The latter is only 4° away and has been identified[5] with the *E*3 galaxy *NGC*4261 in the Virgo cluster. Comparing the opacities makes it unnecessary to know the receiver characteristics extremely accurately. The 21-cm emission we observe with a beam-width of 14′ is uniform over the area around these sources, to that one would anticipate that the opacities would be similar.

A frequency-switching receiver was used to compare the radiation at the frequency of the peak of the line profile, f_1, with that at a frequency f_2, 3 Mc/s lower. If τ is the opacity of the gas in front of 3*C*273, the change in the receiver output between the source and the nearby sky can be written:

$$\Delta = G_1 \cdot e^{-\tau} \cdot T_1 - G_2 T_2$$

where G_1 and G_2 are the gains of the receiver at f_1 and f_2, $e^{-\tau}$, T_1 and T_2 are the antenna temperatures at f_1 and f_2. When $\tau \ll 1$ we can write:

$$\frac{\Delta}{G_1 T_1} \doteq \left(1 - \frac{G_2}{G_1} \cdot \frac{T_2}{T_1}\right) - \tau$$

Similarly for 3*C*270 we have:

$$\frac{\Delta'}{G_1 T_1'} \doteq \left(1 - \frac{G_2}{G_1} \cdot \frac{T_2'}{T_1'}\right) - \tau'$$

Hence:

$$\tau - \tau' \doteq \frac{\Delta'}{G_1 T_1'} - \frac{\Delta}{G_1 T_1} - \frac{G_2}{G_1}\left(\frac{T_2}{T_1} - \frac{T_2'}{T_1'}\right)$$

When $G_2 \approx G_1$, the last term will be about 0·1 per cent (taking spectral indices of 0·2 for 3*C*273 and 0·7 for 3*C*270 (ref. 5)), and this contribution can be ignored.

The measurements were made using a parametric receiver[6] with a noise temperature of 160° K, a bandwidth of 41 kc/s and a time-constant of 20 sec. The 210-ft. telescope was set on the source for periods of 5 min, followed by 5 min on a comparison region. Four comparison regions were used, 15′ away from the source in right ascension and declination. The effective integration time was about 20 min. One series of measurements was made in March 1963, another in June with the receiver tuning modified. The results are shown in Table 1. The error quoted is the standard deviation of the measurements.

Table 1

Date	Quantity	3*C*273	3*C*270
March 17	Δ/G_1	−0·14° ± 0·09° K	+0·15° ± 0·09° K
	Δ/G_1T_1	−0·6% ± 0·4%	+1·2% ± 0·7%
	$\tau - \tau'$	+1·8% ± 1·1%	
June 9	Δ/G_1	−1·61° ± 0·12° K	−0·66° ± 0·04° K
	Δ/G_1T_1	−6·7% ± 0·5%	−5·7% ± 0·4%
	$\tau - \tau'$	+1·0% ± 0·9%	

It will be seen that the opacity of the gas in front of 3*C*273 is not less than that in front of 3*C*270. Unless we invoke the unlikely possibility that there is a minute hole (diam. < 14′) through the galactic hydrogen in the direction of 3*C*270, this result supports Schmidt's suggestion that 3*C*273 is extragalactic.

We thank Mr. J. G. Bolton for suggesting the experiment and Mr. M. B. Mackey for assistance with the observations.

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Radio Emission from Flare Star *V*371 Orionis

During the past three years the Division of Radiophysics, Commonwealth Scientific and Industrial Research Organization, Sydney, Australia, has made a systematic attempt to detect radio emission from a number of the so-called flare stars situated in the Sun's vicinity. Slee, Patston and Higgins[1] have described the earlier work and results. Lovell, Whipple and Solomon[2] have also recently published the results of a similar flare-star survey conducted at Jodrell Bank. Taken together, these two surveys have established with a reasonable degree of certainty that at least some of the flares on the six red dwarfs examined were accompanied by detectable radio emission.

The present communication describes the reception of a particularly intense burst of radio emission from the *dM*3*e* star *V*371 Orionis (Wachmann's star) at the time of an optical flare on November 30, 1962. This occurrence establishes beyond doubt, we believe, that flare stars are powerful radio emitters.

The optical observations were conducted jointly by a Baker–Nunn camera installed at Woomera, South Australia, and by two 10-in. telescopes operated by Australian amateur astronomers. Radio observations were made at frequencies of 1,410 Mc/s and 410 Mc/s with the 210-ft. reflector at Parkes, New South Wales, and at 19·7 Mc/s with the north–south arm of the low-frequency cross situated near Sydney. Each of the higher frequencies made use of a Dicke system in which the feed dipole was switched against a reference load and the receiver gain adjusted to produce a noticeable noise fluctuation-level on the recorder chart with a smoothing time constant of 2 sec. In the 19·7-Mc/s observations, the two halves of the north–south arm of the cross were connected to different total power receivers. One radio beam was directed at the flare star and the other about 3° away in order to identify terrestrial interference.

On November 30, 1962, between 14h40m and 14h54m U.T. the 210-ft. reflector received a burst of radio noise at a frequency of 410 Mc/s. Aural monitoring at the time showed that the deflexion was not caused by any recognizable form of interference. A tracing of the 410-Mc/s recording is given in Fig. 1, which shows a smooth rise in aerial temperature with a peak value of 8·2° K at 14h48m. The descending portion of the burst is characterized by a number of temporary decreases in aerial temperature lasting from 6 sec to about 20 sec.

Shortly before the commencement of the 410-Mc/s burst, two independent teams of amateur astronomers located at different sites near Sydney, observed the flar

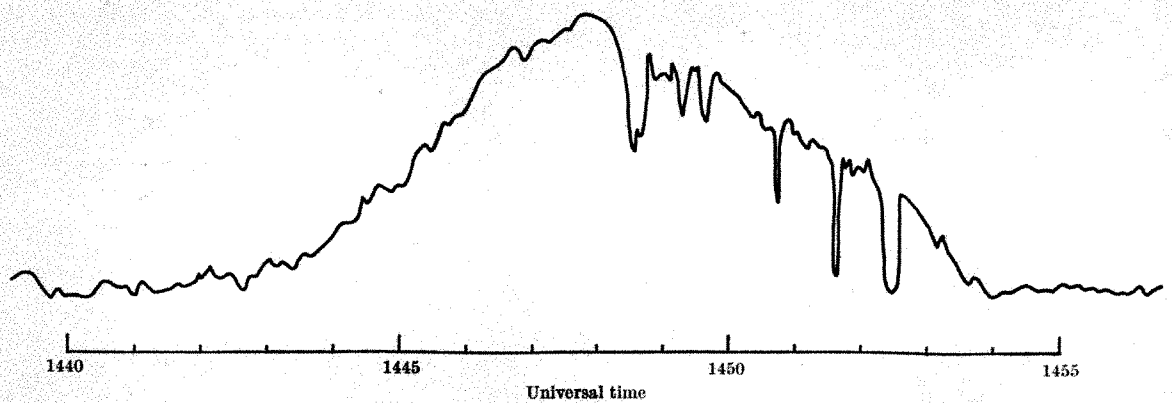


Fig. 1. Tracing of the 410-Mc/s record of November 30, 1962, during an optical flare on *V*371 Orionis

star to increase rapidly in brightness. The more detailed of the visual light curves is presented in Fig. 2*a* and shows a reasonably smooth variation in brightness with peak amplitude of about 0·6 magnitude.

At about the same time, the Baker–Nunn camera, using fast panchromatic film exposed for 8·8 sec in a 2-min cycle, recorded a definite onset of activity. This activity appears to have taken the form of a series of 'bursts' of energy, superimposed on a generally elevated level of brightness. However, it is possible that the increasing haze and cloud conditions (which caused termination of photography at 1507*Z*) and slippage of the film in transport through the camera (causing the photographed clock images to affect the star field) drastically affected the measured brightness of the star on some of these frames. The highest peak appears to have been about 1·0 magnitude above the quiescent brightness-level. Fig. 2*a* gives the results of the plate measurements, based on a modified Argelander estimation system.

The visual and photographic light curves show no detailed correspondence; this may be due in part to the difficulties described here. However, it is also possible that, superimposed on a smooth increase in the continuum in visual light, there were several flashes of blue or ultraviolet light. Alternatively, the flashes of light were in the visual region but of too short a duration to have been noticed by the observers, who were averaging the star's brightness over intervals of three or four minutes.

For the sake of completeness, the smoothed profile of the 410-Mc/s radio burst is shown in Fig. 2*b*. An investigation of the 1,410-Mc/s and 19·7-Mc/s radio records for the same night showed the existence of deflexions that were probably caused by the flare. On 1,410 Mc/s, a group of weak deflexions with peak excess aerial temperature of 0·52° K occurred between 14^h38^m and 14^h41^m U.T. The significance of these deflexions can be judged from the peak amplitude of the noise fluctuations of 0·13° K for the 10 min preceding and following the suspected flare-star radiation. Unfortunately, the 19·7-Mc/s record was marred by interference from a distant electrical storm. However, between 14^h32^m and 14^h48^m U.T. significant increases occurred in the base-level of the receiver connected to the radio beam directed at the flare star. No such radiation was received in the radio beam directed 3° away from the star, and no station interference could be heard in the loudspeaker. The intervals over which the probable flare radiation was recorded at 1,410 Mc/s and 19·7 Mc/s are shown in Fig. 2*b*.

The characteristic common to the radio bursts at all radio frequencies is their occurrence at, or close to, art of the optical flare; in this respect, they are to flare-induced solar-radio outbursts. From spectral data available, there appears to be no tendency for the high-frequency event to e lower-frequency burst as has been demonstra- e II solar outbursts. However, it is well known that the radiations emitted during a solar flare may have a very complex structure in the frequency-time plane, so that observations at widely spaced frequencies cannot be used to deduce the true form of the dynamic spectrum.

Table 1. Energies involved in Flare

Radio frequency or optical wave-length	Peak flux		Integrated flux	
	Density at Earth ($W\,m^{-2}(c/s)^{-1}$)	Radiated by star ($W\,(c/s)^{-1}$)	Density at Earth (joules $m^{-2}\,(c/s)^{-1}$)	Radiated by star (joules $(c/s)^{-1}$)
19·7 Mc/s	$2·3 \times 10^{-22}$	$1·6 \times 10^{14}$	$7·8 \times 10^{-20}$	$5·5 \times 10^{16}$
410 Mc/s	$1·2 \times 10^{-25}$	$8·5 \times 10^{10}$	$4·2 \times 10^{-23}$	$2·9 \times 10^{13}$
1,410 Mc/s	$8·6 \times 10^{-27}$	$6·0 \times 10^{9}$	$5·4 \times 10^{-25}$	$3·7 \times 10^{11}$
5,400 Å (visual)	$6·2 \times 10^{-28}$	$4·4 \times 10^{8}$	$1·6 \times 10^{-24}$	$1·1 \times 10^{12}$

The energies involved in the radio bursts are set out in Table 1, which also includes some estimates of the optical flare energies. Radio energy liberated at the star has been calculated from a recent parallax measurement by Van De Kamp[3] on the assumption that the source radiated uniformly into π steradians. The energy at optical frequencies was estimated from the increase in black-body radiation required to account for the brightness changes recorded by the visual observers. For these calculations, *V*371 Orionis was regarded as a typical *dM*3 star with $Te = 3{,}400°$ K and $\frac{R}{R_\odot} = 0·37$.

We have also estimated the total radio energy liberated during the flare by assuming (according to the data of Table 1) that the integrated flux varied as frequency $^{-2·5}$ over the complete radio spectrum and obtained a value of about 10^{24} joules. The corresponding optical energy radiated per 1000 Å was about 10^{26} joules.

It is interesting to compare these values with the corresponding radio and optical energies released during an intense solar flare. One of the most important solar-radio events was that of February 21/22, 1950, which[4] yielded about 10^{18} joules over the frequency-range 100–10,000 Mc/s, a value which is below the radio energy in the *V*371 Orionis event by a factor of 10^6. The continuum radiation in the optical region cannot be detected during the majority of importance 3 and 3+ solar flares and is estimated[5] to be < 1 per cent of the photospheric continuum emission per unit area. Hence over the area of a large flare that radiates at this rate for 1,000 sec, the optical energy emitted per 1000 Å is $< 10^{23}$ joules. Thus we see that the optical energy liberated by a $0·6^m$ flare on *V*371 Orionis exceeds that of an important solar flare by at least 1,000, and, unlike its solar counterpart, is not a trivial item in the energy balance of the whole star.

The form of the radio spectrum shows conclusively that the intense emission during a flare is due to a non-thermal mechanism. The total radio energy involved is only about

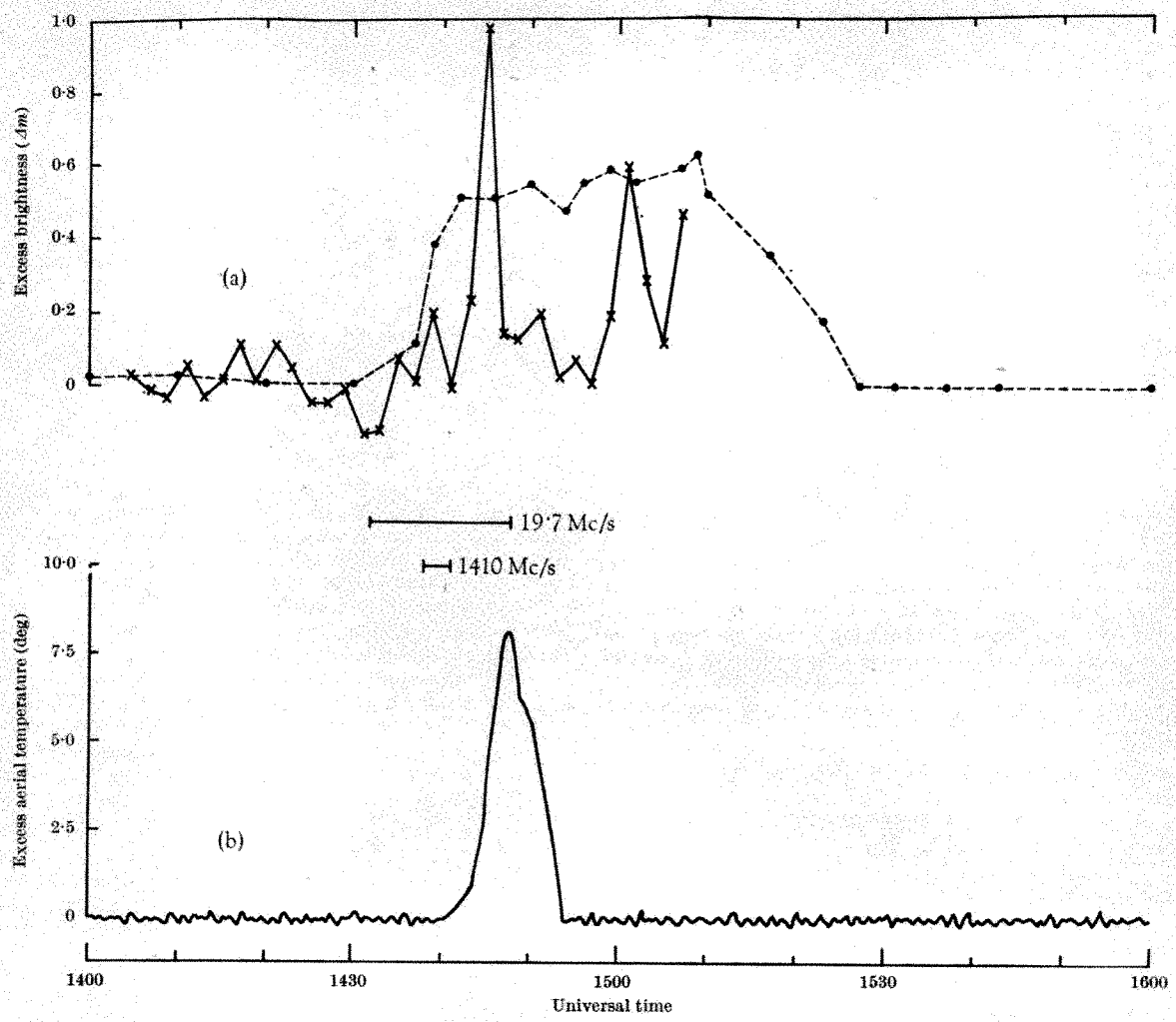


Fig. 2. (*a*) Light curves of the flare on *V*371 Orionis on November 30, 1962. Points marked ● refer to visual observations made by amateurs at Belfield Observatory, Sydney. Points marked × are measurements from the Baker–Nunn camera at Woomera, South Australia; (*b*) Smoothed profile of the radio burst at 410 Mc/s. The durations of probable burst activity at 19·7 Mc/s and 1,410 Mc/s are also indicated

1 per cent of the total optical energy liberated, but is probably a larger proportion of the total energy than the corresponding quantity for a solar flare. It is apparent that we are here dealing with a more efficient method of converting kinetic energy to radio energy. Synchrotron radiation by relativistic electrons gyrating about magnetic field lines is known to yield high ratios of radio to optical energy in some of the discrete radio sources. If such a mechanism is operating here, it may be possible to detect linear polarization in the flare radiation at the higher frequencies.

Finally, it is of interest to compare the radio flux from *V*371 Orionis with that received from the 6 flare stars investigated in the surveys[1,2]. In the Sydney survey conducted mainly at 85·5 Mc/s, 11 visual flares yielded an upper limiting value for the average peak flux density of 95 flux units per magnitude of peak brightness variation (1 flux unit $= 10^{-26}$ Wm^{-2} $(c/s)^{-1}$). The corresponding value obtained from 10 of the brighter flares in the Jodrell Bank survey at 240 Mc/s was 4·2 flux units per magnitude change. If we convert the 85·5 Mc/s value to 240 Mc/s by using a frequency $^{-2.5}$ spectrum found to apply to the *V*371 radio burst, we obtain a value of 7 flux units per magnitude (upper limit), which is in reasonable agreement with the Jodrell Bank result. Using the same spectral law on the *V*371 Orionis outburst, we find that the peak flux density observed at 240 Mc/s would be 45 flux units per magnitude of brightness change. This is an order of magnitude higher than the average value observed during the Sydney and Jodrell Bank flare-star surveys and may be additional evidence for an efficient process of energy conversion in this particular star.

We thank the Australian amateur astronomers, especially Messrs. L. Lockwood, R. Brownett, J. Flavin and K. Lay, who observed the flare visually. The 19·7-Mc/s radio observation was made by Mr. C. S. Higgins, and the Baker–Nunn exposures were made possible by the co-operation of the Australian Department of Supply.

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PHYSICS

Re-entrant Motion in Special Relativity

A recent communication[1] describes three methods of measuring the peripheral velocity of a disk rotating with fixed angular velocity. The purpose here will be to examine methods 'B' and 'C' in a little detail.

Let us suppose that a number (n) of closely spaced fiducial marks are made in the laboratory system all around the periphery of a rotating disk; these are numbered consecutively from 1 to n in the direction of disk rotation. At the location of each mark is a clock. These clocks are synchronized by some convention; the conclusions drawn here are convention-independent, but we may suppose that the usual (Einstein) convention is used. By t_0 will be meant a time at which a single fiducial mark on the disk coincides with mark n in the laboratory, as recorded by the clock located at mark n; by t_i, $i=1, \ldots, n$ will be meant the time first following the coincidence at time t_0 at which the mark on the disk coincides with mark i, as recorded by the clock located at mark i.

Method B may presumably be applied in connexion with any two adjacent marks to find the disk-edge speed v_B. Furthermore, the value obtained for v_B should not depend on which pair of marks is used. Hence it follows that the distance (measured in the laboratory) between adjacent marks i–1 and i (where mark n is also called mark 0) is given by:

$$d_i = v_B(t_i - t_{i-1}),\ i=1, \ldots, n$$

If this is summed from $i=1$ to n, there follows:

$$\Sigma_i d_i = v_B(t_n - t_0)$$

On the other hand, the speed v_C obtained by method C is related to the circumference of the disk $2\pi r$ by:

$$2\pi r = v_C(t_n - t_0)$$

But $2\pi r$ also equals $\Sigma_i d_i$, according to the definition of circumference. Hence v_C equals v_B, contrary to the conclusion reached by Dr. Phipps.

It should now be apparent that Dr. Phipps's analysis of circular motion, based on the assumption that $v_C \neq v_B$, cannot easily be supported by analysis consistent with elementary mathematics; moreover, that a non-equality of v_B and v_C would be most disturbing, in view of the great similarity in the operational definition of the two velocities. Few doubts should remain, for there is nothing in this communication to disprove the applicability of special relativity (with all its traditional idealizations) to problems of re-entrant motion.

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A Fundamental Extension of Einstein's Diffusion–Mobility Relationship

Many readers will no doubt have observed that the concepts of particle-diffusion force and gained energy, which were put forward recently[1] under the above title in these columns, are long familiar in the field of irreversible thermodynamics.

The Einstein relation between diffusion coefficient and mobility is theoretically valid in the limiting case of an infinitely low concentration of diffusing solute. In this limit the 'gained energy' is exactly the chemical-potential difference per particle. The particle-diffusion force is the gradient of chemical potential which at constant temperature is proportional to the generalized thermodynamic force of Onsager[2] conjugate to diffusive flux.

These basic concepts, as is suggested in ref. 1, are indeed essential in the investigation of transport phenomena and have, in fact, a much wider range of validity than has the Einstein relationship. Generalized thermodynamic forces are defined for concentrated and multicomponent solutions, and not only for diffusive transport, but for any irreversible fluxes of energy or particles. Such fluxes J_i are related in a conjugate manner to forces X_i by a calculation of the rate of production of entropy σ in their presence:

$$\sigma = \sum_i J_i X_i$$

For diffusive flux:

$$X_i = -\frac{1}{T}(\text{grad}\ \mu_i)_T$$

where μ_i is the chemical potential of species i.

The thermodynamics of irreversible processes, as a phenomenological theory, has been brought to a satisfactory state of formality over the past thirty years, and there are several excellent texts[3] available. It seems, however, to occupy a 'no-man's land' between chemistry and physics and is not as widely disseminated in schools of either chemistry or physics as it might be. It would provide useful material for a first-year graduate course, and, as a statistical-mechanical theory, is a new and rapidly expanding field for research.

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Suppression of Dust

While examing the beneficiation of fine coal from the Bulli seam, New South Wales, by froth flotation, the hydrophobic characteristics of the coal were observed. Air-borne dust from this seam had an average composition of fixed carbon 60 per cent, volatiles 26 per cent, ash-forming constituents 12·5 per cent and moisture 1·5 per cent. This dust contained a high proportion of vitrain.

Methods of dust suppression which had been examined included water infusion into both pillar and solid coal, water sprays and wetting agents. These methods had proved to be unsatisfactory, and in addition it was considered that their practice would interfere with subsequent beneficiation. It was decided that methods of suppression which made use of the coal characteristics and which may also be useful in subsequent beneficiation work should be examined.

Since the coal was hydrophobic, it appeared that three methods could be used to induce suppression.

Laboratory investigations were carried out using a dust chamber in which dust counts could be made at selected intervals of time to compare the effectiveness of suppression in treated coal. The first method utilized reagents commonly used as depressants in coal flotation to induce hydrophilic surface properties on the coal particles, thereby inducing wettability and subsequent suppression by sprays. Reagents used included potassium permanganate, but results were generally unsuccessful as this coal was difficult to depress. Results using other oxidizing reagents

showed no improvement when compared with water, and in some instances were inferior.

In the second series of tests, various coal flotation reagents (collectors) were used to induce agglomeration by adhesion similar to that achieved in froth flotation. Some success was achieved. With oils and alcohol frothers, particle adhesion and suppression were obtained. Work on the process was discontinued because of the problems which would arise if these materials were to be used in the mine.

For the third method, particle adhesion or agglomeration using flocculating reagents was attempted. Previous work on coal flotation had shown the possibility of floating coal, oxidized coal and ash-forming constituents using amine reagents, which also induced excellent flocculation.

Amine reagents gave satisfactory results and afterwards tests were made using starch, which is commonly used as a flocculant in coal preparation.

Starch is a non-toxic reagent and is therefore suitable for use in a mine. Further, its admittance to the washery circuit water would not influence washery performance. It is already in common use in washeries as a flocculating reagent.

Results obtained when starch was added to the water used for suppression in the dust chamber tests were satisfactory. Further, coal which was treated with starch, when dried and re-wetted by a water spray, retained to a marked degree its reduced dustiness.

It is probable that any non-toxic polyelectrolytes or polyacrylamides commonly used as flocculating reagents would give satisfactory results as dust suppressing reagents.

Adhesion induced by the use of flocculating reagents forms a practical method of dust suppression, especially in handling dust particles which are hydrophobic.

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Size and Growth of Cavitation Bubble Nuclei

Some years ago, one of us (K. S. I.), working in the laboratory of the late Prof. E. G. Richardson (King's College, Newcastle upon Tyne), made use of sound reverberation and optical scattering methods[1] in an attempt to detect the presence and determine the size-distribution of nuclei responsible for the low tensile strength of liquids. During the course of the experiments it became evident that the nuclei existed in two forms in water—as stable air bubbles and as solid suspensions. The bubble nuclei, apart from possessing other unique features, were characterized by measurements of a consistent and reproducible nature when water containing them was subjected to ultrasonic and hydrostatic pressures. The measurements associated with the solid nuclei were erratic and showed considerable scatter.

The bubble nuclei pulsate in an acoustic field and expand rapidly into visible size when the intensity of the field reaches the cavitation threshold. In water saturated with air, a fully grown bubble remains stable while in under-saturated water it quickly reduces in size and disappears when the acoustic field is cut off. Theoretically cavitation, or the growth of a bubble nucleus to visible size, should occur when the ambient hydrostatic pressure falls below the pressure of the air inside the bubble. This can happen during the rarefaction part of the acoustic cycle when the hydrostatic pressure temporarily becomes negative. Since the internal pressure is a function of the size of the bubble and the surface forces acting on it, the cavitation threshold will depend on these factors. The nature of this dependence can be experimentally determined in the following manner.

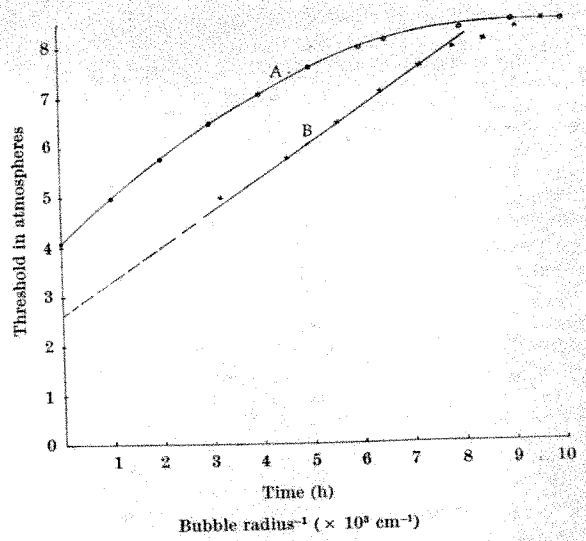


Fig. 1

A convergent beam of ultrasonics is focused on a small region of freshly drawn tap-water kept in a vessel. Initially the water may contain visible bubbles which soon rise to the surface and disappear, leaving a clear liquid without a trace of a rising bubble even when examined under the microscope. If the intensity of the sound is now

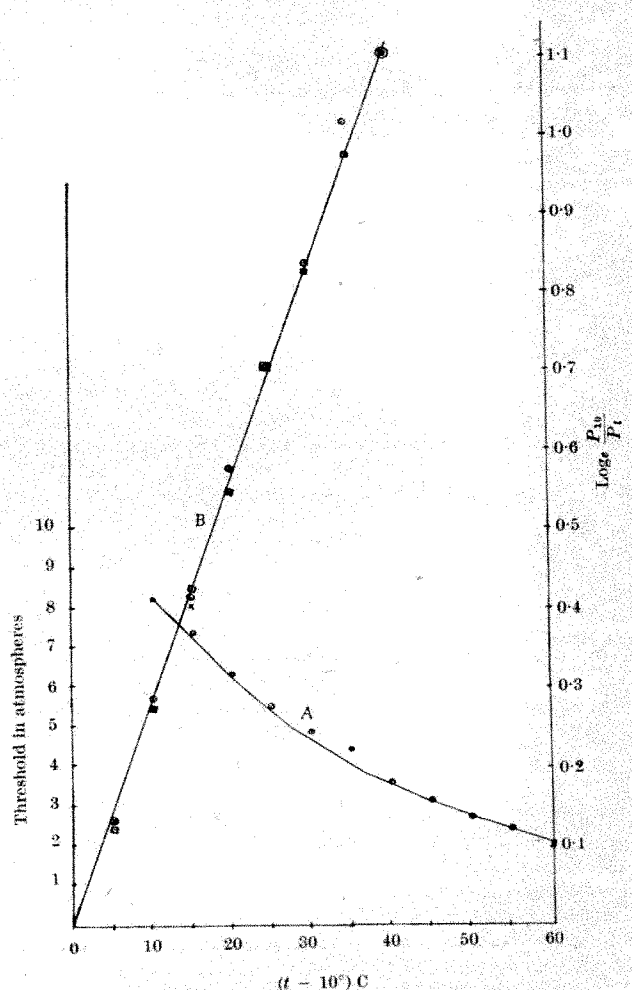


Fig. 2. ×, Conolloy and Fox; ○, fundamental; □, third harmonic

gradually increased, visible bubbles appear at the focus at an acoustic pressure corresponding to the cavitation threshold. If the water remains undisturbed, this threshold pressure rises steadily and becomes constant after several hours as shown in Fig. 1 (*A*). The steady rise in the threshold can be explained as due to the difference in the size of the bubble nuclei, as they ascend through the water, past the focus with a rate of rise given by Stokes's formula $r^2=\frac{d}{2\times 10^4 t}$, the smaller nuclei taking longer time to reach the focus from the base of the vessel. Knowing d, the distance between the base and the focus the radius of the bubble is easily calculated from the time of rise. In Fig. 1 (*B*) the reciprocal of the bubble radius calculated in this manner has been plotted against the corresponding threshold pressure P. The relation which is linear is given by the general expression:

$$P=a+b/r \qquad (1)$$

The constants a and b are affected by the surface contaminations of the bubble and may vary slightly for different samples of water. a is of the order of one atmospheric pressure, and b has a value of about 500 dynes/cm. The plots corresponding to the smaller bubble nuclei shown at the top of the graphs deviate from the straight line relation indicating the failure of Stokes's law when applied to bubbles of 1μ or less. Nuclei of this size remain trapped in water due to the thermal agitation of the molecules. The size of the nuclei reported by other workers[2] are in good agreement with these values.

The cavitation threshold of tap water shows considerable variation in temperature. We have measured the change in the temperature-range 10°–60° C, with a focused beam of ultrasonics generated by a polarized bowl of barium titanate. The behaviour is shown in Fig. 2 (*A*). Plotted on logarithmic scale the threshold varies linearly with temperature. Fig. 2 (*B*) is a plot of $\log_e P_{10}/P_t$ against the temperature interval $(t-10)$ where P_{10} is the threshold pressure at 10° C. The measurements were made with two excitations of the bowl—the frequencies of the fundamental and the third harmonic. The data obtained by Fox[3] at a frequency 1 Mc/s are also shown plotted. All these points lie on a single straight line leading to the expression:

$$P_t=P_{10}\,e^{-\alpha(t-10)} \qquad (2)$$

As the size of the bubble nucleus determines the cavitation threshold the exponential fall of the pressure is indicative of the fact that the growth-rate of the nucleus is exponential when the temperature is raised. Combining expressions (1) and (2) the change in the radius of the nucleus with temperature may be shown to be $r_t=r_{10}e^{\beta(t-10)}$ where $\beta=\left(\frac{ar_t}{b}+1\right)\alpha$. Assuming the value $1\cdot5\times10^6$ dynes/cm^2 for a and 5×10^2 dynes/cm for b, and the measured value 0·02/deg. C of α, the calculated rate of size increase β is 0·026/deg. C when the nucleus is one micron in radius and 0·08/deg. C when the same nucleus grows to 10 times its former size, showing that the growth rate increases with size.

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GEOLOGY

Age Measurements of Antarctic Rocks (Queen Maud Land)

This communication is a report of the age measurem project carried out on rocks from the Sör-Rondane Mo tains (Queen Maud Land, 23° E. to 28° E.). The ro were sampled by Picciotto[1,2] during the 1958 Belg Antarctic Expedition led by Gaston de Gerlache[1]. first set of results has already been published[3]. T biotite strontium/rubidium ages of various eruptive a metamorphic rocks ranged from 460 to 500 million yea

Radiogenic strontium-87 is known to migrate out biotite minerals[4,5]. In the available samples, miner other than biotite have been selected together w whole rocks of sufficient high rubidium/strontium rat Isotopic measurements on zircons were also carried o

The analytical methods used are close to those describ by Aldrich *et al.*[6] and Tilton *et al.*[7]. The geological a petrographic descriptions of the Sör-Rondane Mountai have been published by Picciotto *et al.*[8] and Michot[9,10].

The new results, given in Table 1, confirm the existen of a magmatic and metamorphic activity, roughly 5 million years old (between 460 and 600 m.y.). No great ages have been found in this region.

Table 1. Mineral and Total Rock Ages on Antarctic Rocks (Que Maud Land)

Location*	Sample No.	Source rock	Mineral†	Method	Age (m.y.
Romnoesfjellet	*R1*	Porphyroblastic	B	Sr/Rb§	480 ±
	R1a	granite of intru-	Z	$^{207}Pb/^{206}Pb$¶	540 ±
		sive type		$^{207}Pb/^{235}U$	518 ±
				$^{206}Pb/^{238}U$	514 ±
Nordtoppen 950		Granitic vein in	B	Sr/Rb	463 ±
		diorite	Z	$^{207}Pb/^{206}Pb$	500 ±
				$^{207}Pb/^{235}U$	508 ±
				$^{206}Pb/^{238}U$	510 ±
Nordtoppen 1100	*S9*	Gneiss xenolith	B	Sr/Rb	475 ±
		in diorite	Z	$^{207}Pb/^{206}Pb$	550 ± 1
				$^{207}Pb/^{235}U$	555 ±
				$^{206}Pb/^{238}U$	555 ±
Gunnestadbreen		Granite of intru-	B	Sr/Rb	474 ±
		sive type	F	Sr/Rb	482 ± 1
			Z	$^{207}Pb/^{206}Pb$	575 ±
				$^{207}Pb/^{235}U$	524 ±
				$^{206}Pb/^{238}U$	512 ±
Austkampane	*K16*	Muscovite bio-			
		tite corundum	B	Sr/Rb	492 ±
		gneiss	M	Sr/Rb	510 ±
Strandrudfjellet	*T4*	Fine-grained	B	Sr/Rb	483 ±
		microcline gran-	F	Sr/Rb	460 ±
		ite (anatectic ?)	WR	Sr/Rb	500 ±
Bautaen	*A3*	Fine-grained	B	Sr/Rb	503 ±
		microcline gran-	F	Sr/Rb	476 ±
		ite (anatectic ?)	WR	Sr/Rb	590 ±

* The geographical names refer to the map of the Sör-Rondane Mountai published by the Norsk Polar-institutt during 1957.
† B, Biotite; M, muscovite; F, feldspar; WR, whole rock; Z, zircon.
‡ $\lambda_{^{87}Rb}=1\cdot39\times10^{-11}$ yr.$^{-1}$, rubidium-87 = 0·283 g/g rubidium.
§ Common strontium $^{87}Sr/^{86}Sr$ = 0·709 (measured on calcic feldspar fro Bautaen granite).
¶ $\lambda_{^{238}U}=1\cdot54\times10^{-10}$ yr.$^{-1}$, $\lambda_{^{235}U}=9\cdot72\times10^{-10}$ yr.$^{-1}$.

The small, but systematic, discordance between th younger biotite ages and the older whole rock and zirco ages seems to be significant. It follows a pattern alread described in the literature[11].

In the present case, our tentative interpretation is a follows: the emplacement of the intrusive-type granite (Nordtoppen, Romnoesfjellet and Gunnestadbreen) an the differentiation of the fine-grained 'anatectic' granite occurred between 500 and 600 million years ago. A distinct phase of the geological evolution of this regio might have happened around 480 m.y. ago; it coul correspond to an uplift of the rocks into a superficia zone where radiogenic strontium-87 diffusion has stopped according to the views of Hurley *et al.*[5]. An alternat interpretation would be to assume, at this time, a retro morphose mesozonal episode. Evidence of such a retro morphose has been observed in thin sections[9,10].

We thank Drs. J. Michot and E. Picciotto for stimulating discussions.

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CHEMISTRY

Extensive Reduction of Coal by a New Electrochemical Method

In recent years electrolytic methods have been used in the reduction and structural elucidation of coal[1-2]. The usefulness of these methods is limited by the fact that the benzene ring (that is, the isolated benzene ring as in benzene or tetralin), which may represent a large portion of the coal structure, has not previously been reduced electrolytically. Work here has shown that reduction of benzene can be achieved by electrolysis in ethylenediamine saturated with lithium chloride[3]. The same method has now been successfully applied to the reduction of a high-rank coal and has resulted in the addition of 44 hydrogens per 100 carbon atoms. The best electrochemical reduction achieved[2] prior to this was carried out in dimethylformamide and amounted to the addition of 14 hydrogens.

Six grams of the coal (Pocahontas vitrain ground to pass 325 mesh) suspended in 100 ml. of ethylenediamine containing 1·4 g of lithium chloride was electrolysed at 33° between carbon electrodes at 0·5 amp and 115 V. Apparatus and technique of electrolysis[3] and recovery of the reduced coal[4] were the same as described previously. Current efficiency at the beginning of the electrolysis was 46 per cent and dropped to 10 per cent after 15 h. The electrolysis was continued for an additional 15 h, during which time the current efficiency remained practically constant. The reduced coal was recovered from the solution in 95 per cent yield, analysed and subjected to a second and third electrolytic reduction; it was tan-grey in colour. Analyses of the original and reduced samples are shown in Table 1.

Table 1. Electrochemical Reduction of Pocahontas No. 3 Vitrain

	Grams	C	H	N	S	Ash	H atoms added* per 100 C atoms in starting material
Original vitrain	—	87·66	4·22	1·06	0·65	2·45	—
	—	87·97	4·42	1·08	0·73	2·39	—
First reduction	6·10	83·05	6·43	3·39	0·63	2·55	24·3
	—	84·19	6·18	3·44	0·95	2·52	—
Second reduction	5·80	80·72	6·95	4·47	0·36	2·47	32·4
	—	80·80	7·12	4·32	0·38	2·39	—
Third reduction	3·16	80·73	7·73	4·15	0·39	2·76	43·7
	—	80·48	7·76	4·24	0·25	3·03	—

* Method of calculation as described in ref. 4.

The ratio of lithium chloride used to total hydrogen taken up by the coal was about 1 : 2. This ratio is much more favourable than that obtained in the chemical reduction of the same coal[4] where the ratio of lithium metal to hydrogen added was 1 : 0·1. An interesting resul its the fact that the solubility of the electrolytically reduced coal in pyridine at room temperature (74 per cent) is higher than that of the chemically reduced coal. In the latter case, 45 hydrogens were added but the solubility was only 63 per cent. It is conceivable that chemical reduction at elevated temperature, in contrast to electrolytic reduction at room temperature, is accompanied by polymerization resulting in a decrease in solubility. The advantage of ethylenediamine over dimethylformamide[2] is probably connected with the fact that ethylenediamine can solvate lithium. Experiments[3] indicate that lithium acts as an electron transfer agent in the reduction of benzene in the ethylenediamine–lithium chloride system.

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Interaction of Methyl-substituted Borazines with Various Acceptor Molecules

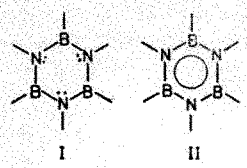
It has been suggested that borazines might be expected to form molecular complexes with electron-pair acceptors[1], and interactions between hexamethylborazine and tetracyanoethylene[2] or iodine[3] have been reported. In further support of this suggestion we wish to report the initial results of a spectrophotometric investigation of the interaction between methyl-substituted borazines and some acceptor molecules. Solutions of B-trimethyl-, B-trimethyl-*N*-methyl-, B-trimethyl-*N*-dimethyl-, and hexamethyl-borazine in carbon tetrachloride and in *n*-heptane exhibit featureless absorption spectra in the range 600–200 mμ, but the spectra of mixtures containing these borazines and typical acceptor molecules in the same solvents exhibit bands which are not characteristic of the acceptors. (See Table 1 for some representative data.)

Table 1. Positions (mμ) of New Absorption Bands exhibited by Mixtures of Some Methyl-substituted Borazines and Various Donors*

	$(Me)_3B_3N_3H_3$	$(Me)_3B_3N_3H_2Me$	$(Me)_3B_3N_3H(Me)_2$	$(Me)_3B_3N_3(Me)_3$
Iodine	290	289, 390	294, 390	291, 390
Picric acid	250†	260†, 433	258†, 455	258†, 455
p-Benzoquinone	none	335	335	335
Chloranil	none	345	350	345
Tetracyanoethylene	—	321	320	320

* 10 : 1 mole ratio of borazine to acceptor, at a concentration of 10^{-3} M.
† Shoulder.

The new bands appear in the spectral region in which charge-transfer bands for aromatic systems occur and can be attributed to molecular complexes formed between the borazine and the acceptors. It is noteworthy that complex formation involving B-trimethylborazine produces the smallest effect on the spectra of the free acceptor molecules, and that mixtures of B-trimethyl-*N*-methyl-, B-trimethyl-*N*-dimethyl-, or hexamethyl-borazine with the same acceptors exhibit substantially equivalent spectra. The spectra of iodine, sulphur dioxide, maleic anhydride, tetracyanoethylene, *p*-benzoquinone, picric acid, chloranil, and iodine monochloride are markedly affected by the addition of tetra-, penta-, or hexa-methylborazine; the last three named acceptors undergo a further slow reaction, and orange-coloured precipitates separate from mixtures containing picric acid on standing.



I II

The formation of molecular complexes containing the borazine nucleus is not necessarily indicative of its 'aromatic nature'. Theoretically, borazines could form complexes with Lewis acids if the π electrons were either localized on the nitrogen atom (I) or if they were partially delocalized (II) as in benzene. In the former case borazine would be formulated as a cyclic triamine and it would be expected to act as a Lewis base. It is well established that amines form molecular complexes with some of the same donors which interact with aromatic systems, for example, sulphur dioxide and iodine. In addition, several strong donors, for example, hydrogen chloride, bromine, and chlorine, are known to add to adjacent boron and nitrogen atoms in the borazine nucleus to form the corresponding analogues of *cyclo*hexane. The solids which separate from mixtures of picric acid and the borazines may have been formed by a similar addition.

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Dissolution of Glass at Elevated Temperatures

The finite solubility of glass containing vessels has long been the concern of chemists interested in the properties of solutions and hence in preparing solutions of accurately controlled composition. By the use of certain types of glass or of silica this problem can, to a large extent, be eliminated at temperatures near ambient. However, recently, certain experimental results have been obtained which have brought to our notice the high solubility of a hard borosilicate glass at elevated temperatures.

An attempt to use glass feed tubes in a boiler operating at 200° C was unsuccessful owing to the high rate of solution of the glass. A stout tube of wall thickness of more than 1 mm was reduced to paper thinness after exposure to oxygenated sea water at 200° C for 16 weeks. Even when exposed for some time to salt solutions boiling at atmospheric pressure, glass beakers have been found to undergo marked etching rendering them partially opaque.

A more striking example of the errors which can be introduced by neglecting glass solubility has recently been obtained during an investigation of the dissolution of zinc in millimolar sodium chloride at elevated temperatures in glass-lined autoclaves. The product obtained was generally found by X-ray analysis to consist of zinc oxide or an unknown compound giving a diffraction pattern identical with one of a series found by Gilbert[1] and designated by the letter *E* (Fig. 1). This compound was found to be insoluble in acids and further examination of the product and comparison of its diffraction pattern with those of the *A.S.T.M. Index* proved it to be a well-crystallized specimen of zinc pyrosilicate hydrate ($Zn_4(OH)_2Si_2O_7.H_2O$, hemimorphite) (Fig. 1). Samples of this material could be prepared at 80° C, 140° C and 200° C by exposure of the zinc to the above oxygenated solution for several hours. In Gilbert's experiments this compound was formed at atmospheric pressure after exposure of zinc

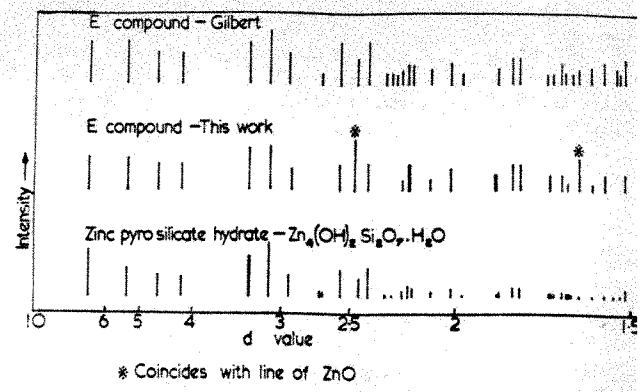


Fig. 1

or zinc hydroxide to distilled water at 85° C for periods of 35 days and upwards. It is quite clearly associated with dissolution of silica in some form from the glass container and interaction with the zinc corrosion product to give the silicate. In a separate experiment the dissolution rate of a borosilicate glass liner was found by loss of weight to be 0·22 mg/dm²/day at 140° C. This agreed closely with the amount of silica found by analysis in the millimolar chloride solution used.

The fact that the dissolution of glass is appreciable in distilled water and dilute salt solutions at temperatures as low as 80° C is noteworthy and should be borne in mind in chemical tests and, in particular, in dissolution tests on metals since silicates are well-known corrosion inhibitors.

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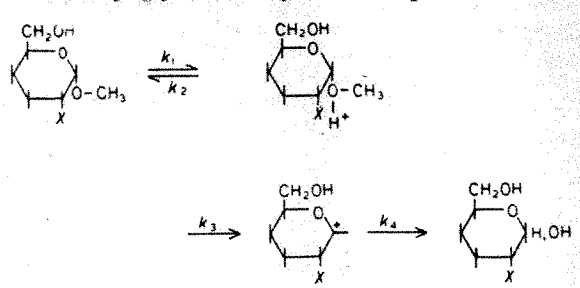
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Rates of Acid Hydrolysis of 2-substituted Methyl Glucopyranosides

The hydrolysis of methyl glycosides probably proceeds through the formation of an oxonium salt as intermediate[1–3]. The steps involved in the reaction of water with a methyl glycoside may thus be represented:



where k_3 represents the rate constant for what is generally believed to be the rate-controlling step.

Shafizadeh[4] has stressed how the inductive effect of a hydroxyl group at C-2 (*X*) may be used to explain the difference in the rates of acid hydrolysis of the methyl glycopyranosides of D-glucose and of 2-deoxy-D-glucose. He has further pointed out the similarity in the effect of a β-hydroxyl group on the stability in acid between these methyl glycopyranosides and the corresponding diethyl acetals.

I wish to indicate how the effect of a C-2 substituent on the rate of hydrolysis of a methyl glycopyranoside may be related in a quantitative way to the *pK* value of the corresponding substituted acetic acid.

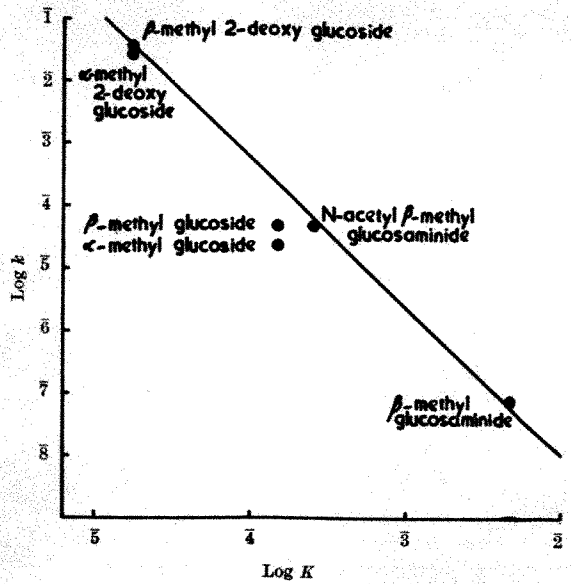


Fig. 1. Relationship between the rates of hydrolysis (k in sec^{-1} units) of various 2-substituted methyl glucopyranosides in N acid at 72·9° and the apparent dissociation constants (K) of the corresponding substituted acetic acid at 25°

If one considers the series of methyl glucopyranosides in which X, the substituent on C-2, is in turn H—, OH—, CH_3CONH— and NH_3^+—, then as X becomes more electron-attracting, the formation of the oxonium salt will be inhibited. The rate constants for hydrolysis (k) at 72·9° in presence of a molar concentration of hydrogen ions for such a series of compounds have been compared with the equilibrium constants (K) for the acid dissociation of the corresponding series of carboxylic acids. Some of the rate constants were obtained in hydrochloric acid and some in perchloric acid, those obtained in the latter tending to be a little larger than those obtained in hydrochloric acid of the same concentration[5], since the reactivity is related directly to the Hammett acidity function. However, the differences in rates of hydrolysis occasioned by the different acids are small relative to those due to inductive effects so that for present purposes they may be ignored. The values are shown in Table 1.

When log k is plotted against log K (Fig. 1), a linear relationship is obtained, indicating a relationship of the form:

$$\log k = \rho \log K + A \qquad (1)$$

the type of relationship envisaged by Hammett[9]. The value of ρ found is $-2{\cdot}34$ (cf. the value $-3{\cdot}65$ for the hydrolysis in acid of various ethyl acetals[10]). It seems reasonable to suppose that since the increased acidity of the carboxyl group of glycollic acid or glycine relative to that of acetic acid is due to the inductive effect of the hydroxyl or positively charged amino group respectively[11], so also is the lower reactivity of β-methyl glucoside or glucosaminide[8] relative to that of β-methyl 2-deoxyglucoside.

Table 1. Rates of Hydrolysis in Molar Acid Concentration at 72·9° for the Series of 2-(X-Substituted)-Methyl Glucopyranosides for Various X Substituents

Methyl glycoside of compound where X =	Anomer	Rate of hydrolysis 10^5 k (sec^{-1})	Corresponding acid	Acid dissociation constant (pK 25°)
—H	α	2·4 × 10^6 (*a*)	Acetic	4·76
	β	3·5 × 10^6 (*a*)		
—OH	α	2·5 × 10^5 (*b*)	Glycollic	3·83
	β	5·0 × 10^5 (*c*)		
—NHCOCH$_3$	β	4·6 × 10^5 (*d*)	Acetyl glycine	3·60
—NH$_3^+$	β	7·6 (*d*)	Glycine	2·35

(*a*) In perchloric acid, extrapolated from data in ref. 5; (*b*) in hydrochloric acid, ref. 6: corresponding value in N perchloric acid = 1·0 × 10^5, ref. 1; (*c*) in hydrochloric acid, ref. 7: corresponding value in N perchloric acid = 2·1 × 10^5, ref. 1; (*d*) in N hydrochloric acid, from data in ref. 8.

The reactivity in acid solution of methyl D-glucoside would be expected to be about 5·3 times less than that of methyl 3-deoxy-D-glucoside using our ρ value in equation (1) together with the pK values (at 25°) for glyceric acid (3·55) and lactic acid (3·86). This value agrees fairly closely with that which has been found for the α-anomers (7·4 times)[12].

For a given aliphatic aglycon it is probable, therefore, that the largest differences in the rates of hydrolysis of the resulting pyranoside are due to differences in the inductive effect of the substituent at C-2. Substituents at the remainder of the carbons have much less effect in altering the reactivity of the methyl glycosides of the pyranoses[4]. The operation of this inductive effect explains why, in naturally occurring compounds, the glycosidic bonds involving neutral sugars are cleaved by acid much more readily than those involving amino sugars.

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Enhanced Photoreactivity of Riboflavin in Aqueous Solution in the Presence of Macromolecules

The photoreduction of riboflavin in aqueous buffered solutions illuminated with visible light is considerably enhanced when colloidal substances such as micellar sodium decyl sulphate, the non-ionic surface-active agent polyoxyethylene 20 sorbitan mono-oleate, and the non-ionic polymer polyvinylpyrrolidone are present. Although a part of the catalysis produced by the non-ionic surfactant and the polyvinylpyrrolidone might be attributed to the

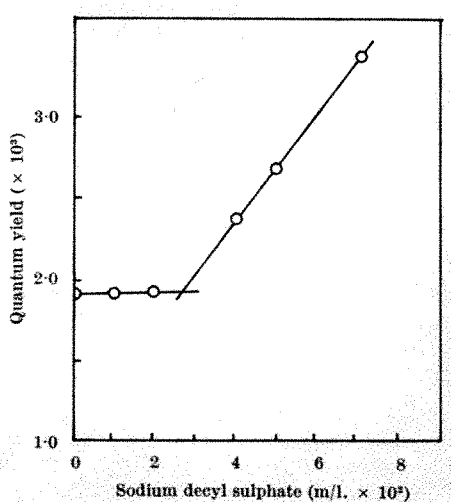


Fig. 1. Effect of sodium decyl sulphate on photoreduction of riboflavin by visible light in 0·05 M phosphate buffer, pH 6·8, 26° C

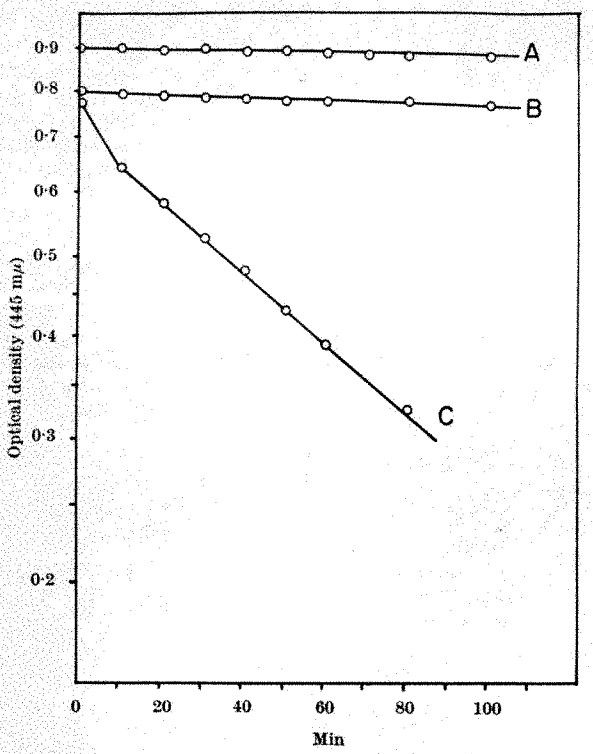


Fig. 2. Influence of micellar sodium decyl sulphate on rate of photo fading of acetyl riboflavin in 0·05 M phosphate buffer, *p*H 6·8, 26° C. Acetylriboflavin in: *A*, 0·07 M sodium decyl sulphate; *B*, 0·01 M ascorbic acid; *C*, 0·07 M sodium decyl sulphate plus 0·01 M ascorbic acid

presence of residual reducing groups, the effect of micellar sodium decyl sulphate appears to be wholly a result of its colloidal nature. Non-micellar sodium decyl sulphate has no effect on the photolysis of riboflavin as illustrated in Fig. 1.

Acetylation of the riboflavin side-chain hydroxyls produces a relatively light-stable compound, since normally the proton involved in the photoreduction originates from the ribityl side-chain[1]. As shown in Fig. 2, the light stability of acetylriboflavin is not influenced by micellar sodium decyl sulphate unless a reducing agent such as ascorbate is added to the system; the presence of the ascorbate without sodium decyl sulphate, however, has little influence on the light stability of acetylriboflavin.

The observed catalytic effects are attributed primarily to interaction of an excited riboflavin species, probably triplet, with the micellar surfactants and polymers[2]. The enhanced photoreactivity in such systems suggests certain similarities between these models and photochemical processes occurring in aggregated states in biological systems.

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BIOCHEMISTRY

The Possible Biological Function of Pseudocholinesterase

Although the main function of acetylcholinestera (AChE) at the synapses of cholinergic nerves has bee fairly well established, the biological role of pseud cholinesterase (PsChE) has not[1–3,13]. It has been pr posed, however, that the latter enzyme is concerned wit relatively slow nerve conduction processes[3], and that th blood cholinesterases might be important in limitin responses to hydrolysable cholinergic agents[4].

Acetylcholine (ACh) is hydrolysed very quickly an butyrylcholine (BuCh) very slowly by AChE whil PsChE hydrolyses BuCh very rapidly, at about 2–3 time the rate of ACh[5]. Burgen and MacIntosh[6] suggested tha the natural substrate for PsChE might be somethin other than ACh. This substrate may be BuCh since i seems to be hydrolysed by PsChE more rapidly than an other substrate[7].

The capacity for ACh synthesis is widely distributed i animal tissue but is predominant in nervous structures and liver tissue (serum PsChE is also formed in the liver[8]) it requires the presence of adenosine triphosphate, co enzyme A (CoASH), acetate or citrate, choline and th enzymes acetyl kinase and choline acetylase[6,9].

In tissues, especially those of the liver, certain othe acyl-coenzyme A derivatives are equally capable of takin the place of acetyl-coenzyme A ($CH_3.CO \sim SCoA$) to giv the corresponding choline esters[9].

In mammals, fatty acid metabolism occurs mainly in th liver. Since the penultimate product of fatty acid degen eration and the primary product of lipogenesis in the fatt acid cycle[10] (Fig. 1, in thin lines) of fatty acids having eve numbers of carbon atoms is butyryl-coenzyme A, w

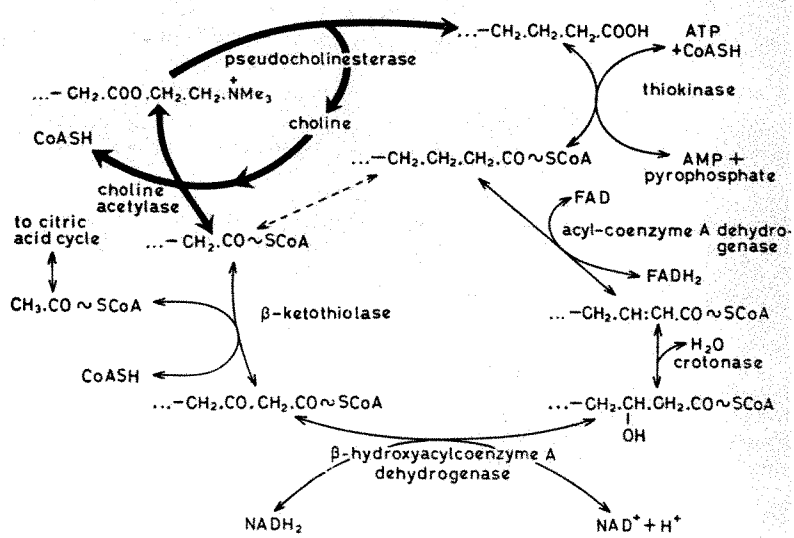


Fig. 1. Pathway of fatty acid metabolism in mammalian liver (—) including postulated synthesis and breakdown of toxic choline esters (—)

postulate that this, and to a lesser extent the acyl coenzyme A derivatives of certain other higher fatty acids, become involved in the choline ester synthetic pathway The products, particularly BuCh, have a powerful nico tinic action[11] and if not quickly destroyed would probably exert undesirable and toxic effects. We consider that the principal biological function of PsChE might, therefore be to hydrolyse this BuCh preferentially, almost at the site of its formation and allow the products of hydrolysis to return to their respective metabolic pathways (Fig. 1 in thick lines).

Some support for this hypothesis is provided by the seasonal variation observed in the PsChE titre of horse

serum, which reaches a maximum in late summer[12] thereafter declining, to parallel closely the seasonal variation in fat metabolism.

γ-Aminobutyrylcholine, isolated from pig brain extracts[13], is a very poorly active cholinergic compound compared with ACh. It is not hydrolysed by AChE and is only poorly hydrolysed by PsChE. Its exact function is unknown but it is possible that it is a functionless by-product of the choline ester synthetic pathway.

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Laminarinases in Soil and Litter Invertebrates

Laminarinases, enzymes hydrolysing β-D-(1→3)-glucans, are widely distributed among micro-organisms[1–3]. Chesters and Bull[1,2] examined their occurrence in 160 fungi. Eighty per cent of the species showed laminarinase activity. Fifty-six per cent of them had laminarinases of the endohydrolytic type (yielding laminaridextrins as initial products), 25 per cent of the exohydrolytic type (yielding glucose as the sole initial product and only later laminaridextrins), and 19 per cent were intermediary, with both laminarinase types.

The presence of laminarinase in the digestive tract of animals has received little attention. Myers and Northcote[4] report a powerful laminarinase from *Helix pomatia*, and Bailey and Clarke[5] from *Entodinium* species of the sheep rumen.

Since, however, snails and slugs seem unique among soil and litter animals in possessing a very wide range of carbohydrases hydrolysing polysaccharides[6] a number of soil and litter invertebrates of widely different systematic position and feeding types were tested for laminarinase activity: Oligochaetes: *Cognettia sphagnetorum* (Vejd.), *Lumbricus rubellus* Hoffm., *L. terrestris* L.; Isopods: *Porcellio scaber* Latr.; Chilopods: *Lithobius forficatus* Koch; Diplopods: *Schizophyllum sabulosum* (L.), *Glomeris marginata* Koch; Insects: *Ectobius silvestris* (Poda), *Pterostichus niger* Fabr., *Philonthus* sp., *Phyllopertha horticola* L.; Gastropods: *Arion ater* L., *A. circumscriptus* Johnst.

Homogenates of guts and gut contents were extracted in phosphate–citric acid buffer, *p*H 4·95, and used as a source of enzymes, insoluble laminarin (Light and Co.) as a substratum. The products of hydrolysis were identified by paper chromatography.

All species showed very strong laminarinase activity yielding glucose as the main product of hydrolysis.

Two samples of laminarinase, associated with other enzymes, were obtained as an almost white powder by half-saturating the homogenates of *Arion circumstriptus* and *Porcellio scaber* with ammonium sulphate, centrifuging, dialysis against tap water, precipitation of enzymes by double the volume of acetone at 0° C, and redissolving the dried powder in water.

Dominant activities of the enzyme solution were laminarinase and β-glucosidase. Laminarinase activity extends over the *p*H-range 2·25–7·6 but is very weak below *p*H 3·0 and above 7·4. The activity curve is unimodal with a peak at *p*H 4·9–5·1 (*Arion circumscriptus*) or 5·2–5·7 (*Porcellio*). The dominant constituent of the laminarinase is of exohydrolytic type yielding large amounts of glucose and only traces of intermediary products (R_{gl} 0·81 and 0·43, descending chromatography, ethyl acetate/pyridine/water, 12 : 5 : 4).

The experiments suggest laminarinases to be widely distributed among soil and litter invertebrates.

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Role of Catalase and Peroxidase in the Metabolism of Leucocytes

Leucocytes are known to be rich in catalase[1] and myeloperoxidase[1–3], yet very little is known concerning the role of these enzymes in leucocyte metabolism. In a recent investigation by Iyer *et al.*[4], evidence was presented for the formation of hydrogen peroxide by guinea pig exudate leucocytes. An obvious physiological function of catalase or peroxidase, therefore, might be to regulate the intracellular concentration of hydrogen peroxide which, if allowed to accumulate, could become toxic to the cells.

In order to assess the role of catalase in the metabolism of leucocytes a relatively specific inhibitor of catalase activity, 3-amino-1,2,4-triazole (AT), was used. This compound has been shown to inhibit the catalase activity of liver and kidney but not of the erythrocyte[5,6].

In the work to be presented here the effect of AT on leucocyte catalase *in vivo* and *in vitro* in comparison with other tissues was examined. An attempt was also made to ascertain a role of catalase in respiration and aerobic glucose utilization of leucocytes.

Male guinea pigs of *NIH* strain, weighing 350–500 g each, were used. Liver and kidney homogenates were prepared as described previously[7]. Erythrocytes were obtained by centrifuging heparinized blood at 100*g* for 15 min. After removal of the plasma and buffy layer, the erythrocytes were washed with saline to remove any traces of AT, and then lysed in 49 volumes of distilled water.

Suspensions rich in polymorphonuclear leucocytes (80 per cent) were obtained from peritoneal exudates of guinea pigs injected intraperitoneally with 10 ml. of a 12 per cent solution of sodium caseinate 16–18 h prior to killing, as described by Stahelin *et al.*[8]. The leucocytes were removed from the exudate by centrifugation at 250*g* for 5 min, washed once with 5 ml. 0·9 per cent saline, and finally suspended in 1 ml. of distilled water and homogenized by 50 passes in a glass-'Teflon' homogenizer with a motor-driven pestle. For enzymatic assays the

homogenates were diluted with distilled water to a final volume of 10 ml.

Respiration and uptake of glucose were measured in Warburg flasks in air at 37° C as described previously[9]. For enzymatic assays the cells were collected quantitatively from the Warburg flasks at the end of the incubation period (2 h), washed once with 5 ml. saline and homogenized as described above for the *in vivo* investigations.

Catalase was assayed spectrophotometrically as described previously[7,10]. Myeloperoxidase was assayed using the guaiacol method, as described by Schultz, *et al.*[11].

Table 1. Effect of AT on Catalase Activity of Guinea Pig Liver, Kidney, Erythrocytes and Leucocytes *in vivo*

Tissue	Catalase activity Control	Catalase activity AT	Inhibition %
Liver*	666 ± 30 (9)†	15 ± 1 (9)	98
Kidney*	67 ± 2 (9)	6 ± 1 (9)	91
Erythrocytes‡	77 ± 9 (6)	75 ± 10 (6)	0
Leucocytes‡	46 ± 2·5 (4)	2 ± 0·7 (3)	96

* Catalase activity is expressed in units/g wet tissue.
† Mean ± standard error of the mean (number of observations).
‡ Catalase activity is expressed in units/ml. packed cells (1,000g/30 min)

The results of the *in vivo* experiments are summarized in Table 1. Injection of 1 g of AT/kg body weight of guinea pig, 12 h prior to death, produced 98 per cent inhibition of catalase activity of the liver, 91 per cent inhibition of the kidney catalase but was without any apparent effect on the erythrocyte catalase. These observations confirm the work of other investigators[5,6,12] who used different species, primarily rats and mice. AT also caused a profound depression of the leucocyte catalase (96 per cent), comparable to that seen in the liver.

Table 2. Effect of AT on Catalase Activity, Oxygen Uptake and Glucose Utilization by Leucocytes *in vitro**

AT concentration (M)	Catalase activity (units/ml. packed cells)	O_2 uptake (μl./h/10^7 cells)	Glucose utilization (μmoles/2 h/10^7 cells)
None	35·0	2·9	0·74
1×10^{-4}	32·5	2·7	0·73
5×10^{-4}	27·0	3·0	0·73
1×10^{-3}	20·0	3·0	0·69
5×10^{-3}	4·0	2·8	0·73
1×10^{-2}	3·0	3·0	0·69

* Each value represents the average of two determinations. The cells were incubated in the presence of 3 ml. Krebs–Ringer phosphate medium, *p*H 7·4, containing glucose, 5·6 μmoles/ml.

The leucocyte catalase was also markedly inhibited by AT *in vitro*, as shown in Table 2. The inhibition was proportional to the AT concentration used, the greatest effect being observed at 10^{-2} M (91·4 per cent) and the smallest at 10^{-4} M (7·1 per cent). The leucocyte preparations treated to remove the few contaminating red cells, by the method of Fallon *et al.*[13], had a level of catalase activity comparable to that seen in the untreated leucocytes.

Inhibition of the leucocyte catalase occurred regardless of whether glucose was present or absent in the incubation medium. Despite the marked effect on the leucocyte catalase activity, AT had no apparent influence on the leucocyte respiration rate and glucose utilization.

Leucocytes (3 different preparations) were also assayed for their myeloperoxidase activity, the mean ($\pm S.E.$) value of which was found to be $0{\cdot}15 \pm 0{\cdot}017$ μmoles of tetraguaiacol produced per min per 10^7 cells. The incubation of leucocytes with 10^{-3} or 10^{-2} M AT, in contrast to catalase, produced no apparent change in the leucocyte peroxidase activity.

On the basis of the evidence presented here with respect to the effect of AT on catalase activity of different tissues, it is evident that leucocyte behaviour is similar to that of the liver and kidney rather than to that of the erythrocyte. Tumour extracts, which are known to depress the catalase activity of the liver but not of whole blood, have also been reported to inactivate leucocyte catalase[14].

The work by Iyer *et al.*[4] suggests that hydrogen peroxide is generated in leucocytes as a product of glucose oxidation via the hexosemonophosphate shunt pathway. Our results also suggest that hydrogen peroxide is formed in leucocytes since the presence of hydrogen peroxide is a prerequisite for the inhibition of catalase by AT[12]. It is also apparent from the work recorded here that leukocytes lack the sulphydryl compound present in erythrocytes that prevents the inhibition of catalase by AT[15]. It is evident that the rates of oxygen and glucose uptake by leucocytes are relatively independent of catalase activity down to as little as 9 per cent of the original activity of this enzyme. It is possible that the residual catalase activity in the presence of 0·01 M AT is sufficient to protect the cell from hydrogen peroxide accumulation. However, a more likely explanation might be that some other hydrogen peroxide destroying system such as myeloperoxidase, which is found in high concentrations (5 per cent of the dry weight) in leucocytes[3], might be of greater significance than catalase in protecting the cell from any toxic effects of hydrogen peroxide. Further work will be necessary, however, before it will be possible to decide between these two alternatives.

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Absence of Free Iodotyrosines from the Plasma of the Rat

Werner *et al.*[1,2] and Beale and Whitehead[3] have recently claimed to have demonstrated the presence under physiological conditions of free 3 : 5-di-iodotyrosine (DIT) and 3-iodo-tyrosine (MIT) in the serum of euthyroid human beings and rats using paper chromatographic separation followed by several methods for the detection and estimation of iodine[1–5], a distinctive feature of these serum iodotyrosines being that they could not be labelled with radioiodine.

From a physiological point of view, the existence of iodotyrosines in blood is difficult to understand because of the following facts: (1) the existence of an active iodotyrosine-dehalogenase in the thyroid gland[6]; (2) the very short half-life of injected radio-DIT (1·5 h) (ref. 7) correlated with the presence in many tissues and especially in liver and kidney of iodotyrosine-dehalogenases; (3) the impossibility of detecting DIT as a metabolic product of injected thyroxine labelled on the four iodine atoms[8]; (4) the inability of these serum iodotyrosines to be labelled after radioactive iodine administration.

By combining the isotopic equilibrium method[9,10] with the gel filtration of plasma[11] we have been able to demonstrate the absence of free DIT and MIT in the plasma of the rat.

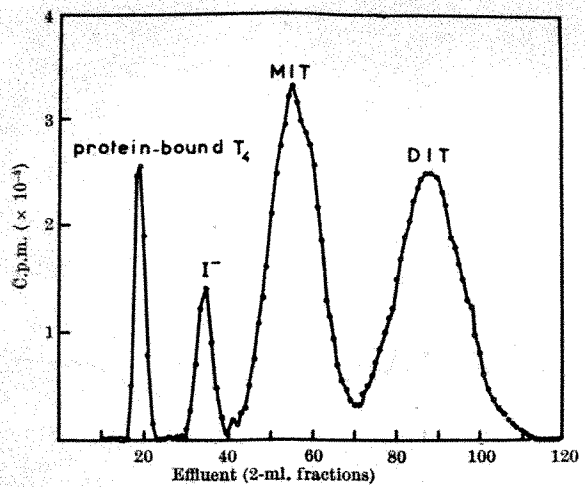


Fig. 1. Filtration on 'Sephadex *G*-25' of plasma supplemented with ^{131}I-labelled iodo-compounds. The gel was equilibrated with 0·2 M ammonium acetate *p*H 5·8 and the elution performed with the same buffer. Column size, 2·5 × 20 cm; fractions of 2 ml. collected at a flow-rate of 1 ml./min. 3 ml. plasma was supplemented with: $Na^{131}I$, 5,400 c.p.m. (tracer); ^{131}I-L-thyroxine, 8,500 c.p.m. (S.A., 25 μc./μg); ^{131}I-di-iodo-L-tyrosine, 47,200 c.p.m. (S.A., 18 μc./μg); ^{131}I-mono-iodo-L-tyrosine, 49,510 c.p.m. (S.A., 15·5 μc./μg)

Male rats (Wistar strain, average weight 280 g) fed on an iodine-free diet were given a regular daily dose of iodine in their drinking water in the form of $K^{127}I$. One group received 5 μg of iodine a day and a second one 50 μg. In animals previously treated in this way an isotopic equilibrium was reached after administration for 50 days of the same drinking water labelled with iodine-125 at a constant specific radioactivity, not however correcting for the decay of the radioisotope (0·10–0·15 μc./μg iodine on the first day according to the group). At isotopic equilibrium, each iodine pool has the same specific radioactivity as the drinking water. Thus, the iodine content of each pool may be calculated by the ratio of its radioactivity to the specific radioactivity of the drinking water.

Gel filtration of plasma was performed as previously described[11] using 'Sephadex *G*-25' equilibrated with 0·2 M ammonium acetate *p*H 5·8.

Fig. 1 shows the results of a gel filtration of rat plasma supplemented with iodine-131-labelled iodide, MIT, DIT and L-thyroxine.

The separation of iodotyrosines from iodide and protein-bound thyroxine is reproducibly unambiguous.

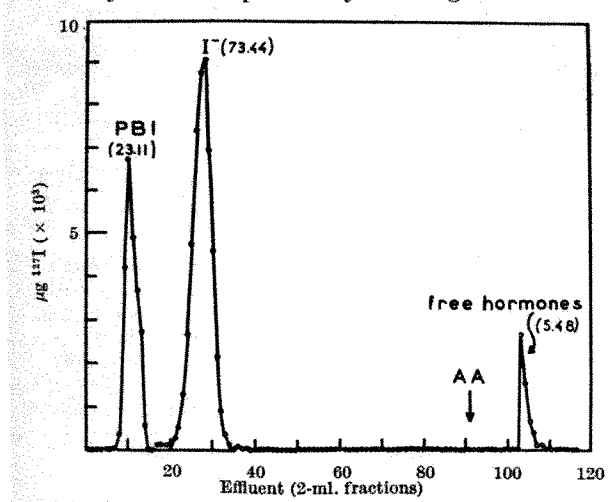


Fig. 2. Filtration on a 'Sephadex *G*-25' column (2·5 × 14 cm) of rat plasma (3 ml.). The sample was obtained from pooled plasma of five rats which were adapted to a daily dose of 5 μg of iodine and maintained in an isotopic equilibrium with iodine-125. Same conditions of buffer and elution as in Fig. 1. *AA* indicates the replacement of ammonium acetate by tertiary amylol saturated with ammonium 2 N. The figures indicate the total amount of iodine-127 calculated from the iodine-125 counting as explained in the text

Fig. 2 shows the analysis of a sample of the pool of equal volumes of plasmas obtained from 5 rats which were adapted to a daily dose of 5 μg of iodine and maintained in an isotopic equilibrium with iodine-125. No radioactivity is detectable in the eluates from the MIT- and DIT-zones.

Other experiments performed in the same way with rats adapted to a daily dose of 50 μg of iodine failed also to show the presence of both free MIT and DIT.

The conditions of isotopic equilibrium used in this investigation, the absence of any treatment of plasma before filtration on 'Sephadex *G*-25' and the good separation of iodotyrosines from iodide and thyroid hormones justify the conclusion that free MIT and DIT are not normal components of the plasma in the rat. Under the conditions of iodine-125-radioactivity counting used, as little as 0·0003 μg of iodine iodine-127 per ml. plasma would have been detected.

Pitt-Rivers and Rall[12], using isotope equilibrium for a shorter period in the rat and anion-exchange resin chromatography for the separation of iodo-compounds, have already concluded that the iodotyrosines were virtually absent in the serum.

Wellby and Hetzel[13] have also concluded that iodotyrosines are absent from the plasma of euthyroid human beings using anion-exchange resin and paper chromatography for the separation of iodinated compounds.

If technical artefacts are not concerned, two main possibilities offer themselves to explain the contrary results of Werner *et al.*[1,2] and Beale and Whitehead[3]:

(1) The degradation of plasma T_4 (and T_3) to DIT during extraction procedures; many oxidizing systems are indeed capable of breaking the diphenylether bridge of T_4 (and T_3); (2) the existence in the serum of DIT- (and/or MIT-) generating compounds which could be degraded into free iodotyrosines during extraction.

However, this does not explain why the iodinated compounds identified as iodotyrosines could not be labelled with radioiodine, a situation which is rather difficult to interpret on a physiological basis.

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PHYSIOLOGY

Central Actions of Psychotomimetics

ALTHOUGH the alterations in human behaviour and mental activity produced by psychotomimetic substances are not necessarily closely related to those observed in the psychoses[1], the central modes of action of these substances are nevertheless of considerable interest. It is possible

that drugs chemically related to tryptamine, lysergic acid and the catecholamines block synaptic excitation in certain regions of the central nervous system, such as has been demonstrated in the lateral geniculate nucleus[2]. These substances may also mimic central inhibitory transmitters[3], since certain derivatives depress the excitability of neurones in the cerebral cortex and thalamus[4].

An interesting series of compounds with psychotomimetic properties are the piperidyl and pyrrolidyl esters of glycolic acid[5]. Although many of these substances have an atropine-like action on the human autonomic nervous system and block the excitant action of acetylcholine on smooth muscle *in vitro*[6], it has been considered unlikely that the central action of the glycolic acid esters is related to the central blockade of acetylcholine or an acetylcholine-like transmitter[5].

Recently it has been reported that *N*-methyl-2-pyrrolidylmethyl α-cyclopentyl-α-phenylglycolate affects the metabolic and functional properties of frog muscle[7]. In particular, at concentrations of 10^{-6} M, this psychotomimetic has a calcium-like action on the excitable membrane and renders it less excitable. Similar depressant actions have hitherto not been reported for nervous tissue[7] and the present results are concerned with the actions of *N*-ethyl-3-piperidyl α,α-diphenylacetate, *N*-methyl-3-piperidyl α,α-diphenylglycolate methochloride, *N*-methyl-3-piperidyl α,α-diphenylglycolate and *N*-ethyl-2-pyrrolidylmethyl α-cylopentyl-α-phenylglycolate upon mammalian neurones. Only the last two of these agents are psychotomimetics[5,6].

The extracellular spike potentials of single neurones in the spinal cord and thalamus of cats anæsthetized with pentobarbital sodium have been recorded by means of the central barrel of five-barrel micropipettes. The glycolic acid esters were ejected electrophoretically as cations from dilute solutions of the appropriate chlorides or hydrochlorides. Spike potentials evoked synaptically or by the electrophoretic ejection of acetylcholine (cation) or L-glutamic acid (anion) were photographed or counted by means of a rate meter and the frequency of firing displayed on a paper recorder. The effect of *N*-ethyl-2-pyrrolidylmethyl α-cyclopentyl-α-phenylglycolate ('*JB* 8099') upon the responses of a spinal Renshaw cell is illustrated in Fig. 1. The frequencies of firing evoked by L-glutamic acid and acetylcholine were approximately equal, but the electrophoretic ejection of '*JB* 8099' depressed the acetylcholine-firing to a greater extent, and for a longer time, than the amino-acid induced firing. Simultaneously (Fig. 1*B*) there was a reduction in the number of repetitive spike potentials elicited by orthodromic stimulation of the cell. All of these agents had a similar action when tested upon Renshaw cells and upon cholinoceptive thalamic neurones[8], and it was usually possible with small electrophoretic currents to reduce selectively the rate of firing produced by acetylcholine without affecting that produced by an excitant amino-acid. Furthermore, the excitation of non-cholinoceptive spinal interneurones by acidic amino-acids was readily reduced by these esters, thus indicating a reduction in the excitability of the post-synaptic membrane of these cells, possibly by a mechanism similar to that proposed for frog sartorious muscle[7]. The actions of all of these substances are thus similar to those of procaine, atropine, hyoscine, benactyzine and 1-(1-phenylcyclohexyl)-piperidine[9].

It is difficult to relate the concentrations of these substances attained electrophoretically to those obtained by systemic administration. However, the results demonstrate that these substituted piperidyl and pyrrolidyl glycolates can reduce the excitability of central neurones and also antagonize the action of acetylcholine upon cholinoceptive neurones. Such effects upon electrically excitable membrane and acetylcholine receptors appear to be common to many agents having local anæsthetic or atropine-like actions. It is possible that alterations in human behaviour and mental activity may be produced in

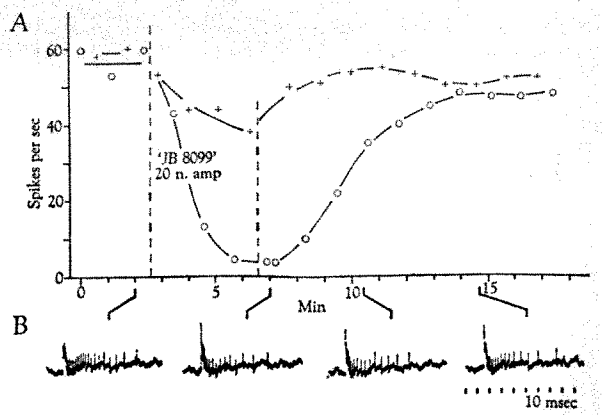


Fig. 1. *A*, Extracellular spike responses of a single Renshaw cell were counted by means of a rate meter and the symbols indicate the maximal frequency of firing evoked by alternating 15 sec ejections of L-glutamate (+) and acetylcholine (○) using electrophoretic currents of 70 n.amp and 10 n.amp respectively. Between the vertical broken lines '*JB* 8099' was ejected from another barrel of the five-barrel micropipette, using a current of 20 n.amp. *B*, Synaptic responses of the same Renshaw cell, recorded during the series plotted in *A*, and evoked by maximal stimulation of the segmental ventral root. The lines indicate the times at which these responses were photographed. Time, 10 msec

at least three ways, if and when these glycolic acid ester reach central neurones. In the first place transmission i small diameter presynaptic fibres may be modified c blocked, thus affecting transmitter release at centra synapses. Secondly, the depression of neuronal excita bility can affect the repetitive responses of nerve cells t synaptic stimulation. Thirdly there may be antagonism towards the synaptic action of acetylcholine; and in thi respect the degree of antagonism of these agents toward acetylcholine upon smooth muscle may not necessarily b related to this central action.

We thank Dr. L. G. Abood for samples of the glycoli acid esters used in this work.

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Anorexic Effect of Intraperitoneal Glucose in the Hypothalamic Hyperphagic Cat

In previous communications[1,2] one of us reported result leading to the hypothesis that peripheral glucoreceptor (hepatic and, perhaps, muscular) play an important par in the regulation of intake of food. The classical gluco static theory of the regulation of intake of food[3] postulate that the ventromedial hypothalamic area has a mechanism for glucostatic regulation of appetite and feeding. Th lack of ventromedial glucoreceptors is regarded b proponents of this theory as being instrumental in th development of the hyperphagia observed when th ventromedial hypothalamus is lesioned bilaterally. Thi communication presents data concerned with the possibilit of glucoreceptors regulating appetite as being locate elsewhere than in the ventromedial hypothalamic area.

A total of 23 experiments were performed in 2 adul cats weighing 2·8 and 3·4 kg respectively at the beginnin

of the work. In these two animals bilateral ventromedial hypothalamic lesions were electrolytically produced. Following these lesions both cats became hyperphagic, increasing their weights by 1·1 and 1·4 kg in the first month post-operatively. Intraperitoneal glucose injections in doses of 0·4–2·0 g/kg were administered during the active phase of hyperphagia. The injections were administered in a sound-proof chamber via a polyethylene tube attached to a syringe on the outside. The injection needle was fixed in place by means of an abdominal belt at the beginning of the experiment, thus avoiding extraneous stimulation during the actual entrance of the solution. The animals were always tested after a 12–24 h fast. Four adult intact cats served as controls.

In a control period of several test days the hyperphagic cats were observed to consume freely more than 10 approximately 10-g portions of raw horse meat given at 1–3 min intervals. Often these animals ate more than 200 g of meat before showing any signs of satiation.

When glucose was injected in sufficient doses (0·6–1·0 and 1·0–2·0 g/kg respectively) the cats refused to feed for periods of 15–45 min after having eaten only 2 or 3 portions preceding the injection. After this period of time had elapsed the animals began first to feed irregularly and finally with the usual voracity. The latency of this anorexia was 1–2 min in the first cat and 1·5–4 min in the other. Smaller doses of glucose (0·4–0·5 and 0·8–0·9 g/kg) did not inhibit intake of food, thus providing suitable controls showing that entrance of the hypertonic solution into the peritoneal cavity does not in itself produce anorexia.

In the 4 intact cats the minimum intraperitoneal doses of glucose producing anorexia were from 0·5–0·9 g/kg. The latency in these animals varied between 1 and 3 min and the anorexia persisted for 10–25 min. Thus, the sensitivity to glucose of the cats deprived of the ventromedial hypothalamic area is not significantly different from that of intact animals, and the anorexic effect seems to be even more prolonged.

At the completion of the experiments, the brains of both cats were perfused *in situ* with 10 per cent formalin, frozen, and sectioned at 40μ, and stained by the Kluver–Barrera method, in order to identify the symmetry, extent, and location of the lesions. Fig. 1 shows the lesions in one of the cats.

The foregoing results provide some evidence for the existence of glucoreceptors outside the ventromedial hypothalamic area. Integrating these findings with the presumed organization of the hypothalamic 'appestat', our hypothesis assumes that a tonic discharge originating in hepatic, and perhaps muscle glucoreceptors, might be responsible for the tonic activity of the lateral hypothalamic 'feeding centre'. This discharge should decrease when intracellular glucose or some related metabolite increases in hepatic and muscle cells. It is possible that the ventromedial hypothalamic 'satiety centre' represents an inhibitory loop functioning in a manner similar to inhibitory loops from caudate and red nuclei on muscle tonus. When these inhibitory loops are suppressed the excitatory mechanisms are liberated producing exacerbated reactions such as decerebrate rigidity or hyperphagia. Peripheral glucoreceptor signals may inhibit the 'satiety centre' as well as excite the 'feeding centre' directly. A small reduction of these signals could liberate the satiety mechanisms thus inhibiting the activity of the 'feeding centre' in addition to diminishing the direct excitatory input to the 'feeding centre'. When the 'satiety centre' is removed the decrease in peripheral glucoreceptor signals produced by feeding is no longer sufficient to suppress activity of the 'feeding centre' since the inhibitory loop is lacking, thus resulting in hyperphagia. However, a stronger reduction of these signals as produced by a large dose of glucose going directly to the liver results in a sufficient reduction of the excitatory input to the 'feeding centre' to provoke anorexia, both in the normal intact and hypothalamic hyperphagic animal.

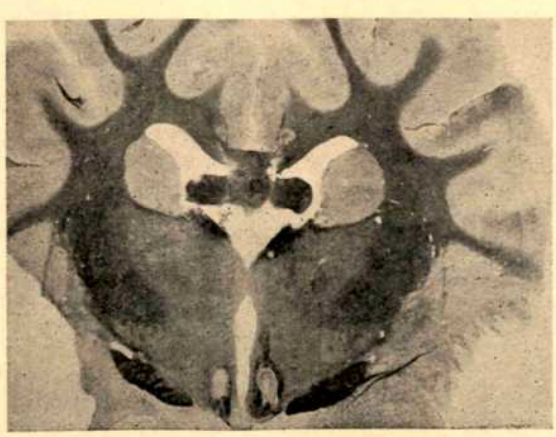


Fig. 1. Frontal section through the brain of the cat at anterior stereotaxic plane 12·0 indicating the bilateral electrolytic lesions in the ventromedial hypothalamic area

We thank Dr. R. Hernandez-Peon, director of the Brain Research Unit, for use of facilities where this work was carried out.

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HISTOLOGY

Localization of Anti-human Growth Hormone Serum within the Human Placenta: Evidence for a Human Chorionic 'Growth Hormone-prolactin'

Josimovich and MacLaren[1] have recently reported the presence in human placenta of a component which cross-reacts with rabbit anti-human growth hormone serum. This protein, which gave a reaction of partial identity with human growth hormone, exhibited prolactin activity and was detected in peripheral venous serum at term and in retroplacental serum by the Ouchterlony gel diffusion technique. Further immunochemical, physicochemical, and biological characterization of this cross-reacting placental protein, detectable in fresh saline homogenates of human and simian placenta, has been described by Kaplan and Grumbach[2]. Immuno-electrophoresis in agar gel (barbital buffer, *p*H 8·2) and starch gel electrophoresis (discontinuous buffer system of Ferguson and Wallace) indicate that the migration of the placental component differs from that of native and purified human growth hormone. A partially purified preparation of the cross-reacting placental component contains significant growth-promoting activity[2].

Localization of fluorescein-labelled rabbit anti-human growth hormone serum has been demonstrated in the acidophils of the human anterior pituitary gland and in eosinophilic adenomas associated with acromegaly[3,4]. In view of the evidence indicating a protein in human and simian placentæ which partially cross-reacts with rabbit antiserum to human growth hormone, the Coons labelled antibody technique[5] was applied to determine the localization of this component within the human placenta.

Representative areas of the villous portion of first, second and third trimester human placentæ were processed

by the freeze-drying method and sectioned at 1·5μ. Cryostat sections of fresh tissue and formalin fixed deparaffinized sections gave the same localization of fluorescence, but the specificity of the cytological detail was superior in the freeze-dried material and this was used for most observations. Both the indirect and the direct immunofluorescent techniques were employed. For the indirect technique fluorescein conjugated sheep anti-rabbit γ-globulin (SA-RGG-F) was used, while for the direct technique a highly potent anti-HGH[6] was conjugated with fluorescein isothiocyanate (A-HGH-F). Fluorescein conjugated antisera were purified further by filtration through a 'Sephadex *G*–25' column. The antisera were absorbed with rat and rabbit liver powder. Sections were incubated for periods of time varying from 45 min to several hours, and then viewed under the standard fluorescence microscope. Placental autofluorescence was not a complicating factor. Heterologous tissue controls and heterologous antibody controls were performed. Absorption of the conjugated antiserum with HGH abolished the specific fluorescence and pre-incubation of the tissue sections with unlabelled anti-HGH blocked the specific fluorescence of A-HGH-F used in the direct procedure.

Multiple sections from six placentæ were examined, ranging in gestational age from 12 weeks to term. In all instances specific fluorescence was localized to the cytoplasm of the syncytiotrophoblastic villous epithelium. Fluorescence was not present in the cytotrophoblastic layer. Cytoplasmic fluorescence was extensive and not localized to any specific sub-area of the syncytial cytoplasm, although occasional perinuclear areas and fragments of the syncytial brush border gave strong fluorescence. A section of a mid-trimester placenta is shown in Fig. 1 which illustrates fluorescence in two strips of adjacent syncytiotrophoblastic cytoplasm. Presented in Figs. 2*a* and *b* are phase contrast and fluorescence photomicrographs of the identical areas of a section of term placenta, again indicating fluorescence in the cytoplasm of the syncytiotrophoblast. Rabbit anti-human chorionic gonadotropin sera did not inhibit the fluorescence.

These findings indicate that the human placenta as early as the twelfth week of gestation contains a protein localized in the syncytial cytoplasm of the villous trophoblast which immunologically partially cross-reacts with rabbit anti-HGH serum. Studies to be reported[2] elsewhere suggest that this protein is a chorionic 'growth hormone-prolactin' and may represent an important metabolic hormone of pregnancy. It is noteworthy that immunofluorescent investigations of human chorionic gonadotropin in the human placenta by Midgley and Pierce[7] and similar investigations by Sciarra and Tallberg[8] indicate that this hormone is also present in the syncytiotrophoblast.

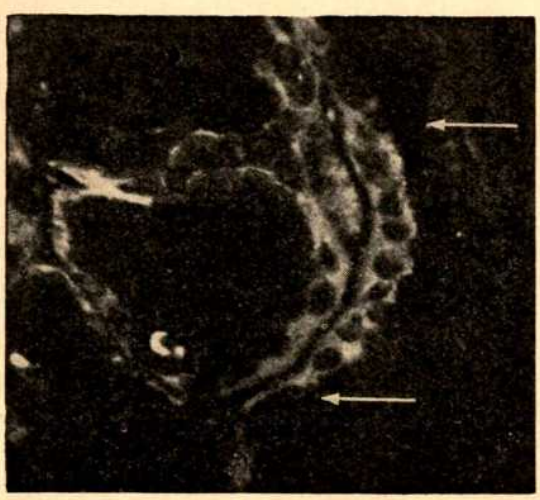


Fig. 1. Fluorescence photomicrograph of a section of a 19-week placenta prepared by freeze-drying and incubated with rabbit anti-HGH γ-globulin and subsequently with SA-RGG-F. The syncytiotrophoblastic cytoplasm exhibits marked fluorescence. Areas occupied by the syncytial nuclei are non-fluorescent. The location of non-fluorescent Langhans cells of the cytotrophoblast are indicated with arrows. (× *c.* 650)

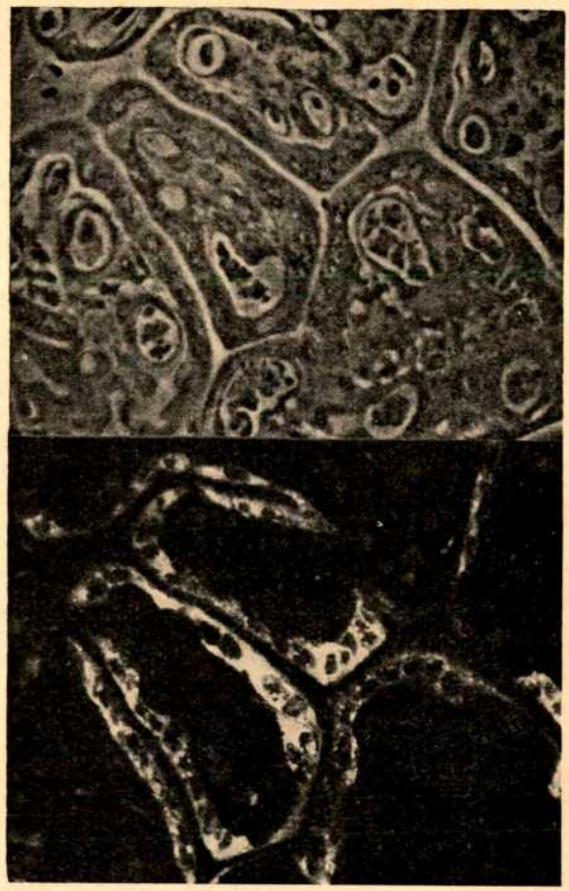


Fig. 2. *a*, Phase contrast photomicrograph of a section of the villous portion of a term human placenta prepared by freeze-drying. Notice the extensive layer of the syncytiotrophoblastic epithelium. Cells of the cytotrophoblastic layer, although rare, are present but are not identifiable at this magnification (× 500). *b*, Fluorescence photomicrograph of the identical area pictured above after incubation for 45 min with fluorescein-labelled anti-HGH illustrating the fluorescence localized to the cytoplasm of the syncytial layer of the villous trophoblast (× 560)

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RADIOBIOLOGY

Barium Content of Different Parts of the Choroid of the Bovine Eye

Sowden and Pirie[1] analysed the barium and strontium content of different parts of the eye and confirmed earlier work showing that cattle choroid contained large amounts of barium. These authors did not, however, separate the tapetal fibres from the pigmented choroid and determine whether barium was also present in the tapetum. Weitzel *et al.*[2] have shown that zinc is an integral part of the tapetum of carnivores, and although the structure of the tapetum of the cow is quite different from that in carnivores we thought it would be interesting to determine whether there was any particular concentration of barium in the tapetum of the cow. This is a specialized part of the central choroid lying immediately under the retina and made up of fine parallel fibres which act as a diffraction grating and reflect light[3]. It is backed by pigmented choroidal tissue.

To separate the tapetal fibres, eyes from the slaughterhouse were opened by an equatorial cut and the front half, to which the whole vitreous body remains attached, was discarded. The retina was then removed leaving the choroid in position on the sclera and showing the tapetum as a brilliant blue-green area[4]. A cut was then made through sclera and choroid around the outline of the tapetum thus separating the choroid into tapetal and non-tapetal parts. The non-tapetal choroid was then scraped off the sclera and collected. The tapetal fibres were separated from the underlying choroid by gentle scraping with a scalpel under the dissecting microscope. The pigmented choroid underneath was then scraped off the sclera. Fig. 1 shows the tapetal fibres prepared in this way and then shaken in saline to get a fine suspension. They are remarkably uniform, a necessary attribute for fibres making a diffraction grating.

The three separated parts of the choroid were collected from several eyes and the barium in them estimated by activation analysis[5].

Samples were dried at 100° C for 12 h, weighed and transferred to a muffle furnace and ashed at 500° C overnight. Each ashed sample was powdered, mixed and weighed before being stored in a glass-stoppered bottle. An aliquot of about 5–20 mg from each ash was activated by irradiation in the Harwell pile. After irradiation barium was separated from the ash and the concentration estimated by comparison of radioactivity with that of the irradiated barium standard. The barium fraction was counted consecutively over a period (4 h) in a thallium activated sodium iodide crystal type gamma scintillation counter.

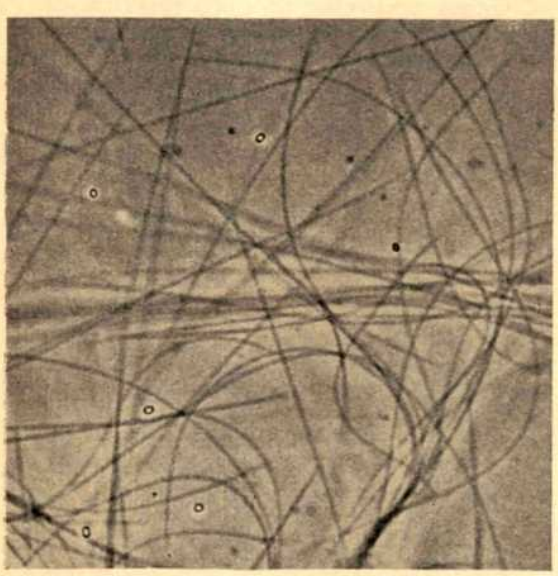


Fig. 1. Fibres of tapetum lucidum of cow. (× *c.* 890)

Table 1. Barium Content of Bovine Choroid and Tapetum

	Non-tapetal choroid	Choroid under tapetum	Tapetum
Cow			
mg Ba/100 g wet weight	97, 111, 91, 94·5	103, 93, 86, 95	17, 17, 17, 25
mg Ba/g ash	67, 76, 63, 71·5	84, 73, 76, 72	17, 17, 17, 27
Calf			
mg Ba/100 g wet weight	10·4	6·6, 5·9, 7·8	3·1
mg Ba/g ash	11·0	10, 9, 11·8	2·9

Table 1 shows that barium is associated with the pigmented choroid, not with the tapetal fibres. It appears to be distributed evenly throughout the pigmented choroid, whether this is under the tapetum or peripheral to it. The analyses confirm that the choroid of the cow is richer in barium than the choroid of the calf, nearing the extraordinary concentration of 1 per cent of the total ash. The human choroid normally contains traces of stable barium[1].

Garner[6] showed that when barium-140 was administered to cows it became concentrated in the choroid of the eye, the concentration reached being 10-fold that in the femur. Accumulation of barium-140 also occurred in the choroid of rabbits, animals which have no tapetum. It is possible that barium-140 produced in nuclear explosions concentrates in the choroid. Anderson[7] detected traces of barium-140 in deer in New Mexico, in a cow, and in milk samples in the United States. Since the half-life of barium-140 is about 12 days it will only occur in fall-out deposited shortly after an explosion. But that this can happen on a considerable scale was shown by Fisher *et al.*[8], who found that after the Russian nuclear explosions in the autumn of 1961, barium-140 contributed about 15 per cent of the total β-activity in air measured at Chilton, Berkshire. The maximum barium-140 on grass measured at the same place was 16,900 pc./m² in mid-November 1961. The fact that barium-140 is one of the more abundant fission products and that it becomes so markedly concentrated in the choroid of bovines makes it possible that this tissue should be considered when estimating the hazard from fall-out.

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Reversal of Gamma-ray-induced Susceptibility to Decay of Potato Tubers and Tomato Fruit by Methyl Ester of Indolyl-3-Acetic Acid

Potatoes. 'Up-to-date' potatoes were washed after lifting and stored for one week at room temperature. They were not completely cured at the end of this period. On the eighth day, they were packaged individually in

Table 1. No. Decayed out of 40 Potatoes in Every Treatment

Storage period (weeks)	Control		Irradiated	
	IAA	Distilled water	IAA	Distilled water
3	nil	4	nil	9
6	4	4	3	15
9	9	22	5	20
12	18	22	10	25
15	18	22	15	30
18	18	27	15	30

Table 2. Percentage Cumulative Physiological Losses in Weight*

Storage period (weeks)	Control		Irradiated	
	IAA	Distilled water	IAA	Distilled water
3	2·5	2·8	2·3	3·2
6	4·6	4·9	4·0	5·4
9	5·7	6·0	5·3	6·0
12	7·0	7·6	6·6	7·7
15	9·8	10·5	8·0	9·0
18	12·8	13·9	9·3	10·9

* Determinations were made on samples of 5 potatoes

Table 3. No. Sprouted out of 40 Potatoes in Each Treatment in Control Series

Storage period (weeks)	IAA	Distilled water
12	nil	nil
13	8	30
14	8	40
15	24	40
18	24	40

Table 4. No. Decayed Out of 40 Tomatoes in Every Treatment

Storage period (days)	Control		Irradiated	
	IAA	Distilled water	IAA	Distilled water
5	nil	nil	nil	4
10	nil	1	nil	5
15	2	3	nil	8
20	2	4	1	8
25	3	10	2	11
30	9	18	3	16
35	15	23	9	20
40	22	27	14	25
45	24	30	20	28

Table 5. Percentage Cumulative Physiological Losses in Weight*

Storage period (days)	Control		Irradiated	
	IAA	Distilled water	IAA	Distilled water
5	4·4	4·8	3·8	5·6
10	6·5	7·5	5·8	8·0
15	8·8	9·7	7·6	10·3
20	11·0	12·2	9·8	12·5
25	13·8	15·2	12·0	15·4

* Determinations were made on samples of 5 tomatoes. These determinations were discontinued after 25 days of storage due to the decay of some of the fruits in the samples.

Table 6. No. in Green Stage out of 40 Tomatoes in Every Treatment

Storage period (days)	Control		Irradiated	
	IAA	Distilled water	IAA	Distilled water
5	33	30	35	24
10	20	14	32	9
15	8	6	12	5
20	nil	nil	nil	nil

perforated polyethylene bags and half the tubers were irradiated with 10 krad of cobalt-60 γ-rays at the rate of 2·5 krad/min. One day after irradiation, half the irradiated and a similar number of control tubers were dipped in a 1,000 p.p.m. solution of methyl ester of indolyl-3-acetic acid (IAA) for 25 min. Simultaneously, the remaining tubers from both the irradiated as well as the control lots were dipped in distilled water for the same period. Tubers belonging to all the four treatments were afterwards stored at room temperature (23°–35° C; relative humidity, 50–85 per cent).

Irradiated, distilled-water treated potatoes were more susceptible to decay during the initial six weeks of storage (Table 1). Similar results were obtained by Rubin and Metlitsky[1] and Lewis and Mathur[2]. Treatment with methyl ester of indolyl-3-acetic acid resulted in a reduction in the decay rate both in irradiated as well as control tubers (Table 1). Physiological losses in weight (that i losses due to transpiration and respiration) were low in the potatoes treated with indolyl-3-acetic acid bo in irradiated as well as control lots (Table 2). Sproutir was completely prevented in the irradiated potatoe However, indolyl-3-acetic acid inhibited sprouting to considerable extent in control potatoes (Table 3).

Tomatoes. Tomatoes (var. 'Ox Heart') were picked i the green stage, packaged individually in perforate polyethylene bags and half of the fruits were irradiate with 10 krad of γ-rays at the rate of 2·5 krad/min. Ha of the fruits from each of the irradiated and the contr lots were dipped in a 1,000 p.p.m. solution of meth ester of indolyl-3-acetic acid for 25 min. Simultaneousl the remaining fruits from each of the irradiated and t control lots were dipped in distilled water for the sam period. The operations of picking, irradiation and trea ment with indolyl-3-acetic acid were done on the sam day. Fruits belonging to all the four treatments we afterwards stored at room temperature (24°–34° C relative humidity, 45–70 per cent).

The results obtained are recorded in Tables 4–6. Th rate of decay was the highest in irradiated, distilled-wat treated tomatoes, during the initial 20 days of storag Treatment with indolyl-3-acetic acid reduced very co siderably this initial high decay rate (Table 4). Th physiological losses in weight (that is, losses due to tran piration and respiration) were the lowest in irradiate indolyl-3-acetic acid treated tomatoes (Table 5). The ra of ripening was the slowest in irradiated, indolyl-3-aceti acid treated tomatoes during the initial 15 days of storag (Table 6).

It appears that γ-irradiation reversibly inactivat the endogenous indolyl-3-acetic acid synthesizing syste in the tomato fruits and potato tubers and that th effects of this inactivation can be counterbalanced b exogenous indolyl-3-acetic acid.

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Changes occurring in the Excretion of Radiorubidium by Saliva in Rats after Whole-body Irradiation

Changes occurring in the excretion of electrolytes b saliva in irradiation sickness have so far received litt attention. In a previous report[1] we concentrated on th excretion of radiosodium by saliva in irradiated rats. I the work recorded here we investigated the excretion c rubidium the metabolism of which resembles that c potassium and which consequently occurs mostly in th intracellular compartments.

In the experiments to be reported use was made of 4 white rats of laboratory breeding divided into 4 groups c 10 animals. The rats were given water and a standar diet *ad lib.* All rats received concurrently 25 μc. rubidium 86/kg live body-weight, intraperitoneally, in 1 ml. c physiological saline. The first group served as a contro the remaining 3 were irradiated, 24 h following the admin istration of radiorubidium, with a dose of 1,000 r. c rœntgen radiation on the apparatus Makrophos, at 1 m.amp, 150 kV, filter 0·5 mm aluminium, 0·5 mm copper distance 120 cm. The dose rate was 10 r./min. In th individual experimental groups of rats, saliva was collecte under thiopental anæsthesia after the administration o pilocarpine at time-intervals of 6, 24 and 48 h followin

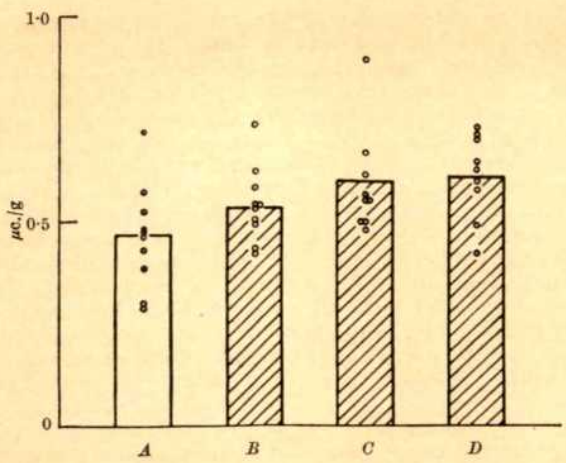


Fig. 1. Concentration of radiorubidium in saliva after irradiation (μc./g dry weight). *A*, Control group; *B*, 6 h after irradiation; *C*, 24 h after irradiation; *D*, 48 h after irradiation

irradiation. In the control group, saliva was collected in a similar fashion. The collection technique, according to Bernard *et al.*[2], was the same as that utilized in our previous investigation[1]. The samples of collected saliva were dried and weighed and their activity determined by measuring with a window Geiger–Müller tube for the measurement of β-activity using a standard.

From the resulting values the concentration of radio rubidium in the samples of saliva was determined in μc./g dry substance. The results, after correction has been made for excretion equally as in the previous investigation[1], are shown in Fig. 1. The heights of the columns express the mean values of radiorubidium concentration found in the individual groups, the points represent individual values. The column *A* belongs to the control group, the columns *B*, *C* and *D* consecutively to the groups of animals in which saliva was collected, respectively, 6, 24 and 48 h following irradiation. The last 2 columns include only 9 individual values since in each of the corresponding groups 1 animal had succumbed prior to the collection of saliva.

From Fig. 1 it is obvious that there occurs, after irradiation, in all the groups examined an increase in excretion of radiorubidium by the saliva as compared with the controls. On statistical evaluation we have found that the probability of insignificance of the differences in the values found in the controls and in the individual irradiated groups was: 6 h after irradiation more than 5 per cent, 24 h after irradiation less than 2·5 per cent, and 48 h after irradiation less than 1 per cent.

The significant increase in excretion of rubidium by saliva occurring 24 and 48 h after irradiation may be explained by a rise of its concentration in blood. It is known that the excretion of the analogous element potassium by saliva is directly dependent on its concentration in blood[3], and that there occurs, after irradiation, potassium depletion from a number of organs and consequently a rise in its blood-level[4].

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BIOLOGY

Root Hair Zone of Soil-grown Roots

Pathogens which invade the roots of higher plants from the soil, in general, enter through the root hair zone of the seedling or the feeder roots of the older plant. Recent examination of certain common soil-grown species of dicotyledons and monocotyledons, namely, broad bean (*Vicia faba*), castor bean (*Ricinus communis*), mustard (*Brassica nigra*), purslane (*Portulaca oleracea*), radish (*Raphanus sativus*), squash (*Cucurbita pepo*), willow (*Salix* sp.), onion (*Allium cepa*), and wheat (*Triticum aestivum*), indicates that the description of the root hair zone in current text-books is inadequate and requires considerable modification in respect to the extent of the region of absorption and the details of tissue structure[1]. In present-day diagrams of the primary root, the root hair zone is invariably short. The xylem elements are devoid of contents. It is generally stated that the epidermis of the root differs from that of the shoot in the absence of a cuticle. It is somewhat difficult to reconcile the limitation of the absorptive region with the extent of the root systems which have been examined in detail by numerous investigators[2]. In rye grass, for example, the estimated total length of the $4{\cdot}5 \times 10^9$ root hairs amounts to 6,603 miles, with a surface area of 432 sq. ft. (ref. 3).

Extent of root hair zone. In dicotyledons the root hair zone, as described, occurs on the tap and lateral roots of the main root system which undergo secondary thickening. In addition, however, the absorptive region is supplemented by a wealth of slender filamentous roots which arise from tap roots and laterals, generally, but not invariably, in acropetal succession. In seedlings and in feeder roots these filamentous roots may measure 20 cm or more in length and are covered from tip to origin with living nucleated root hairs. Their increase in thickness is barely appreciable, with 2–4 xylem elements near the tip, and 6–8 at the point of origin. When filamentous roots first emerge on the root surface, the adjacent epidermal and cortical cells which have been forced apart are somewhat crushed but are already well cutinized or suberized. The healing of the surface tissue is thus concomitant with root emergence. Moribund roots become brown and somewhat shrunken and, after death and disintegration, a well-suberized root scar remains on the parent root until sloughed off during secondary thickening. The actual life duration of the filamentous roots is not known in the species examined.

In the soil-grown monocotyledons, onion and wheat, all the fibrous roots are much branched. The long and very slender laterals, like the filamentous roots of the dicotyledons, are covered throughout their length with living nucleated root hairs.

Epidermis. The surface of soil-grown roots is covered with soil particles, among which fungal hyphæ, bacteria and occasional other micro-organisms are visible. The walls of the epidermal cells, piliferous and non-piliferous, consist of a predominantly cellulose framework with a surface film of mucilage and a cuticle[4,5]. Pits and plasmodesmata occur on radial and periclinal walls, and, under the electron microscope, are evident also in the root hairs[6]. In the outer tangential walls the plasmodesmata appear to be anchored in the cuticle. The epidermis of the monocotyledons examined resembles that of the dicotyledons.

For comparison with soil-grown roots, those of a willow which extended from a stream bank into running water were studied. They resemble soil-grown roots in the presence of mucilage and a cuticle on the outer epidermal wall, including the root hairs.

The outer epidermal wall of the root is thus a complex stratified and perforate membrane. The plasmodesmata presumably play a part in the absorption of solutes and in the transport of the precursors of mucilage and of cutin essential during the growth in thickness of the wall, and



Fig. 1. Filamentous root of radish, living material slightly crushed, stained neutral red, showing protoplasts in the xylem elements, the diameter of which ranges from 3 to 5μ. The root hairs are not in focus

also in the exudation of specific substances in certain plants.

Cortex. Near the root tip, the meristematic cortical cells are polyhedral and are pitted on all cell faces. In the root hair zone the mature cortical cells are cylindrical or ellipsoidal and are partially separated by intercellular spaces. Intercellular contact is maintained through larger and comparatively fewer pits and plasmodesmata which are aligned vertically in 5 or 6 rows on the cylinder[7]. The ubiquitous pitting in the circular end walls is similar to that in the meristematic cells. Intercellular spaces arise as soon as cell growth in volume begins and the cell corners of the polyhedral cells are rounded off. The spaces form a continuum throughout the plant and the entire surface is coated with a lipid film, termed 'suberin' in this and in previous papers[9].

Endodermis. The innermost layer of the cortex has been discussed by many investigators, including Priestley[10], in relation to the entrance of water into the stele. More recently, Van Fleet has considered the significance of the biochemical changes which occur during differentiation[11]. Pits and plasmodesmata are numerous in the outer and inner periclinal walls and are also evident on the radial walls. In the latter, however, under the electron microscope, the pits and plasmodesmata are seen to be partially or completely embedded in the Casparian strip, a fact which explains the characteristic pattern of plasmolysis in the endodermis. The outer and inner rims of the Casparian strip are first delimited as fine lines of thickening on the radial walls next to the air-filled intercellular spaces. It therefore appears that suberin deposition begins next to the intercellular spaces and coincides with the initiation of wall thickening.

Pericycle. The pericycle is responsible for the origin of branch roots. The details of root initiation have not been worked out in the species at present examined, but they presumably resemble that of the strawberry[12].

Stele. During stelar differentiation, annular and spiral cells with pitted walls[13] are followed by reticulate and pitted vessel elements. These elements range in length from 50 to 1,000μ and in diameter from 4 to 25μ. In the transparent, filamentous, living roots, 2–4 annular or spiral elements are seen near the tip. Slight pressure frees the entire stele from the enveloping cortex. With or without the addition of neutral red or other aqueous stains, the majority of the xylem elements are seen to contain living nucleated protoplasts (Fig. 1). Isolated protoplasts with their projecting plasmodesmata are demonstrated when xylem elements, unlignified, or semi-lignified are treated with $I_2KI—H_2SO_4$ with the resultant disintegration of the cellulose walls[14]. In older roots the empty xylem elements are lined with a suberin film similar to the osmiophilic membrane or the warty layer of other authors[15,16]. It is thus evident that water and solutes pass directly into living protoplasts in the root hair zone, from which they are released into the transpiration stream on the death of the xylem protoplasts at a higher level in the root.

In conclusion, the extent of the root hair zone, the cuticle of the outer epidermal wall, the lipid 'suberin' coating of all intercellular spaces, the pitting of all cell walls including root hairs and xylem elements, and the presence of living protoplasts in the xylem elements require consideration in relation to absorption of solutes, root exudation, the invasion of pathogens into living cells, and the transport of certain viruses.

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A New Cycadeoid Plant from the Permo-Carboniferous Coal Measures of the Transvaal

A portion of a slender stem, with two pedicellate flower-like structures in organic attachment, shown two-thirds natural size in Fig. 1, was recently discovered in Lower Permian beds at Vereeniging, Transvaal. Remains of *Glossopteris* spp. leaves and a petiole of a leaf of *Noeggerathiopsis* sp. occur on the same slab of red ferruginous shale. The original organic material was replaced by ferric oxide and the plant is preserved in the form of a compressed cast of red ochre.

The much-flattened slender stem is striated longitudinally and has a uniform width of 6·5 mm. The thickness is approximately 1·5 mm which is also the total thickness of the woody zone surrounding the central pith, which is not preserved.

One of the two solitary and alternately arranged fructifications, shown near the upper end of the stem, is badly damaged and provides little detail. The more conspicuous second specimen, lower down the stem, is beautifully preserved, however. The axis of this flower-like structure is composed of a fleshy receptacle, borne on a strong woody pedicel, which is swollen in the region of its junction with the receptacle. The presence of the latter is inferred from



Fig. 1

the swollen nature of the basal portion of the structure. The true nature of the receptacle is unknown since it is obscured by a whorl of petal-like organs inserted round the base; apparently in several series.

There is a gradual decrease both in the width and thickness of these organs towards the apices and there are indications that they were traversed longitudinally by several vascular strands.

The general appearance of the fructification is that of a hypogenous angiospermous flower in which there is no distinction of calyx and corolla. Among fossil plants the fructification is reminiscent of the bisexual flowers of *Williamsoniella*. Whether the petal-like structures and receptacle of the new fructification actually functioned as staminate and ovulate organs, respectively, remains an open question, however.

Large flower-like fructifications, referred to the Bennettitales, were recently described by Plumstead[1] from the same beds in which the new plant was discovered. Although the two plants were evidently related, the former differed from the latter in that the flowers were much larger and were borne on very thick stalks.

The new plant has not been studied in detail yet, but the small size and general appearance of the flower in addition to the slender stem suggest classification under the Microflorae. The preliminary evidence afforded by the external morphological features seems to carry sufficient weight to suggest the separation of the plant, not only specifically, but also generically, from the known cycadeoids.

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A Sensitive Method for Determination of Low Concentrations of Diquat and Paraquat

Determination of residues has become an important aspect of herbicidal research. Efforts are continually being made to increase the sensitivity of known tests for the chemical analysis of a large number of herbicides. Aquatic herbicides must be effective and used in small quantities (usually 1·0–5·0 p.p.m.) to be economically feasible; consequently, their residues are usually very small.

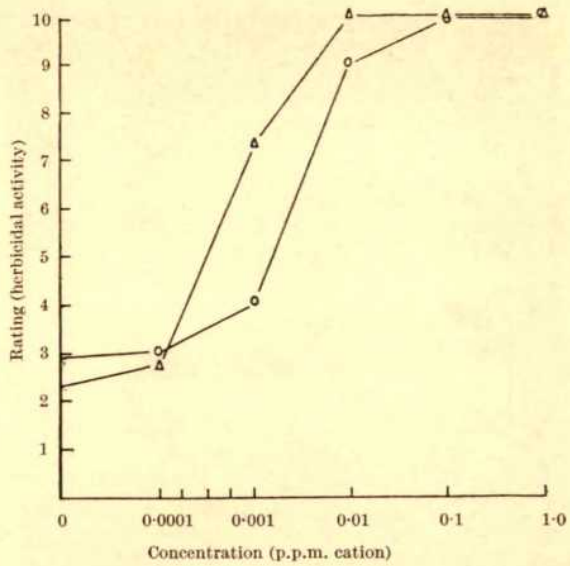


Fig. 1. Effect of various rates of diquat and paraquat on the chlorosis of duckweed. △, Diquat (11 days); ○, paraquat (11 days)

New herbicides which control a number of aquatic weeds at exceedingly low rates are 1,1′-ethylene-2,2′-bipyridylium dichloride (diquat) and 1,1′-dimethyl-4,4′-bipyridylium dichloride (paraquat). In preliminary experiments, it was observed that these chemicals caused a general and finally complete chlorosis of several aquatic weeds. One of the weeds, which was completely bleached by these herbicides, was duckweed (*Lemna minor* L.)[1]. This communication presents evidence of the extreme sensitivity of duckweed to diquat and paraquat.

All experiments were conducted in a growth-chamber where the conditions were as follows: 500 ft.-candles of light, 75° F, 14-h day and a 10-h night. Test plants were maintained in the same growth-chamber. They were grown in aerated 0·1 strength Hoagland and Arnon's No. 2 nutrient solution in a plastic dish-pan which measured 14 in. × 10 in. × 8 in. A 2-in. layer of sand and soil (50–50 mixture) covered the bottom of the dish-pan. A chelated source of iron was added periodically.

Approximately 50 fronds of duckweed were placed in each of nine 400-ml. clear plastic cups which contained 50 ml. of 1·0, 0·1, 0·01, 0·001, 0·00075, 0·0005, 0·00025, 0·0001, and 0 p.p.m. (cation) (since the cation is the herbicidally active portion of the molecule, all data are presented in p.p.m. cation) of either diquat or paraquat. The plastic container was covered with a clear, plastic lid. The condition of the duckweed was rated every 24 h for 11 days by the following system: 1–3 = control, 4–6 = herbicidal activity, 7–9 = definite herbicidal activity, and 10 = dead. Each test was duplicated and then replicated four times.

The effect of *p*H on herbicidal activity was also checked. Fifty ml. of a 0·01 M phosphate buffer (KH_2PO_4 for *p*H's 4–6 and K_2HPO_4 for *p*H's 7–10) which contained 0·1 p.p.m. diquat or paraquat was used for this work. The check was buffered at *p*H 7. The condition of the fronds was rated every 24 h for 3 days.

Duckweed was very sensitive to diquat and paraquat (Fig. 1). The bleaching effect caused by 1·0 p.p.m. is very rapid (24 h), while the appearance of chlorosis in plants subjected to decreasing rates of herbicide required a longer period of time. The herbicidal activity ratings for both chemicals at the end of 72 h was high for concentrations of 0·01, 0·1, and 1·0 p.p.m. It takes considerably longer (approximately 8 days) before the effect of the lower rates becomes evident. From the

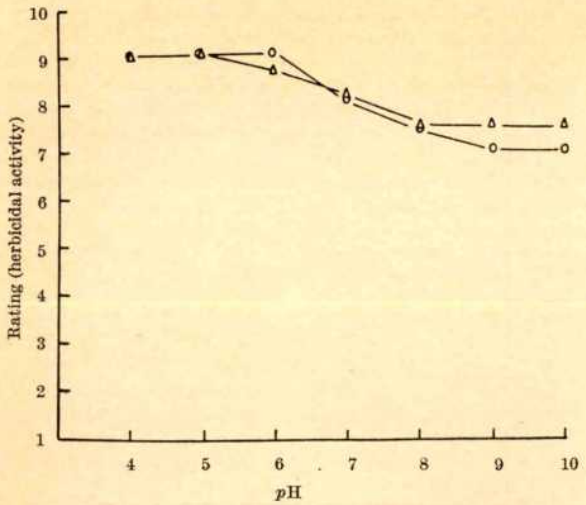


Fig. 2. Effect of diquat and paraquat on the chlorosis of duckweed at various *p*H's. ○, 0·1 p.p.m. diquat (72 h); △, 0·1 p.p.m. paraquat (72 h)

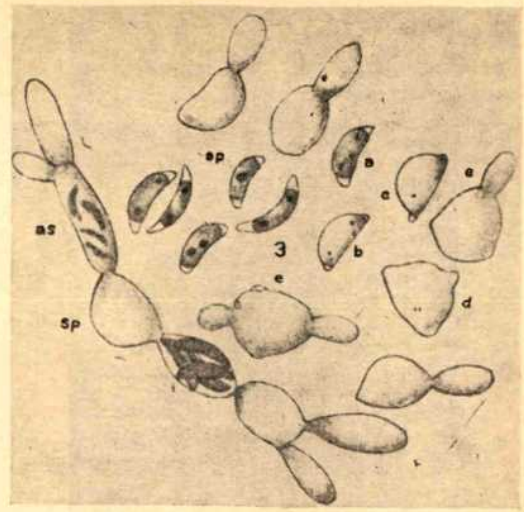


Fig. 1. *Guilliermondella selenospora*, from Nadson and Krassilnikov (ref. 1)

results presented in Fig. 1, it appears that 0·0005 p.p.m. diquat and 0·00075 p.p.m. paraquat can be detected by bioassay with duckweed. The sensitivity of this test is as good or better than many of the more common bioassays[2–5]. There is an excellent analytical procedure for determination of these compounds[6], but it has a maximum sensitivity of 0·01 p.p.m. The best results from this bioassay have been obtained when test plants from a young, actively growing culture were used.

The bleaching effect by both herbicides was more rapid at lower *p*H's (4–6) than at higher *p*H's (8–10) after 72 h. Forty-eight h later, or after a total of 120 h, no difference among the various *p*H's could be detected. The masking of the *p*H difference with time was expected, since 0·1 p.p.m. is a rather high concentration for this sensitive bioassay (Fig. 1). Diquat is unstable at high *p*H's, but paraquat is rather stable up to *p*H 11 (ref. 7). Therefore, the stability of these chemicals at various *p*H's does not explain the results presented in Fig. 2. The bioassay has been used to determine residues of diquat and paraquat in water collected from treated ponds and streams. It has also been used to detect the presence of these herbicides in the expressed cell sap from submersed aquatic weeds.

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Ascospores of *Endomycopsis selenospora* (Nadson et Krassilnikov) Dekker

In 1928 Nadson and Krassilnikov[1] described a yeast isolated from the exudate of oaks in Kalouga, U.S.S.R. According to the original description, the yeast formed true mycelium as well as budding cells. Its asci contained four falcate spores. Nadson and Krassilnikov further stated that the ascospores appeared to have a double membrane, the latter being visible as two colourless appendages at the extremities of the spores (Fig. 1). On account of the characteristic ascospore shape the authors erected a new genus for this yeast and named it *Guilliermondella selenospora*.

Stelling-Dekker[2] transferred this species to the genus *Endomycopsis*, maintaining that *Endomycopsis* and *Guilliermondella* differed only in the shape of the ascospores, a criterion which she considered insufficient for separating these two genera. Stelling-Dekker's classification was accepted by Lodder and Kreger-van Rij[3].

The ascospore structure of *Endomycopsis selenospora* has recently been investigated in somewhat greater detail by ourselves. We have examined by means of the light microscope the spores of two strains of *E. selenospora* cultivated on malt agar and on corn-meal agar, and have been able to confirm Nadson and Krassilnikov's description. In several instances transparent appendages were visible at the extremities of the spores. However, there was considerable variation in spore shape. The spores, especially when still in the asci, may assume a more or less spheroidal to ellipsoidal shape. When liberated from the ascus the falcate shape was more pronounced, and so were the appendages.

An electron microscope examination of ultra-thin sections of the spores fixed with osmic acid and uranyl acetate according to the procedure described by Ryter

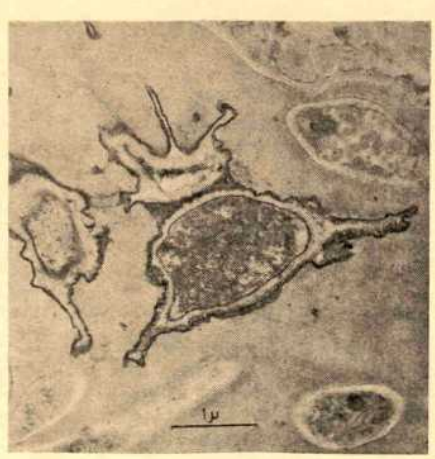


Fig. 2. *Endomycopsis selenospora*

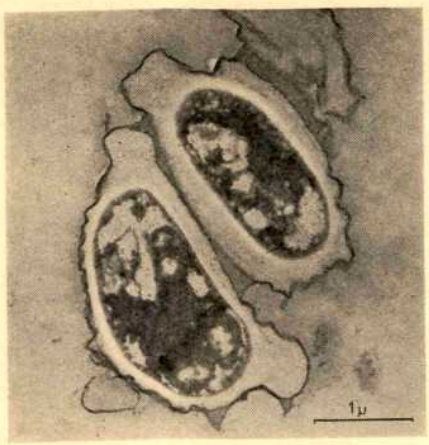


Fig. 3. *Endomycopsis selenospora*

et al.[4], revealed that the transparent parts were in fact outgrowths of the spore wall and comparable with the ledge structure on the spores of *Pichia* and *Hansenula* species. However, in the case of *E. selenospora* the outgrowths were restricted to the opposite poles of the spore and did not form circumfluent rims as is the case with the hat- or saturn-shaped spores of *Pichia* and *Hansenula*.

Cross-sections showed the spore wall either as a ring, more or less dented on the outside, or as a round to oval ring with two processes at the opposite poles (Figs. 2 and 3). The processes varied from thin protuberances of variable length to broad outgrowths.

Analogous investigations of the ascospores of other yeasts revealed that the internal structure of the spore of *E. selenospora* wall did not differ from that of, for example, *Saccharomyces* and *Pichia* species. The expression 'double membrane' does not, therefore, seem applicable to the spores of *E. selenospora*.

A comparison of the spores of *E. selenospora* with the crescentiform or reniform spores of *Sacch. fragilis* (Fig. 4) and *Sacch. delphensis* showed that in the last two species the spore wall had no outgrowths. The wall appeared thin and slightly notched, and this was also the case with the spores of *Sacch. cerevisiae* when observed under similar conditions.

Boidin *et al.*[5] have recently redefined the genus *Guilliermondella* so as to include budding yeast species forming 1–4 kidney- or crescent-shaped—more rarely spheroidal—ascospores which are liberated from the ascus at maturity. *Guilliermondella* would thus include, besides *E. selenospora*, species from the genera *Saccharomyces*, *Fabospora* and *Zygofabospora*.

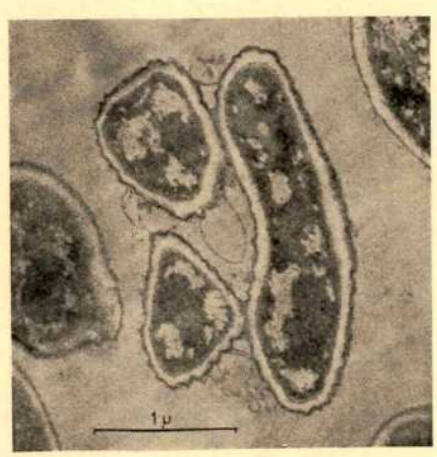


Fig. 4. *Saccharomyces fragilis*

From the findings we have described it is apparent that the resemblance in shape of the spores of *E. selenospora* to those of, for example, *Sacch.* (*Fabospora*) *fragilis* is superficial and does not constitute valid grounds for assigning *E. selenospora*, which produces an abundance of true mycelium, to a genus which, for the rest, consists entirely of budding species. We therefore regard *E. selenospora* as distinct from those species with crescenti- and reniform spores and which multiply exclusively by bud-formation, and prefer its provisional classification in the genus *Endomycopsis*.

We thank Miss J. M. Kempers for the preparation of the ultra-thin sections.

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Effect of Scabs on the Rate of Epidermal Regeneration in the Skin Wounds of Guinea Pigs

The effect of scab on the healing of a wound is not well understood. MacCallum's[1] idea that the scab may store nutritive material for the granulation tissue is not generally accepted. James[2] demonstrated a high concentration of hydroxyproline in the scab hydrolysates but this was shown to be derived from the collagen fibres which had been incorporated into the scab[3]. Winter[4] put forward some experimental evidence designed to show that the scabs retarded the rate of epidermization of wounds in the skin of pigs. During an examination of the various factors which influence the rate of wound healing, observations have been made which are very different from those of Winter's.

Ten male guinea pigs, each weighing 200–250 g, were used. The skin of their dorso-lumbar region was shaved by an electric clipper. Twenty square pieces of rubber, each $3{\cdot}5 \times 3{\cdot}5 \times 0{\cdot}5$ cm in size, were taken and square holes of $2{\cdot}5 \times 2{\cdot}5$ cm were made into them. These rubber frames were used as splints. One of the splints was fixed in each flank of the guinea pigs by means of an adhesive paste. A wound of $1{\cdot}5 \times 1{\cdot}5$ cm was then made inside each splint. The wound area was first marked by a stamp and the skin was then removed along with the panniculus carnosus by means of a sharp scalpel, leaving deep fascia in the base of the wound. Immediately after a wound was made, its margins were traced on a tracing paper. Afterwards, a piece of polythene was fixed to each left-side splint in order to prevent drying of wounds on this side. The wounds on the right side were left uncovered. The animals were examined on alternate days and the wound margins traced again. The wound tracings were enlarged in an enlarger and retraced again on a Bristol board from which they could be cut out and weighed. By knowing the weight of the Bristol board per cm^2 it was easy to calculate the wound area.

The effectiveness of these splints in preventing wound contraction had been demonstrated in many experiments

Table 1

Days after wounding	Mean wound area with its standard deviation Left	Right
0	2·20 ± 0·06	2·21 ± 0·04
2	2·13 ± 0·11	2·09 ± 0·10
4	2·06 ± 0·09	2·00 ± 0·11
6	1·87 ± 0·12	1·68 ± 0·07
8	1·56 ± 0·10	1·49 ± 0·06
10	1·32 ± 0·07	1·31 ± 0·12
12	1·02 ± 0·07	1·10 ± 0·11
14	0·93 ± 0·12	0·88 ± 0·05
16	0·67 ± 0·13	0·75 ± 0·04
18	0·37 ± 0·05	0·43 ± 0·06
20	0·13 ± 0·08	0·20 ± 0·05

not mentioned here. It is therefore reasonable to assume that all these wounds healed almost exclusively by epidermization. The exposed wounds formed a dry and tough scab over them while the covered wounds had only a very thin and soft scab. Two of the covered wounds became infected—a phenomenon exceedingly rare in the exposed wounds of guinea pigs. Table 1 compares the mean area (with its standard deviation) of the left and the right side wounds on alternate days in eight guinea pigs. It is clear that there is no significant difference in the rate of wound healing on the two sides. The mean time taken by wounds to heal was 18·3 days on the left side and 18·5 days on the right, the difference being again insignificant.

A histological examination of both covered and uncovered wounds was also carried out in a separate experiment. It was found that even in an uncovered wound, where a thick scab formed, there was always a layer of fresh exudate under the scab. This exudation was a continuous process. The edge of the newly regenerating epidermis crept forward through this exudative layer and therefore encountered minimum resistance. It occasionally happened that the epidermal edge split into two branches, one spreading superficially and the other deeper to the scab. It was always the deeper branch which survived. In the uncovered wounds of the guinea pigs the first scab was usually discarded after the first five to seven days. A second scab promptly formed, but this again had a layer of exudate under it through which the epidermal edge continued its progress. Some necrosis of the wound edge always occurred[3] but this was traumatic in nature and had nothing to do with the drying of the wound. The epidermal edge seemed to have no difficulty in undermining this necrotic edge.

I thank Dr. A. H. T. Robb-Smith for introducing me to this subject, which is part of the work carried out during the tenure of a Rhodes Scholarship.

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CYTOLOGY

Interaction of Chromatid Breaks induced by Three Different Radiomimetic Compounds

Quite a few physical and chemical inducers of chromosomal aberrations have been tested for interaction of chromatid breaks induced by different agents, the product of interaction being chromatid translocations (exchanges). On the basis of the breakage-reunion hypothesis presence or absence of interaction may be expected to give some hints on the bonds broken by the treatments being identical (full interaction) or not (no interaction, additive effect), provided time (different stages of maximal sensitivity against the agents) and space factors (different distribution of aberrations within and between chromosomes) can be ruled out as influencing the experimental results.

Table 1. Presence (+) or Absence (−) of Interaction of Chromatid Breaks induced by Different Agents in *Vicia faba* Root Tip Meristem

Agents used for treatment	Interaction	Refs.
X-rays and KCN	+	1, 2
X-rays and β-propiolactone	+	1, 2
X-rays and 8-ethoxycaffeine	+	1, 2
X-rays and maleic hydrazide	+	1, 2
X-rays and diepoxybutane	−	3
X-rays and 2,2′bipyridine	−	3
X-rays and 5-fluorodeoxyuridine	−	16
Myleran and ethylmethanesulphonate	+	4, 5
Myleran and ethyl alcohol	+	4, 5
Ethylmethanesulphonate and ethyl alcohol	+	4, 5
KCN and β-propiolactone	−	1, 2
KCN and diepoxybutane	+	3
KCN and 2,2′bipyridine	+	6
Diepoxybutane and 2,2′bipyridine	+	6
Maleic hydrazide and 8-ethoxycaffeine	−	1, 2
5-Fluorodeoxyuridine and 8-ethoxycaffeine	−	16
Maleic hydrazide and ethyl alcohol	−	This repor
Maleic hydrazide and TEM	−	This repor
TEM and ethyl alcohol	+	This repor

Table 1 summarizes the results of interaction experiments done by various authors using meristems of *Vicia faba* root tips as test materials for their investigations. Obviously it depends on the specific attributes of the agents combined or administered one following the other whether interaction of chromatid breaks induced by different agents appears or not. Both in combination experiments with X-rays and radiomimetic compounds or with different radiomimetic chemicals cases of interaction and non-interaction have been found (Table 1). Absence of interaction may be the result of various mechanisms[1–3].

We treated main root meristems of *Vicia faba* var. *minor* ('Dornburger Ackerbohne') with 5×10^{-4} M maleic hydrazide (MAL), 5×10^{-5} M triethylenemelamine (2,4,6-tri(ethyleneimo)-1,3,5-triazine, TEM) and 2×10^{-1} M ethyl alcohol (EA). Treatment time and temperature were 30 min. and 24° C in the first two cases and 4 h and 30° C in case of EA. Temperature during recovery time (time between treatment and fixation) was 24° C. Prior to fixation (3 parts ethyl alcohol, 1 part glacial acetic acid) the roots were immersed in 0·05 per cent colchicine for 2 h. Permanent Feulgen squashes were prepared by the dry ice method. The three agents, which are known chromosome breakers in *Vicia*[7–10], were administered one following the other. They are inducing preferentially localized aberrations on chromatid basis only, the distribution pattern of maleic hydrazide induced aberrations being characterized by significant differences in comparison with those of the two other agents. MAL, TEM and EA belong to the class of radiomimetic agents with delayed effects. No structural chromosomal changes can be observed the first 8 h after treatments with these agents. Thus it seems as if these compounds are unable to induce aberrations in cells where DNA synthesis is completed[11,12]. Different modes of action of MAL, TEM and EA are evident from experiments testing the influence of the metabolic state of the cells during treatment on aberration production.

The results of our experiments with the three compounds are given in Table 2 for 24-h recovery time which gave the

Table 2. Behaviour of Chromatid Breaks induced by Maleic Hydrazide (MAL), Triethylene Melamine (TEM) and Ethyl Alcohol (EA) in Exchange Production (400 Metaphases Scored, 24 h Recovery Time)

Treatment	Abnormal metaphases (%)	Isolocus breaks (%)	Exchanges % calculated Observed	additive	interaction
EA	18·25	9·75	6·75	—	—
TEM	12·25	7·75	4·0	—	—
EA—TEM	42·25	17·75	18·75	10·75	21·0
MAL	17·75	9·0	7·5	—	—
TEM	11·5	7·5	3·75	—	—
MAL—TEM	30·75	18·0	11·5		
TEM—MAL	30·0	17·25	11·25	11·25	21·5
EA	17·5	9·0	7·5	—	—
MAL	17·25	8·75	7·75	—	—
EA—MAL	27·25	15·0	13·25	15·25	30·5

aberration peak. Scoring of aberrations after 30-h recovery time gave lower aberration yields but the same results so far as interaction is concerned.

Interaction of chromatid breaks induced by EA with those induced by TEM is evident from Table 2. A comparison of the frequency of chromatid translocations (exchanges) scored and theoretically expected after treatment of root tip meristems with both EA and TEM indicates practically complete interaction of the breaks produced by the two agents. For isolocus breaks (intrachanges) the minimum expected frequency from the EA–TEM treatment would be that indicating additivity of the values found in the treatments with EA and TEM alone (controls) and this is, in fact, the case. From the interaction of chromatid breaks induced by EA and TEM it may be concluded that the breaks produced by these agents are of a similar or identical nature provided the breakage-reunion hypothesis is a correct interpretation of the events leading to aberration production[13].

Different results have been obtained when MAL and TEM or EA and MAL were administered one following the other. In these circumstances the percentage of both iso'ocus breaks and chromatid translocations was not h'gher than additive in comparison with the control values from the treatments with MAL, TEM and EA alone, indicating absence of interaction of the chromatid breaks in exchange production. Since the period of maximal sensitivity of the interphase nucleus is in all probability identical for all three compounds used the time factor—restitution or rejoining of breaks induced by one agent before break induction by the other—cannot be used for an interpretation of the absence of interaction.

Considering the fact that there is only a restricted number of possible sites in the interphase nucleus where two chromosomes are so close together as to be able to produce an exchange[14] when two breaks occur in this area, an interpretation of the absence of interaction in our experiments may be seen in the different localization of aberrations induced by MAL[7] on the one hand and TEM[8,9] as well as EA[10] on the other. But since we found in fractionation experiments that breaks induced by TEM and EA seem to stay open at least for 8 h[15] whereas those induced by MAL are closed already after a time span of less than 3 h (unpublished results) the breakage of differential bonds by the agents may also play some part if the differential rejoining time really reflects a different nature of the bonds broken. It is not possible at present to point out the relative significance of these two factors which may be responsible for the absence of interaction between chromatid breaks induced by the combinations MAL–EA and MAL–TEM.

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VIROLOGY

Interference Experiments relating to whether Tobacco Mosaic Virus and Cucumber Viruses 3 and 4 are Strains of the same Virus

THE problem of whether cucumber viruses 3 and 4 (CV3, CV4) and tobacco mosaic virus (TMV) are different viruses or strains of the same virus has been examined in detail by Knight[1]. On the basis of several criteria such as host-range, chemical composition, electron microscope morphology, etc., he concluded that they were different viruses. However, two criteria, serology and the ability to exhibit cross-protection, still support the idea that they are strains of the same virus. In regard to cross-protection, it was first found by Fulton[2] that prior infection of cucumber by CV3 and CV4 did not protect against subsequent TMV infection, but later Rochow[5] was able to demonstrate a high degree of protection. Apparently, the difference between Fulton's and Rochow's experiments is shown in the length of time the plant is allowed to sustain a CV3 and CV4 infection before it is challenged by a second inoculation with TMV.

Another method for assessing virus relationship has come from experiments which utilize mixed inocula[3]. This system of experimentation has shown that strains of TMV will markedly interfere with each other's lesion production when they are present in the same inoculum. The degree of interference is a function of the ratio of the two strains as well as the absolute concentration of the two strains in the mixed inoculum. In contrast, when the same system is used to test interference between tobacco necrosis virus and TMV, no interference is detected[4]. In addition to the mixed inoculum test, all other criteria indicate that the spherical tobacco necrosis virus is completely unrelated to the rod-shaped TMV. It was of interest, therefore, to investigate whether two rod-shaped viruses, CV4 and TMV, would display interference when analysed by the mixed inoculum system.

We have repeated the experiments of Fulton and Rochow and have confirmed their results. Cotyledons of 7–8 day old cucumber seedlings (*Cucumis sativus* L., var. Improved Long Green) were used. One cotyledon was inoculated with 1 mg of CV4/ml., and the opposite cotyledon was rubbed with phosphate buffer without virus. At different days after the inoculation, both cotyledons were re-inoculated with 0·5 μg of *U*1 (Common) TMV/ml. Four days after the TMV inoculation, the plants were placed in a dark room for 24 h, and then the starch lesions were developed and counted according to the method of Lindner *et al.*[5]. Since CV4 does not produce starch lesions on cucumber cotyledons, all lesions were scored as TMV lesions. The result of one such experiment is shown in Table 1. When the interval between CV4 and TMV inoculations was 6 days or less, virtually no interference with TMV lesion formation was observed. However, if the interval extended to 10 days, protection against TMV lesion formation was almost complete.

The possibility of interference between CV4 and TMV present in a mixed inoculum was also tested. A typical result was the following. One half of the cotyledons on 65 cucumber seedlings was inoculated with 0·5 μg TMV/ml. The other half was inoculated with a mixture that contained 0·5 μg TMV/ml., together with 250 μg CV4/ml. The total number of starch lesions that developed was 2,939 for TMV alone, and 3,001 for the mixture of TMV and CV4. Thus, no interference with TMV lesion formation by CV4 was detected in this or several other experiments. It may be recalled in this connexion that had 0·5 μg of one strain of TMV been present along with 250 μg/ml. of another strain, interference to the level of about 93–98 per cent would have occurred[3]. It also seemed possible that CV4 might be behaving like the *U*2 or *VM* strain of TMV on pinto bean leaves[3]. Under our environ-

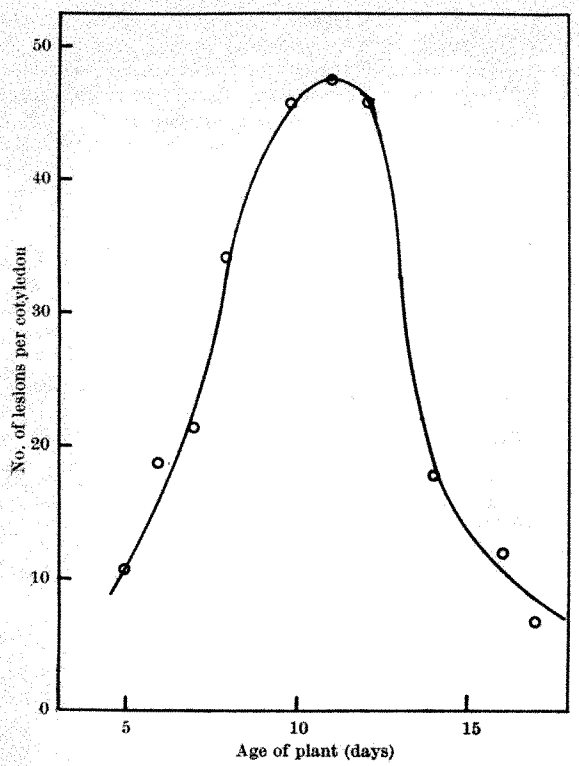


Fig. 1. Relationship between number of starch lesions produced by TMV and the age of cucumber cotyledons. Days indicate the time of inoculation after sowing the seeds. 0·5 μg TMV/ml. was used as an inoculum

mental conditions, *U2* and *VM* do not produce visible indications of having infected pinto bean leaves. However, the presence of *U2* or *VM* in a mixed inoculum with *U1* will strongly suppress lesion formation by *U1*. We therefore tested a mixed inocula containing *U1* and CV4 and found no suggestion of interference with *U1* lesion formation on pinto bean, scotia bean and *N. glutinosa*.

Thus, the additional criterion of absence by mixed inocula supports the view that CV4 and TMV are different viruses. But the question remains as to how to account for the cross-protection exhibited in Rochow's experiments. One possible explanation is that infection by CV4 alters the physiology of the host to a degree where a subsequent infection by TMV cannot proceed to the formation of starch lesions conspicuous enough to be detected by iodine staining. From Table 1, it is apparent that the number of starch lesions produced by the same concentration of TMV decreases as the age of the cotyledons increases. Additional experimental data plotted in Fig. 1 show this effect in greater detail where it was observed that cucumber cotyledons were maximally sensitive to TMV about 10 days after germination, and then rapidly lost their sensitivity. We have also observed that as sensitivity decreases with age, the TMV starch lesions also decline in size until they disappear entirely about 20 days after germination. Thus, the explanation we propose is that the presence of CV4 in a cucumber cotyledon merely accelerates the ageing process which normally operates to make the cotyledons resistant to TMV lesion formation. In this view, the cross-protection observed is not the direct result of two different viruses competing for the same virus synthesizing machinery within the same host cell. Rather, the presence of CV4 in some cells hastens the development of resistance in these and other cells that TMV may have invaded, but the resistance prevents the TMV infection from continuing to a visible stage. If sufficient time were allowed for the cotyledons to age, the resistance would have arisen in the absence of CV4.

Table 1. Effect of Previous CV4 Infection on the Number of TMV Starch Lesions

Age of seedlings at the time of TMV inoculation (days)	Interval between CV4 inoculation and TMV inoculation (days)	Total No. of lesions on 13 cotyledons not infected with CV4	Total No. of lesions on 13 cotyledons infected with CV4	Percentage protection of CV4 against TMV
11	4	651	700	0
13	6	456	371	18·7
17	10	127	8	93·7

We thank Dr. C. A. Knight, of the Virus Laboratory of the University of California, for supplying the strain of CV4. In this connexion, this strain did not prove to induce chlorotic and starch lesions on *N. tabacum* var Samsun and var. Xanthi-nc, and thus is different from the CV4 strain reported by Brcak *et al.*[7].

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Transformation of Bovine Tissue Culture Cells by Bovine Papilloma Virus

Several viruses of the papovavirus group, namely polyoma, *SV*–40, and rabbit papilloma viruses, produce hereditable morphological alterations—'transformation'—of appropriate tissue culture cells[1]. We have found that another member of this group, the bovine papilloma virus produces cellular alterations and proliferation in certain bovine tissue culture cells after a very short latent period.

The changes in the cells occurred soonest and were most readily recognized in a semi-continuous diploid cell line of fœtal bovine conjunctiva (strain *DBC*). (All tissue cultures were obtained from Microbiological Associates, Inc. Bethesda, Maryland.) Tube cultures were held stationary at 36° C and maintained in medium N.C.T.C.–109 (ref. 2) with 10 per cent unheated agammaglobulinic calf serum ('Agamma Calf Serum', Hyland Laboratories, Los Angeles), penicillin, and streptomycin. Petri dish cultures were grown and maintained at 36° C in a humidified atmosphere of 5 per cent carbon dioxide in air; the medium was Eagle's basal medium with 10 per cent unheated fœtal bovine serum, and antibiotics. Freshly planted cultures of uninfected *DBC* cells consist of triangular, elongated cells of epithelioid or fibroblastic appearance; within several days, the cells form a continuous monolayer of polygonal cells resembling epithelium, with a few of the triangular cells at the periphery of the cell sheet. These control cultures remain unchanged with respect to cell type, and except for increasing granularity and non-specific degenerative changes, may survive for periods as long as 120 days. Cultures inoculated with 20 per cent extracts of three bovine papillomata (from cattle in Maryland, Wisconsin (supplied by Dr. Carl Olson, jun.), and Michigan (supplied by Dr. R. A. Tjalma, East Lansing)) consistently show distinct morphological changes within 3–10 days. The earliest change is the appearance of rather short, thin, spindle cells scattered over the cell sheet; in tube cultures, many long, thin, spindle cells appear at the periphery of the cell sheet. The majority of the epithelial cells then elongate, and

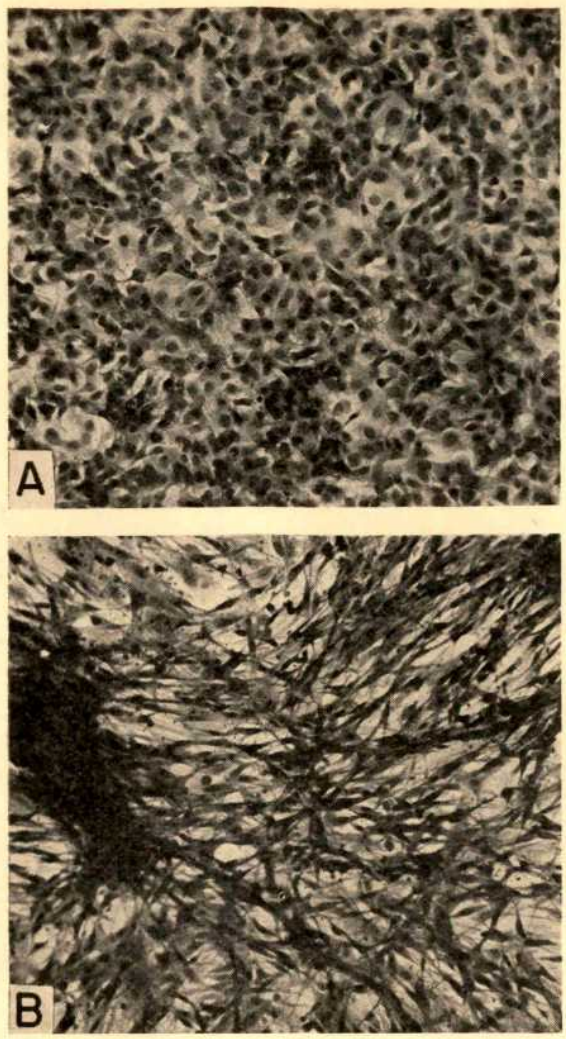


Fig. 1. Comparison of normal diploid bovine conjunctiva cells with transformed cells at third passage-level (hæmatoxylin and eosin, × *c.* 128). *A*, Control cells, primarily epithelial in morphology; *B*, transformed culture, consisting of spindle-shaped cells growing in disorganized pattern

within 10–14 days become long, thin, spindle cells, which grow in disorganized patterns, grow over the top of one another, and form a grossly thickened cell mass. In tube cultures, localized areas of more intensive proliferation may occur, which appear macroscopically as thick, whitish plaques. In cultures infected with a 10^{-2} dilution of a low potency extract, the changes were restricted to focal areas. Infected cultures acidify the medium more rapidly than do the controls.

The usefulness of the system was enhanced by the fact that within an experiment cultures inoculated with the same material showed almost identical changes.

When infected and control cultures are serially passaged by trypsinization, the morphological differences are maintained (Fig. 1), and the altered cells show a more rapid growth rate. One line of transformed cells is at present in the twenty-third serial passage.

The morphological transformation occurs most rapidly when freshly trypsinized *DBC* cells in suspension are exposed to virus. Cultures 6–11 days old are rinsed with Eagle's basal medium, and the cells dispersed with 0·25 per cent trypsin solution; an equal volume of growth medium is added, and the cells sedimented by centrifugation at 900 r.p.m. for 5 min. The cells are suspended in growth medium to give a concentration of 10^6 cells/ml. One volume of virus material is mixed with 10 volumes of cell suspension; after 1 h at room temperature, the mixture is diluted 10-fold, and 3·5 ml. added to Petri dishes (60 mm. diam. Falcon Plastics, Los Angeles). The cultures infected with undiluted extracts are clearly different from control cultures by the time the latter have formed confluent monolayers, at 2 or 3 days. Cultures infected with a 10^{-3} dilution of the most potent wart extract grew out as normal appearing epithelial cells, but by the fifth or sixth day underwent the characteristic sequence of change to abnormally growing spindle cells. In a comparative experiment, this wart extract, used undiluted, produced recognizable transformation within 3 days in cells infected in suspension, but required 10 days to induce definite changes when inoculated on to established cell sheets.

The ability of wart material to produce transformation was not destroyed by passing the extract through a bacteria-tight filter or by exposing the extract to 10 per cent chloroform for 10 min at 0° C. When a portion of this extract was fractionated by ultracentrifugation at 59,000*g* (avg.) for 2½ h, the washed pellet, resuspended to one-fifth the original volume in Eagle's basal medium, produced cellular alteration in 4 days, while the supernatant fluid produced changes only after 10–15 days. In the same test, the uncentrifuged extract produced transformation in 4 days.

Virus neutralization tests using the cellular transformation as the end-point gave clear-cut results. These tests were made by mixing serial dilutions of serum with a 10^{-1} dilution of wart extract; after 1 h at room temperature, the mixtures were added to cell suspensions as already described here. Paired sera from a heifer with naturally occurring papillomata showed a rising antibody titre; a 1 : 10 dilution of serum taken shortly after warts were detected did not affect the rate of transformation, while a 1 : 40 dilution of serum taken a month later completely prevented the changes. Serum (supplied by Dr. Olson) from a cow hyperimmunized with bovine papilloma suspension neutralized at a dilution of 1 : 5,120.

Other bovine tissue cultures have shown similar reactions to inoculation with the undiluted wart extracts. A diploid, epithelial-appearing, cell line of bovine kidney cells was infected in suspension; cellular elongation occurred in 4 days, but the early alterations were less easily discerned than in *DBC* cultures. Secondary cultures of fœtal bovine heart also responded, but with longer and more variable incubation periods. Primary bovine kidney cultures infected in suspension or as established cultures showed no alteration during observation periods of 34–108 days.

Preliminary evidence indicates that cultures of hamster embryo tissues show similar alteration to spindle cell morphology when inoculated with bovine wart extracts. No evidence of cytologic changes, altered growth pattern, or proliferation was observed after infection of primary, trypsin-dispersed, monolayer cultures of human embryonic skin-muscle, or kidneys of human embryos, rabbits and *C3H* mice, during observation periods of 38–53 days.

Attempts to induce transformation of *DBC* cultures with extracts of transformed *DBC* cells and of hamster fibrosarcomata induced by bovine papilloma virus[3] have been negative.

The alterations in cell morphology, growth-rate and pattern, and rate of acid-production suggest that the changes produced by the bovine wart extracts are comparable to the cultural transformation induced by other papovaviruses. Whether the altered bovine cells have malignant potentialities is not known; transformed *DBC* cells of the fifth serial tissue culture passage inoculated subcutaneously and into the conjunctiva of two calves 4 months of age have produced no evidence of tumour formation by the ninth week after injection.

That the agent responsible for the transformation is actually the bovine papilloma virus is indicated by the serological data; the chloroform resistance, filterability, and sedimentability of the agent; and by the occurrence

of positive results with wart extracts from cattle in three geographical areas.

Studies of wart viruses have been hampered by lack of convenient assay systems; none has been propagated in tissue culture, and no hæmagglutination has been detected. The bovine wart virus produces lesions in cows, horses[4], and hamsters[3]; but these systems are either cumbersome or insensitive. Since transformation was induced by virus dilutions as high as 10^{-3}, the highest dilution tested, it is possible that the system described here may provide a relatively sensitive assay for virus. However, its sensitivity relative to inoculation of cows or horses remains to be determined. The *DBC* cells do not appear to provide a method for propagating the virus, and in this respect the cell-virus relationship resembles some of the transformation systems with other papovaviruses[1].

The high percentage of *DBC* cells that are transformed by the bovine wart viruses suggests that this may be a particularly useful model system for studies of cellular transformation by viruses.

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PSYCHOLOGY

Use of 3-Dimensional Shapes for Investigation of Learning Sets in the Rat

In a series of experiments, Harlow[1] and other investigators have studied learning-sets in a number of species. Their evidence demonstrates that the ability to form learning sets is related both to the phylogenetic 'level' of a species and to the individual animal's ontogenetic development. When trying to establish learning sets in such an animal as the rat, the difficulty has been that the learning of individual problems has taken so long that it was not possible to present sufficient problems to examine this phenomenon of learning to learn.

In an effort to achieve faster learning, the following apparatus was constructed using 3-dimensional shapes as discrimination stimuli. In this situation a rat not only sees an object but also touches and moves it. The procedure is sufficiently close to that of Harlow's work with primates to allow a comparison of learning-set formation with other species.

The animal was placed in a wooden box 12 in. × 10 in. At one end were two apertures 6 in. across and 4 in. high. The floor of each aperture was wooden and raised towards the back wall at an angle of 10°. In the centre of the floor of each aperture was a food-well ¼ in. × ½ in. over which a wooden 3-dimensional shape was placed. There were two screens dividing the box from the apertures. A transparent inspection screen allowed the animals to see both shapes but prevented any approach to them until it was raised; a second opaque screen prevented animals from observing the change-over of shapes and from correcting their responses. The inside of the apparatus, with the exception of the white wooden shapes, was painted black.

Twelve male black hooded Norway rats were used; seven were 4 months and five were 11 months old at the beginning of the experiment. An animal was first trained to eat pieces of spaghetti from the food-wells and then learned to 'nose' off the shapes to find food beneath. In the experiment itself a rat, after being deprived of food for 20 h, was placed in the box with both screens 'down'. The stimulus shapes were placed in position, the 'correct' one was baited and the opaque screen was then raised for an inspection period of 5 sec, after which the inspection screen was raised so that the animal could respond. If the animal made a correct response, time was allowed for it to take the food and eat; if incorrect, then the screens were immediately lowered. An error was recorded when the animal entered the wrong aperture, and a correct response when the shape over the baited well was pushed away. The inter-trial interval was approximately 10 sec.

Each animal was given 210 trials on problem 1 (Fig. 1), and each achieved more than 75 per cent correct responses on the trials for the last day. Problems 2–5 were then presented for 100 trials at 25 a day. Once again all animals reached the criterion of 75 per cent correct responses. At this point it seemed that animals might reach this criterion in fewer than 100 trials, so problems 6–18 were run at 25 trials a day and changed immediately after passing the criterion. Most animals did in fact reach criterion on all problems in 50 trials. In order to present more problems to each animal, problems 19–58 were given at 2 a day and 10 trials per problem.

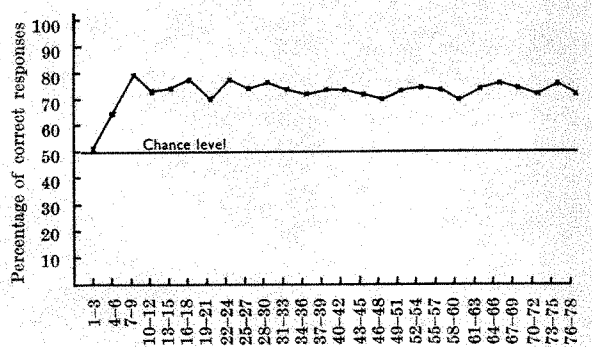


Fig. 1. No. of correct responses for first ten trials on problems 1–78

The animals again scored above chance; but towards the end of the problems scores were fluctuating and the latency of responses was increasing. Since further improvements with this rapid changing of problems seemed unlikely, the final problems, 59–78, were presented for 25 trials each at 1 problem a day. There was no marked change in performance for these last 20 problems though the scoring was again well above chance.

The scores for the first 10 trials for all problems are presented in Fig. 1. These animals had clearly learned how to react to this rapid change of pairs of stimuli and achieved scores which indicate some mastery of the task. By using three-dimensional shapes it was possible for these rats to learn 78 discrimination problems in a period of less than 3 months. This was considerably faster than with experiments using two-dimensional shapes, and gives some hopes that with this type of apparatus it should be possible to present a sufficient number of problems to these animals to make phylogenetic comparisons. So far, on the evidence of the present results, the rat learned quicker than with two-dimensional shapes, but, as a species, it was not so rapid as other animals higher in the phylogenetic scale.

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FORTHCOMING EVENTS

Monday, September 9—Friday, September 13

INSTITUTE OF PHYSICS and the PHYSICAL SOCIETY (at Imperial College of Science and Technology, London, S.W.7)—Conference on "The Liquid State".

Tuesday, September 10

SOCIETY FOR ANALYTICAL CHEMISTRY, ATOMIC ABSORPTION SPECTROSCOPY DISCUSSION PANEL of the PHYSICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.40 p.m. —Discussion Meeting initiated by an introductory paper by Dr. J. B. Willis (C.S.I.R.O., Australia).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in mathematics, and an interest in, and knowledge of, statistical methods) AT THE ROAD RESEARCH LABORATORY, D.S.I.R., Harmondsworth, Middx, to undertake research in the field of road transport in tropical and sub-tropical countries—(the work involves regular visits overseas)—The Director, Road Research Laboratory, D.S.I.R., Harmondsworth, Middx (September 12).

RESEARCH ASSISTANTS (at least two, with a first degree in physiology, biochemistry, chemistry, or zoology) IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY, to join research groups studying the endocrinology of hypothermia, biochemical aspects of food processing, or invertebrate neurophysiology—The Secretary and Registrar, The University, Southampton (September 13).

CHIEF TECHNICIAN (with qualifications in histology)—Head, Department of Physiology and Pharmacology, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (September 14).

D.S.I.R. RESEARCH ASSISTANT (with training in zoology or physiology or psychology) IN THE DEPARTMENT OF ZOOLOGY, to assist in research in the neurology and behaviour of birds—The Registrar, The University, Liverpool, quoting Ref. CV/399/N (September 14).

LECTURER (preferably with an interest in control systems, data processing or communication theory and the application of electronic circuits to these or related fields) IN ELECTRICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (September 14).

SENIOR BIOCHEMIST (with at least five years previous experience of hospital work) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY in the Group Laboratory, for duties principally in laboratory supervision—The Group Secretary, Epsom Group Hospital Management Committee, Epsom District Hospital, Epsom, Surrey (September 14).

LECTURER IN BIOCHEMISTRY (Veterinary Biochemistry)—The Secretary of University Court, The University, Glasgow (September 15).

TECHNICIAN or ASSISTANT TECHNICIAN (preferably with experience in a microbiological laboratory) IN THE BOTANY DEPARTMENT, University of Canterbury, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 18).

SENIOR EXPERIMENTAL OFFICER (with a degree or equivalent qualification in physics and engineering, several years experience in a research laboratory, and preferably a thorough practical knowledge of the design and operation of electronic equipment) IN THE DEPARTMENT OF NUCLEAR ENGINEERING, University of Manchester, and based at the University Reactor Centre at Risley, near Warrington (operated jointly by the Universities of Liverpool and Manchester), to be responsible, under the Research Manager, for planning and safety evaluation of work on the reactor, and also for the design and manufacture of equipment used in conjunction with it—The Registrar, The University, Manchester 13 (September 20).

LECTURER IN THE DEPARTMENT OF EDUCATION—The Secretary, The University, Aberdeen (September 21).

RESEARCH ASSISTANT (honours graduate in botany or biochemistry, and interested in working towards a higher degree) IN THE DEPARTMENT OF BOTANY—Prof. Carr, Department of Botany, David Keir Building, The Queen's University of Belfast, Stranmillis Road, Belfast 9, Northern Ireland (September 21).

SENIOR DEMONSTRATOR (honours graduate in botany or biochemistry) IN THE DEPARTMENT OF BOTANY—Prof. Carr, Department of Botany, David Keir Building, The Queen's University of Belfast, Stranmillis Road, Belfast 9, Northern Ireland (September 21).

SENIOR LECTURER IN CHEMICAL PATHOLOGY—The Registrar, The University, Sheffield (September 21).

SENIOR RESEARCH FELLOW (with post-doctoral experience using particle counting techniques) and a RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSICS, to join a group developing and testing satellite equipment for cosmic ray recording—Dr. P. L. Marsden, The University, Leeds (September 23).

SENIOR LECTURER and a LECTURER IN MATHEMATICS at the University College of Townsville, University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, September 27).

RESEARCH OFFICER (Biochemist) (with a Ph.D. degree, or postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability, and preferably training and experience in mammalian biochemical research) IN THE McMASTER ANIMAL HEALTH LABORATORY, Division of Animal Health, Commonwealth Scientific and Industrial Research Organization, Sydney, to undertake research in some aspects of chemical pathology, particularly in relation to the effects of parasitism—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 202/214 (September 28).

ASSISTANT LECTURER (preferably with special training in either mathematics or psycho-pharmacology) IN EXPERIMENTAL PSYCHOLOGY—The Registrar, The University, Manchester 13 (September 30).

ASSISTANT LECTURER (with a good honours degree in metallurgy or chemistry) IN CHEMICAL METALLURGY in the Department of Metallurgy in the Faculty of Science—The Registrar, The University, Manchester 13 (September 30).

ASSISTANT LIBRARIAN (graduate with a professional qualification) at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 30).

CHAIR OF BIOCHEMISTRY at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

D.S.I.R. RESEARCH FELLOW (with a Ph.D. or equivalent postgraduate experience in experimental geophysics, and preferably experience in the maintenance or development of electronic equipment) IN SEISMOLOGY IN THE DEPARTMENT OF ASTRONOMY, to undertake field and laboratory studies of seismic waves and to relate their observations to earth structure and tectonics—Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh (September 30).

LECTURER IN BIOCHEMISTRY IN THE DEPARTMENT OF CHEMISTRY in the Faculty of Science—The Registrar, The University, Manchester 13 (September 30).

LECTURERS (2) IN RADIO-ASTRONOMY IN THE DEPARTMENT OF PHYSICS of the Faculty of Science, to pursue research at the Nuffield Radio-astronomy Laboratories, Jodrell Bank—The Registrar, The University, Manchester 13 (September 30).

READER (with appropriate academic qualifications and teaching experience) IN PHILOSOPHY at the University of Hong Kong—The Secretary, The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, September 30).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in biochemistry or a closely related subject, and preferably experience of the chemistry of carbohydrates or of the use of radioactive carbon) IN THE DEPARTMENT OF BIOCHEMISTRY, for work on the nature and origin of the carbohydrate fraction of soil organic matter—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (September 30).

CHEMIST (Corrosion) (preferably with an honours degree and some research experience) in the Dominion Laboratory, Department of Scientific and Industrial Research, Lower Hutt, New Zealand, to work with a small team undertaking investigational work on corrosion of metals and related problems such as corrosion and diffusion of hydrogen in metals exposed to geothermal steam, sulphide stress cracking, delayed hydrogen fracture, mechanisms of corrosion in water supply systems, corrosion and stress corrosion in aircraft constructional materials—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B.13/18/17/2331 (October 2).

CHAIR OF SURGERY at the University of Hong Kong—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, October 5).

LECTURER/ASSISTANT LECTURER (preferably with special interests in the fields of plant anatomy and morphology, angiosperm taxonomy or phycology) IN BOTANY at the University of Hong Kong—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, October 5).

CHAIR OF BOTANY; and CHAIR OF EDUCATION at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 10).

CHAIR OF PURE MATHEMATICS—The Registrar, The University, Manchester 13 (October 12).

UNIVERSITY LECTURER IN BIOCHEMISTRY, for duties which will include teaching and research—T. D. Kellaway, Secretary of the Appointments Committee, Department of Pathology, The University, Tennis Court Road, Cambridge (October 14).

LECTURER or ASSISTANT LECTURER IN GEOLOGY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 20).

ASSISTANT (with experience in technical information work, and preferably a qualification in science or engineering) TO THE EDITOR OF *Fuel Abstracts and Current Titles*, to be responsible for the subject indexing of this monthly journal—The Secretary, The Institute of Fuel, 18 Devonshire Street, London, W.1.

ASSOCIATE PROFESSOR (preferably working on the integrative aspects of mammalian physiology, especially in the neuroendocrine, gastro-intestinal or cardiovascular fields) IN PHYSIOLOGY—Prof. J. W. Pearce, University of Alberta, Edmonton, Alberta, Canada.

CHEMIST (Veterinary) (under 45 years of age, with an honours degree in chemistry, preferably with some veterinary/agricultural or medical experience, and experience in biochemistry) IN THE DEPARTMENT OF AGRICULTURE, Tanganyika, to supervise the Chemical Section of the Veterinary Research Laboratory, including frequent safaris for experiments in the field—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S.W.1, quoting Ref. RC/Cl. 213/145/05.

FOREST TREE BREEDER (not over 40 years of age, with an honours degree in forestry, or equivalent, postgraduate ezperience in forest tree breeding, and preferably with a Master's degree and experience in silviculture or wood anatomy) IN THE EAST AFRICAN AGRICULTURAL AND FORESTRY RESEARCH ORGANIZATION, to undertake an approved programme of tree breeding and selection, in which the principal species concerned will be pines (particularly *P. radiata* and *P. patula*) and cypruses of the *C. lusitanica* group—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S.W.1, quoting Ref. C1/RC. 264/348/08.

LECTURER (medical or non-medical) IN BACTERIOLOGY—The Secretary, The Welsh National School of Medicine (University of Wales), 34 Newport Road, Cardiff.

LECTURER (with at least a good honours degree in chemistry, some teaching and research or industrial experience, and an active interest in research) IN INORGANIC CHEMISTRY, to teach to Grad. R.I.C., B.Sc. General and H.N.C. standards—The Principal, South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex.

ORGANIC- or BIOCHEMIST (young graduate), to join a team studying the relationship between wheat variety and growth conditions and lipid composition—The Secretary, British Baking Industries Research Association, Chorleywood, Rickmansworth, Herts.

RESEARCH ASSISTANT for wind tunnel research in the field of industrial aerodynamics—Head, Aeronautics Department, Imperial College of Science and Technology, London, S.W.7.

RESEARCH ASSISTANT IN THE DEPARTMENT OF CHEMISTRY, to investigate the surface properties of clean metals and semiconductors and their subsequent reactions with gases—Dr. M. W. Roberts, Department of Chemistry, The Queen's University, Belfast, Northern Ireland.

RESEARCH ASSISTANT (with a knowledge of mathematics up to at least first-year university honours standard, and preferably experience in statistical and bibliographical work) IN THE RESEARCH TECHNIQUES DIVISION, to assist with the preparation of a bibliography of statistical literature—Prof. J. Durbin, The London School of Economics and Political Science, Houghton Street, London, W.C.2.

RESEARCH OFFICER (national of, and permanently resident in, Great Britain or the Republic of Ireland, medical qualifications registrable in the United Kingdom or an honours degree in zoology or allied subject, an aptitude for research, and preferably research experience in protozoology, bacteriology or virology) AT THE LABORATORIES OF THE EAST AFRICAN TRYPANOSOMIASIS RESEARCH ORGANIZATION, to undertake research on human trypanosomiasis and its wild animal reservoirs, mainly from the protozoological and immunological aspects, in association with field studies by other officers on the epidemiology of the disease—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S.W.1, quoting Ref. RC.264/309/02.

SENIOR TECHNICIAN (with experience in a similar post) IN THE DEPARTMENT OF GEOLOGY—The Professor of Geology, The University, Leicester.

SUPERINTENDENT (with a degree in biological science or equivalent qualification, for example, F.A.T.A., and experience in some branch of animal work), to take charge of the Physiology Department's goat and small animal house—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 63/2.

TECHNICAL OFFICER (with a degree in agriculture or agricultural botany, or a National Diploma) IN THE ROOT AND FODDER CROPS SECTION OF THE TRIALS BRANCH—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

TECHNICIAN FOR A.R.C. UNIT OF FLOWER CROP PHYSIOLOGY at Shinfield, to maintain controlled environment equipment and develop research apparatus—The Assistant Bursar, The University, Reading.

REPORTS and other PUBLICATIONS

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Great Britain and Ireland

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 20: Rearing Chickens for Egg Production. Pp. 16. (Belfast: Ministry of Agriculture, 1963). [127

Department of Scientific and Industrial Research. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country Around Maidstone. (Explanation of One-inch Geological Sheet 288, New Series). By B. C. Worssam, with contributions by Dr. J. Ineson, the late F. H. Edmunds, D. A. Gray, J. G. O. Smart, C. P. Chatwin, Dr. R. Casey, Dr. F. W. Anderson, R. W. Elliot and Dr. P. A. Sabine. Pp. viii+152+5 plates. (London: H.M. Stationery Office, 1963.) 25*s*. net. [127

Political and Economic Planning. Annual Report, 1962–63. Pp. 20. *Planning*, Vol. 29, No. 473, (8 July 1963): Transport in the Common Market. By Sylvia Trench. Pp. 223–286. (London: Political and Economic Planning, 1963.) 6*s*. [127

The Royal Institute of Chemistry. Lecture Series, 1963. No. 1: Living Molecules. By Prof. J. N. Davidson. (Twelfth Dalton Lecture). Pp. 17. 4*s*. No. 2: Quantitative Evaluation of Substituent Effects by Electronic Spectroscopy. By Dr. J. N. Murrell. Quantitative Aspects of Aromatic Substitution. By Dr. R. O. C. Norman. (Meldola Medal Lectures). Pp. 32. 5*s*. No. 3: Agriculture and Some of Its Chemical Problems. By Dr. E. G. Cox. (Fifth P. F. Frankland Memorial Lecture). Pp. 12. 3*s*. (London: The Royal Institute of Chemistry, 1963.) [127

The Overseas Development Institute. British Aid-3: Educational Assistance—a Factual Survey of the Government and Private Contribution to Overseas Development through Education and Training. Pp. 125. (London: The Overseas Development Institute, 1963.) 7*s*. 6*d*. [125

Committee on the Problems of Noise. Noise: Final Report. Pp. xii+235. (Cmnd. 2056). (London: H.M. Stationery Office, 1963.) 13*s*. net. [127

Colonial Development Corporation. Annual Report and Statement of Accounts for year ended 31st December 1962. Pp. vi+95. (London: H.M. Stationery Office, 1963.) 6*s*. 6*d*. net. [127

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United Kingdom Atomic Energy Authority. Ninth Annual Report for the period 1st April, 1962–31st March, 1963. Pp. ix+87+7 plates. (London: H. M. Stationery Office, 1963). 6*s*. 6*d*. net. [157

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Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 47: Sheep Husbandry. Pp. 27. (Belfast: Ministry of Agriculture, 1963.) [177

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Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Comptes Rendus de Recherches, No. 30, (Mai 1963): Travaux du Comité pour l'Étude des Maladies et de l'Alimentation du Bétail (C.E.M.A.). Pp. 142. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1963.) 200 francs. [27

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Maps—Preliminary Series. Map 39–1961: Virden Manitoba and Saskatchewan. Paper 62–31: Structure, Isopach, and Facies Maps of Upper Cretaceous Marine Succession, West-Central Alberta and Adjacent British Columbia. By C. F. Burk, Jr. Pp. 1+10. 50 cents. (Ottawa: Queen's Printer, 1961 and 1963). [27

United States Department of the Interior: Office of Saline Water. Saline Water Conversion Report for 1962. Pp. xv+163. (Washington, D.C.: Government Printing Office, 1963.) 1.25 dollars. [57

Unesco South Asia Science Cooperation Office, New Delhi. Indian National Scientific Documentation Centre, New Delhi. Scientific Documentation on South and South East Asia: a Survey of a Regional Seminar held in New Delhi from 7th to 16th March, 1961, under the auspices of Unesco and the Government of India. Pp. iii+47. (New Delhi: Unesco South Asia Science Cooperation Office; and Documentation Centre, 1963.) [57

National Science Foundation, Washington. United States Antarctic Research Program. Pp. 32. (NSF 63–14.) (Washington, D.C.: National Science Foundation, 1963.) [57

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Centre National de la Recherche Scientifique, Paris. Rapport National de Conjoncture, 1962/1963. Pp. 264. (Paris: Centre National de la Recherche Scientifique, 1963.)

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturals. Dundo—Lunda—Angola. Museu do Dundo. Subsidios para a Historia, Arqueologia e Etnografia dos Povos da Lunda. Publicacoes Culturais, No. 62: Prehistoric Cultures of Northeast Angola and their Significance in Tropical Africa (Part 1). By J. Desmond Clark. With an Appendix on the Analysis of Pollen Samples by E. M. van Zinderen Bakker. Pp. 222. (Lisboa: Companhia de Diamantes de Angola, 1963.) [57

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CENTRALIZING BRITAIN'S DEFENCE ORGANIZATION

THE White Paper on *Central Organization for Defence** demands some attention from scientists and perhaps also a close scrutiny from the Parliamentary and Scientific Committee. It details proposals for a reorganization which were foreshadowed in the speech of the Minister of Defence, then Mr. P. Thorneycroft, when moving the approval in the House of Commons of the *Statement on Defence 1963*† on March 4. In that speech, Mr. Thorneycroft expressed doubts as to whether the Ministry of Defence possessed sufficient effective power to make the defence budget a real synthesis of defence problems and whether, when resources were under pressure, the interests of individual Services did not prevent the formulation of a real central policy. More significant still for the scientist and technologist, he questioned whether research and development could be really effectively devised and controlled, and admitted that this was one of the most difficult problems of all.

How important this last matter has become is sufficiently illustrated by the figures given in the *Statement on Defence*. For 1963–64 there is shown in the Defence statistics a separate item of £5·4 million for the cost of defence research undertaken and financed by the Atomic Energy Authority. This item is included in the total net estimate of £769·8 million for research and development in a net budget of £1,837·7 million, although research and development are not separately distinguished. For the Admiralty the net figure is £235·1 million; for the War Office, £93·4 million; for the Air Ministry, £244 million; and for the Ministry of Aviation, £530·95 million gross or £191·87 million net.

The memoranda accompanying the separate estimates of the Navy, Army and Air Force, which are included with the *Statement*, all emphasize the importance of continuing research and development. The memorandum accompanying the Navy Estimates, for example, besides referring to the value of the International Indian Ocean Expedition and of the National Institute of Oceanography —which are financed partly from the Admiralty—stresses the importance of the shore-based prototype nuclear submarine installation at Dounreay. This is regarded as essential for future development of submarine reactors and training personnel; note is made of the considerable effort being devoted to ship-borne automatic data-processing and display systems for the overall defence of the fleet. The memorandum on the Army Estimates refers to the new system of radio communications known as the Clobart plan now under design study. This aims at rationalizing and integrating the new generation of radio equipment, taking account of improved engineering techniques, and using computer techniques which would enable subscribers in the field to achieve rapid, automatic inter-connexion wherever they are. This is, of course, apart from the effort which has gone into the development of new anti-tank weapons, the General Purpose Machine Gun and a new battle tank. Long-term projects include research into more advanced armoured fighting vehicles, the development of high strength and temperature-resistant metals, and work on improved ammunition fuses, engines and bridging.

Although the memorandum on the Air Estimates makes no specific reference to research and development, the vital importance of such work for the development of the new aircraft required is just as apparent as it is for the Navy and the Army. Nevertheless, for all Mr. Thorneycroft's observations in the House of Commons, the *Statement on Defence 1963* is no more than a compilation of the Estimates from the three Service Ministries and the accompanying separate memoranda. There is no covering statement from the Ministry of Defence and the shortcomings of the existing organization, and the virtual bankruptcy of the arrangements made in 1958 as regards co-ordinated policy on research and development or on any other aspect of defence could scarcely have been made plainer.

Mr. Thorneycroft, in announcing on March 4 that the Government had agreed that there should be one unified Ministry of Defence on a Service basis, with the one Department of Defence responsible for advising the Government on defence policy, and housed in one building, rejected the idea of an amalgamation of the Services. However, neither then nor in the White Paper now issued can reference be found to the undoubted advantages which some measure of joint training for junior officers would offer, particularly in its ultimate effect on integration and co-operation. He pointed out, however, that most of the research and development work of the Ministry of Aviation was done for the Ministry of Defence and the Service Ministries on an agency basis, and he thought that it was desirable to keep military and civil aviation research and development together. No final decision had then been taken as to whether the Ministry of Defence should take over all such research and development and carry out the rather smaller civil part on an agency basis for another Ministry.

The fact that research and development could not well be separated from production was against such a step. However, the question was not examined further either by Mr. Thorneycroft or in the subsequent debate, and this solution has now been rejected. Nor, apart from Lord Robertson of Oakridge's reference, was the question of research organization raised in the House of Lords debate on March 14. According to the White Paper, the Ministry of Aviation will retain its wide responsibilities for civil as well as for military research and development as an independent department responsible for executing programmes of defence research, development and production formulated and approved by the Secretary of State for Defence. The Minister of Aviation will not be a member of the new Defence Council which replaces the Defence Board, but will attend the Defence Council whenever meetings affecting his Department are under consideration. The Ministry of Aviation will be fully associated with the new arrangements for formulating operational requirements, for controlling defence research and development, and for evaluating weapons systems. The Ministry of Defence will in future be represented on the main departmental committees of the Ministry of

* *Central Organization for Defence*. Pp. iii+14. (Cmnd. 2097.) (London: H.M.S.O., 1963.) 1*s*. 6*d*. net.

† *Statement on Defence 1963*, including Memoranda to Accompany the Navy, Army and Air Estimates, 1963–64. Pp. 87+24 plates. (Cmnd. 1936.) (London: H.M.S.O., 1963.) 8*s*. 6*d*. net.

Aviation responsible for managing defence research, development and production programmes.

The omission of the Ministry of Aviation from the formal structure of the new organization is to be further balanced by ensuring the freest possible communication at all levels between the Ministry of Defence and the headquarters and establishments of the Ministry of Aviation. Scientists in the latter Ministry will be freely available to serve on panels in the Ministry of Defence and free interchange is also proposed for senior staff. This will be particularly so for defence scientists, of the administrative and other Civil Service classes in the two Ministries. To assist close consultation, the Minister of Aviation and those senior officials who are mainly or exclusively concerned with defence projects will be accommodated in the same building as the staffs of the Ministry of Defence who are responsible for policy. Arrangements are to be made for the regular and easy interchange of staff between the two Ministries, particularly in the scientific field.

These measures, together with new and improved procedures for controlling the research and development programme, are expected to result in closer monitoring of the progress of individual major projects, so that technical and cost factors which seem likely to cause unforeseen difficulties will be identified with greater speed and certainty, enabling decisions to overcome such difficulties to be taken promptly. In this, however, the decisive factor is likely to be the Defence Scientific Staff, now to be established. Before discussing this, however, the broad structure of the new arrangements should be described.

They comprise absorption in the new Ministry of Defence of the present Ministry, the Admiralty, War Office, and Air Ministry—authority and responsibility being vested in a single Secretary of State for Defence with complete control both of defence policy and the administrative organization. However, the Services will preserve their separate identities and the Chief of the Defence Staff and the three Chiefs of Staff will retain their right of access to the Prime Minister. The new Defence Council will comprise, besides the Secretary of State for Defence, the three Ministers of State for the Navy, the Army and the Air Force, the Chief of the Defence Staff, the three Chiefs of Staff, the Chief Scientific Adviser to the Secretary of State for Defence and the Permanent Under-Secretary of State for Defence, and will deal mainly with major defence policy.

The Chief Scientific Adviser's work covers all aspects of defence which are affected by scientific advance, and, to enable him to play his full part in the formulation and control of the defence research and development programme, several changes are being made. First, the establishment of the Defence Operational Requirements Staff, under an assistant chief of the Defence Staff to review long-term requirements, who will sponsor long-term studies on future concepts of war, and examine and harmonize all major new weapon requirements. This should improve the arrangements for formulating military requirements, and it is proposed that whenever the problems are of a Defence and not a single Service character, the scientific staffs of the present Ministry of Defence and the present Service Departments will work together under the Chief Scientific Adviser as the Defence Scientific Staff. Next, the present Defence Research Policy Committee will be replaced by two new Committees, of both of which the Chief Scientific Adviser will be chairman. These Committees will work closely with the Defence Operational Requirements Staff and the Defence Secretariat, but no reference is made to relations with the Advisory Council for Scientific Policy, and it would seem improbable that the chairman of that Council can, in future, also be chairman either of the new Defence Research Committee or of the Weapons Development Committee.

The Defence Research Committee will oversee all military research which does not directly support approved weapons and equipment projects. It will advise the Secretary of State and the Chiefs of Staff on all scientific and technical matters which may affect defence research policy, and will keep the defence research programme under review so as to ensure that it is appropriate to defence needs and is matched by the resources available. In particular, it will be responsible for bringing to notice, in good time, scientific developments which may be of military interest. Its membership will be mainly scientific and will include independent scientists.

Closely linked to this Committee by a substantial degree of common membership will be a new Weapons Development Committee. This Committee will draw to a larger extent on military and administrative staffs and will advise the Secretary of State and Chiefs of Staff on what major developments should be included in the development programme. It will keep that programme under review so as to ensure that it is properly matched to available resources, current defence policy and operational requirements agreed in the light of what is technically feasible, and it will be responsible for the prompt detection of any difficulties which may rise in the execution of the programme. The Ministry of Aviation will be represented on both these Committees, and a third committee, consisting of the directors of operational research of the three Services, with a member of the Chief Scientific Adviser's staff as chairman, will co-ordinate operational research activities.

The staff under the Chief Scientific Adviser will be increased primarily to provide adequate service for these Committees, to undertake and develop techniques for the study and evaluation of weapons systems, undertake cost-effectiveness studies where appropriate and to investigate longer-term problems of a scientific nature which have a bearing on defence policy. The increase is also needed in order to develop close association at all levels between the Defence Scientific Staff and the Defence Staff and Defence Secretariat. The normal method of working contemplated under the direction of the Chief Scientific Adviser is to form *ad hoc* panels, or working groups, to deal with specific problems, drawing on appropriate specialists from the military, scientific and civil administrative staffs of the Ministry, the Ministry of Aviation and the Services, and on outside advisers.

Co-ordination is the responsibility of the Permanent Under-Secretary of State, who will be assisted by four Second Permanent Under-Secretaries of State, one of whom will be specifically responsible for identifying fields in which administration might with advantage be placed on a Defence rather than a single Service basis and for recommending any appropriate changes in Department organization for this purpose. It is fully recognized, however, that any long-term financial planning and control of the defence programme and allocation of resources, which will be a primary responsibility of the Permanent Under-Secretary himself, must take into account political, military, scientific, technical, financial and economic

factors. Constant collaboration between military, scientific and civil administrative staffs will be required, besides a sound appreciation of each other's problems and a common effort to improve planning and control techniques. The co-ordination of the views of the military, scientific and civil administrative staffs is a further responsibility of the Permanent Under-Secretary. There is also a reference to the importance of effective management so as to safeguard the career interests and welfare of all staff, so far as possible, on a defence basis. It is recognized that it is important that civil administrative staff in the Defence Secretariat, who are specially concerned with a particular Service, together with the scientific staffs of the Services, should continue to make a major contribution to management.

It could be urged that the reorganization proposed, which leaves the three Services intact, does not go far enough, and admittedly the proposals fall far short of what has already been put into practice in the United States and advocated in Britain by Lord Montgomery. Nevertheless, it leaves the way open for further changes and it seems improbable that inter-service training of officers can long be delayed. The system may well work, though that will obviously depend, as in fact the White Paper admits specifically in respect of defence research, on the men it can attract and the judgment they can bring to bear on problems which are seldom amenable to straightforward analysis and accounting and which involve, in varying degrees, military, scientific, technical, financial and economic content.

On the scientific side the changes are indeed considerable. Bearing in mind the criticism of the Select Committee on Estimates, it may reasonably be doubted whether with the establishment of a scientific staff in the Defence Ministry, the retention of independent scientific research staffs in the separate Service Departments is justified. However, the spirit of the White Paper is encouraging and goes some way to meet criticisms which have been advanced and hesitations on the part of scientists to commit themselves to research on defence problems. The decision regarding the Ministry of Aviation is a matter of opinion, but the proposals have been at pains to meet possible objections and it would seem that they would also provide for any appropriate co-operation with such bodies as the Institute of Strategic Studies.

Where they seem most open to criticism on the scientific side is in relation to scientific and technological research generally. The absence of any link with the Advisory Council for Scientific Policy has already been noted, and similarly, when the relation of the new Ministry to the Cabinet is considered, there is no reference to the Minister for Science, in spite, too, of his formal responsibility for the Atomic Energy Authority. Some formal link with the civil research organization seems desirable, if not essential, if the Defence Research Committee is to discharge one of its specific responsibilities efficiently, and the omission is the more pointed in view of the elaborate arrangements regarding the Ministry of Aviation. Nor is the contact of importance in one direction only. Any considerable disarmament effort could make such a link imperative if the full possibilities in the civil field of any advances in defence research are to be realized. This may become the more important as the Ministry of Defence acquires effective control over the Services.

It is in such respects as these that the White Paper does not seem to look sufficiently to the future. It is at pains to remove some of the obstacles to co-operation which have existed in the past and some of the factors which have discouraged able minds from participating in defence research and development. Even as yet, however, the magnitude of the problem of communication has not been fully grasped. There is, none the less, a framework which should be welcomed and which, if wisely and imaginatively used, could eliminate mistakes of the magnitude which have marred Britain's defence effort in the past. Moreover, it could also contribute to the more effective use of Britain's resources, whether of manpower or materials, alike in defence itself or in the run-down with all the economic and technical problems which will be posed if disarmament on any real scale once becomes feasible. Last, but not least, what is here set forth offers ample scope for taking defence policy and expenditure out of the arena of party politics—an essential condition alike for achieving a really effective defence policy and securing the whole-hearted participation of the scientist.

BACCHANALIAN ADDICTION

Alcoholic Addiction

A Psycho-social Approach to Abnormal Drinking. By Howard Jones. Pp. x+209. (London: Tavistock Publications, 1963.) 30*s*.

THIS book, giving in the main a very stimulating account of research carried out in Canada in a residential treatment unit and in a prison, is concerned with "Anglo-Saxon alcoholism". It claims that the "size of [the] alcohol problem and extent of its consequences for society and individual more than justify research into its causes and treatment". How far is this statement true for England? On p. 7 the book shows a table compiled by a Canadian authority which, like a similar table published previously by the World Health Organization, ranks England and Wales last but one among twelve listed countries with regard to the estimated prevalence of alcoholism. But, even so, the author quotes a Ministry of Health source that this estimate for England and Wales represents a proportion more than forty times as great as the proportion of ascertained mental defectives in the whole population of the country in the same year; and in England and Wales in the decade 1951/1961 nearly one in 10 prison receptions consisted of men or women sentenced for inebriate offences (excluding offences such as assault and vagrancy, which may often result from drinking). There has been in Britain recently a spectacular rise in convictions for drunkenness—the author quotes a figure of a 250 per cent increase between 1946 and 1960, and the rise has been continuing since. There are other indirect signs that alcoholism in Britain may well be on the increase. It is clear that research into alcohol problems—such as described by Dr. Jones—is a vital necessity also with regard to conditions in Britain, and his results and conclusions deserve the greatest interest.

The author, a sociologist, uses a social criterion to define alcoholism: ". . . a chronic behavioural disorder manifested by repeated drinking of alcoholic beverages . . . to an extent that interferes with the drinker's health or his social or economic functioning". He has adapted this definition from one proposed by Keller in 1958, but it also resembles that given by the World Health Organization in 1952. But unlike the World Health Organization (and unlike Jellinek, who is frequently referred to in this volume) he does not use medical criteria ('loss of control' and 'inability to abstain') to define 'alcohol addiction', but the social criterion—the 'addict' continues to drink excessively even though, as a result, his social life and his happiness are being gradually

destroyed. Dr. Jones disagrees with Jellinek's criticism of an entirely psycho-social explanation of the nature of alcohol 'addiction'. According to Jellinek, physical withdrawal symptoms are the basis of alcohol 'addiction'; against that, Dr. Jones argues that the addict will resume drinking again even after being 'dried out' thoroughly: "this must be one manifestation of his 'addiction', in any normal usage of this word"; and this phenomenon is "hardly consistent" with a physiological explanation, but "readily explainable in psycho-social terms". Dr. Jones also states that there is, so far, no evidence to support Jellinek's hypothesis of a virtually irreversible metabolic abnormality to explain why the alcoholic sets off on a new bout once he resumes drinking. He rather favours psychosomatic explanations: physical withdrawal symptoms may be psychological in origin. "The persistence of unsatisfied psychological needs in the alcoholic may also have its counterpart in the physical experience of the withdrawal phase, both being directed towards the same aim of continued drinking. The long-lived physical dependence . . . may then be seen as a translation into bodily terms of such a persisting psychological dependence".

Discussing the ætiology of alcoholism, the author remarks that physical explanations of disease are usually preferred to psychological ones, and he quotes in this connexion the concept of 'physical allergy' so popular with 'Alcoholics Anonymous' (although in fairness one has to add that the latter couple it with the additional one of a 'mental obsession'). Striking cultural variations in the prevalence of alcoholism among different communities are "perhaps the most telling argument against exclusively physiological causation". In this part of his discussion, the author largely follows Jellinek's hypotheses. Excessive drinking may stem from the life of the addict's original and present community. Thus where heavy drinking is part of the 'way of life', as in France, the heavy drinker is likely to be the conformist, and "many French alcoholics are . . . initially . . . unlikely to be strikingly abnormal". On the other hand, in countries or in groups where heavy drinking and drunkenness are less widely accepted "it is, perhaps, only the deviant, the disturbed or abnormal personality, whose needs are strong enough to drive him on nevertheless". In spite of similar factors in France and Italy—which, like France, is a strong wine-drinking country—Italy has a low alcoholism rate, probably because of different popular sentiment and different dietary habits. In general, this exposition rings true, but it is only fair to add here the very insistent attempts made nowadays in France in spite of all the difficulties to fight alcoholism; to mention that according to several Italian psychiatrists alcoholism, at least in northern Italy, is by no means as uncommon as generally accepted and, finally, that in Britain a considerable proportion of alcoholics seen in out-patient clinics and hospitals seem to be basically relatively 'normal' personalities and not 'deviants', although drunkenness and heavy drinking are not generally accepted.

While an "ambivalent attitude (of society) towards alcohol is . . . relevant to the problem of Anglo-Saxon alcoholism", one has to look at personal problems to understand how particular individuals within this culture become alcoholics. Is there a specific 'alcoholic personality'? The author found even in his small sample of 72 alcoholics quite a number of different personality types, and, as he himself rightly stresses, his list is by no means exhaustive. Most common among his cases was the dependent type of alcoholic, the person who in his childhood was dependent on his mother, later on often married a woman older than himself (mother substitute) and then became dependent on her. Frequently in such cases acceptance by the alcoholic's wife of his dependence on her helped to maintain the marriage, but also his drinking "at a level consistent with the maintenance of the marriage itself". Slightly less common than 'maternally dependent' were 'socially insecure' alcoholics who, lacking the close relationship with their mother or mother-substitute, were concerned with their wider social relationships. Dr. Jones divides this group into those suffering from a general feeling of personal inferiority or inadequacy ('ego-need'), those feeling badly about themselves when participating in social situations ('socially inadequate'), and finally those who, driven by a strong need for acceptance, become extreme conformists ('socially dependent'). Common to the whole 'social insecurity' group of alcoholics is their own feeling of emotional deprivation in childhood.

The 'escapists' among the author's alcoholics—the 'narcosis' drinkers—came from varied backgrounds, but their drinking arose from an inability to tolerate difficulties, from an "unbearable stress caused to certain personality types by certain kinds of environmental experience". In a different environment such people might not have become alcoholics at all or different types of alcoholics. Finally, Dr. Jones found some alcoholics in whom sexual factors seemed to have been responsible for causing alcoholism, such as unresolved Oedipal situations or unconscious homosexuality.

Thus many different kinds of personality can become alcoholics, and, moreover, there is some overlap in background, etc., between them. Not only personality factors but also environmental conditions must be considered and studied in a 'socio-personal' approach. Alcohol, for the alcoholic, is a means of adjustment. "Alcoholism is not the invariable result of particular kinds of personality constellation, but the solution found when problems of adjustment arise because certain types of personality are confronted by certain types of environmental stress".

Early environmental factors were also found to be of great importance in the differences found between clinic alcoholics and prison alcoholics. In general, the prisoners lacked even the limited success enjoyed by the clinic patients, which the author ascribes mainly to the prisoners' lack of social assets such as education, vocational training and marriage.

The author draws certain conclusions from his investigation of prevention and treatment. Prophylaxis requires better education in the handling of family relationships. in upbringing of children, in changing of parents' emotional attitudes, as excessive drinking arises on the basis of problems dating from experience in early life. In regard to therapy, Dr. Jones favours an integration of social and personal therapy in a background of a 'therapeutic community', with group therapy, and social workers helping the alcoholic in relation to his outside environment.

The author himself stresses the limitations set by the relatively small size of his sample and its lack of being 'representative'; however, the great majority of alcoholics also in Britain do not enter prison and do not come for treatment until they are in fairly advanced stage. Many data given by the author closely resemble those found from investigations in Britain. Thus the average age of alcoholics (44), the ages at which they reached the prodromal stage (30), the 'incubation period' between first drink and start of the prodromal phase (15 years), all closely resemble the average ages found in English alcoholics. Many of the differences between clinic and prison samples described by Dr. Jones correspond to those found in Britain between alcoholics admitted to an alcoholic hospital unit on one hand, and excessive drinkers and alcoholics found in a reception centre for the homeless on the other (Glatt and Whiteley, 1958). Personality types, as described by Dr. Jones, commonly occurred among much greater numbers of alcoholics closely observed during the past ten years in Britain. The type of treatment he suggests—such as the 'the-

peutic community' approach, group therapy, the use of social workers as group therapists, etc.—has been practised in alcoholic units in Britain with success for the same period, and is the basis of the management of regional alcoholic units now recommended by the Ministry of Health. It is therefore clear that the author's findings, discussions and conclusions also are of great relevance and interest to Britain. He has certainly succeeded in his aim of studying closely the part which addiction plays in the lives of a limited number of alcoholics; and, by the presentation of data in what he calls a qualitative rather than a quantitative form, and the adoption and description of the life-history approach, he gives the reader many fascinating glimpses into the lives, individual problems and worries of alcoholics. At the same time, he is careful not to draw too far-reaching conclusions and to point out the limitations which are inevitable when studying samples of such a diversified population as formed by alcoholics. Alcoholism is a very complex subject requiring a multi-dimensional interdisciplinary approach. Anyone whose work brings him in contact with alcoholics or who is concerned with alcohol problems in general will find close study of Dr. Jones's *Alcoholic Addiction: A Psycho-social Approach to Abnormal Drinking* extremely interesting and highly rewarding.

M. M. GLATT

TWENTY YEARS OF VITAMINS AND HORMONES

Vitamins and Hormones

Advances in Research and Application. Vol. 20. Edited by Robert S. Harris and Ira G. Wool: Consulting Editors: G. F. Marrian and Kenneth V. Thimann. Pp. xvii + 707. (New York and London: Academic Press Inc., 1962.) 114*s*. 6*d*.

WITH this, the twentieth volume, *Vitamins and Hormones* celebrates twenty years of continuous publication, and the editors have appropriately invited P. Karrer and G. Pincus to mark the occasion by writing guest prefaces in which they express their appreciation of the service this publication has given to all of us working in the field. The past twenty years cover a period of extraordinary growth and development both in vitamin research and in endocrinology, and there can be little doubt that the many authoritative reviews published in *Vitamins and Hormones* have made a valuable contribution to progress, both through their critical assessment and clarification of the available information on a topic, and in providing the stimulus for further investigation.

The present volume fully maintains the standards of its predecessors, and it follows the example set two years ago and is enlarged to contain the papers contributed to the symposium on "Vitamin E and Metabolism" held in honour of H. M. Evans at Zurich in June 1962. This collection of some twenty papers and discussion by many of the leading workers on vitamin E gives a comprehensive picture of present-day knowledge of this vitamin. A felicitous account of its early history by H. M. Evans is followed by descriptions by members of the research staff of Hoffmann-La Roche of the chemistry, physico-chemical properties and assay of the tocopherols, and of the absorption and distribution in tissues of the vitamin. Probable steps in the metabolic degradation of α-tocopherol are described by C. Martins. R. S. Harris has prepared a valuable summary of the influences of storage and processing on the content of vitamin E in foods. He emphasizes that much of the published data must be considered to be qualitative, or at best semi-quantitative, for it is only recently that precise methods of analysis have become available.

The metabolic function of vitamin E is considered in detail. K. Schwarz discusses the interrelationships between vitamin E, trace elements and sulphydryl groups in the respiratory decline characteristic of liver tissue from vitamin E-deficient animals during the early stages of diet-induced liver necrosis; J. Green considers the mutual interactions of vitamin E and other vitamins and the ubiquinones; A. L. Tappel reviews some of the more recent work on the biological function of vitamin E and of selenium compounds as lipid antioxidants; J. S. Dinning summarizes the findings from his own laboratory on the influence of vitamin E deficiency on nucleic acid metabolism; H. Dam considers the relationships between vitamin E and poly-unsaturated fatty acids in experimental animals; M. K. Horwitt describes the long-term experiments he is carrying out with his colleagues on the tocopherol requirements of men and animals; H. M. Nitowsky, K. S. Hsu and H. H. Gordon discuss the vitamin E requirements of human infants; and J. Marks presents a critical evaluation of the therapeutic value of α-tocopherol in various disorders. The three remaining articles deal authoritatively with the probable metabolic functions of vitamin E in farm animals: poultry by M. L. Scott, cattle and sheep by K. L. Blaxter, and pigs by O. Swahn and B. Thafvelin. Nevertheless, neither E. C. Slater and P. György, who contribute their comments on the other papers, nor any of the authors of these papers, claim yet to see the solution of the problem of the mechanism of action of vitamin E, and the complexities of its interrelationships with selenium and unsaturated fats.

Six chapters are devoted to hormonal problems: A. I. Winegrad considers the effects of hormones on the metabolism of adipose tissue, with particular reference to the use of *in vitro* techniques for the investigation of the effects of hormones on glucose utilization, on the synthesis of fatty acids and triglycerides and on the release of free fatty acids by adipose tissue; P. J. Randle and H. E. Morgan contribute a comprehensive review of studies of hormonal and other factors which regulate the uptake of glucose by muscle; D. E. Tapley and W. B. Hatfield discuss present views of the mode of action of thyroxine; H. Breuer considers the biosynthesis, intermediary metabolism, conjugation and degradation of natural œstrogens of animal origin; A. D. Lerner and T. H. Lee contribute a short account of the studies leading to the isolation and determination of the structure of the peptides that function as melanocyte-stimulating hormones, and J. P. Mascaren and L. Machlis discuss the probability that hormonal control contributes to the directional growth of pollen tubes.

Two chapters deal with vitamins other than tocopherol: L. J. Reed gives a comprehensive account of lipoic acid and of its biological function as a prosthetic group in α-keto-acid dehydrogenase systems in animal and bacterial cells, and discusses possible other roles for this growth factor; T. B. Morgan and J. Yudkin contribute an excellent discussion of the intriguing phenomenon of the vitamin-sparing action of dietary carbohydrates. Their own studies with sorbitol have done much to clarify the situation, and it is now evident that coprophagy supplies the vitamins spared not only with sorbitol and ascorbic acid-containing diets, but also with starch-containing diets. Thus coprophagy provides the explanation of the condition of refection in rats fed on diets containing high levels of potato or rice starch.

In the remaining chapter, J. E. Kirk spans the fields of both vitamins and hormones in making a useful compilation of the available information on the content of these substances in the tissues of man and animals of various ages.

It is thus abundantly clear that this volume of *Vitamins and Hormones* worthily upholds its own high standards. May succeeding volumes continue to do so.

J. W. G. PORTER

INDUSTRIAL CANCER OF THE BLADDER

Carcinogenic and Chronic Toxic Hazards of Aromatic Amines

By T. S. Scott. (Elsevier Monographs on Toxic Agents.) Pp. xi+208. (Amsterdam and New York: Elsevier Publishing Company, Inc., 1962.) 30*s*.

THE development of the synthetic dye industry is a fascinating story full of romantic and also tragic events. As in many other passages of history, the full implications are not yet clear. As all the dyes are aromatic compounds, and so benzene derivatives, the story really began with the discovery of benzene by Faraday in 1825. Twenty years later, Hofmann isolated benzene from coal-tar. The young W. H. Perkin, working in Hofmann's laboratory in London, obtained a mauve dye by oxidizing aniline in an attempt to synthesize quinine. Perkin sent a sample of this dye, which he called mauveine, to the dyer Sir Robert Pullar of Perth. As a result of this the manufacture of the dye on a commercial scale started, and Perkin took out a patent in 1856. The red dye, magenta or fuchsin, was discovered in France in 1859. During this period Griess developed the diazo reaction while working in a Burton brewery, and the Russian chemist Zinin discovered benzidine and 1-naphthylamine. Lieberman and Schneiding prepared 2-naphthylamine in 1879.

During the past hundred years the synthetic dyestuffs industry, which is largely dependent on aromatic amines, has expanded enormously. At first the expansion was mainly in Germany and Switzerland, in spite of the fact that the first synthetic dye was discovered, manufactured and used in Britain. During the First World War, however, Britain, the United States and other countries started their own dyestuffs industry because the German products were not available. The manufacture of synthetic dyestuffs is now carried on in many countries throughout the world. Many of the intermediates are also used in the manufacture of rubber products, paper, textiles, cables, pigments and plastics.

The first fifty years of the development of this side of the organic chemical industry were full of hope and success. Occasional toxic effects in workmen were seen, due to aniline and other amines causing methæmoglobinæmia and cyanosis. Sometimes the compounds caused irritation of the bladder, but provided that the working conditions were not exceptionally bad the effects were not considered serious.

In 1895, however, Rehn, a surgeon from Frankfurt-am-Main, which was a centre of the dyestuffs industry, pointed out the incidence of bladder cancer in men employed in the industry. Rehn discussed the frequency of hæmaturia and cyanosis among the workers and considered that the bladder cancers seen in three men who had worked on the manufacture of magenta for periods varying from 15 to 29 years were due to exposure to aniline. He called the disease aniline cancer. It is now considered that aniline itself does not cause cancer, but the aniline used in the past century contained many other amines as impurities or contaminants. Magenta could not have been formed if the aniline used had been pure. As other countries took up the manufacture of synthetic dyes the industrial bladder cancer appeared some decades after the commencement of manufacture.

As the result of much long and careful investigation it was established that although aniline did not cause cancer, exposure to any of three aniline derivatives—1-naphthylamine, 2-naphthylamine and benzidine—was associated with cancer of the bladder. Later it was shown that men exposed to xenylamine (4-aminobiphenyl) or the dyestuff 'Auramine' also developed the disease. The risk from 2-naphthylamine appeared to be greatest. The manufacture of 2-naphthylamine has ceased in most countries; this is due in part to the fact that many dyes can be obtained without using the base itself. The other amines are now manufactured under improved conditions where the exposure to workers is minimal. There was, however, a delay of about half a century between the first suggestion that aromatic amines could cause cancer in man and action to prevent the hazard.

The delay was in part due to the difficulty in inducing cancer of the bladder in animals. The first species in which the carcinogenic aromatic amines induced bladder cancer was the dog. Hueper, working in the United States, produced bladder cancer by dosing dogs with 2-naphthylamine for two years. The evidence for the induction of experimental bladder cancer with benzidine is less certain; but all the amines known to cause bladder cancer in man induce cancer at other sites in rodents.

As a result of the knowledge of the hazard from these compounds the exposure has been reduced. The Association of British Chemical Manufacturers invited a committee to draw up a "code of industrial practice to cover the manufacture and use of known or suspected bladder carcinogens". The code which was drawn up, mainly by Dr. T. S. Scott and the late Dr. Michael Williams, has been approved and widely adopted. Dr. Scott, who has himself played a major part in the development of the control of these dangerous compounds, has produced an interesting and authoritative account of the chemical, technical and medical aspects of the subject.

E. Boyland

NUCLEAR ENERGY ON THE DESK

Newnes Concise Encyclopaedia of Nuclear Energy

Advisory Editors: D. E. Barnes, R. Batchelor, Dr. A. G. Maddock, J. A. Smedley and Dr. Denis Taylor. Pp. x+886+8 plates. (London: George Newnes, Ltd., 1962.) 160*s*. net.

THIS *Encyclopaedia of Nuclear Energy* has been produced "under the leadership and guidance of five Advisory Editors" all of whom are well known in their own fields. There is a foreword by Dr. H. Kronberger, but no editorial introduction nor any indication of who bears the overall editorial responsibility—a pity, since the job seems to have been well done. It is necessary, therefore, to turn to the dust cover to learn that the book provides information on "theory, techniques and applications of nuclear science", is intended for "scientists, research workers, laboratory assistants and sixth form science students for A level", and that "over seventy scientists have contributed".

Nuclear energy is a wide topic; its applications involve some nuclear physics, engineering, biophysics, solid-state physics, metallurgy, and so on. Indeed its coverage might be expected to include something from most branches of science. This expectation is well realized and the topics treated do indeed give a fair picture of the scope of nuclear energy. It is no serious criticism to say that, occasionally, as in some of the biological articles, the bounds of nuclear energy appear to be exceeded but, even so, there are one or two gaps that merit filling in a second edition. For example, I could find no article on radiation damage, although there is one on the annealing of radiation damage. Nor is there anything about neutron radiography or non-destructive testing.

There are many competent articles and some very good ones, for example, on atomic structure, atomic weapons and their effects, biological effects of radiation reactors, nuclear fission, controlled thermonuclear research, to name a few. Some of the shorter definitions (for example, those of atomic interaction and neutron diffraction) are rather trivial, and one at least, that of cold neutrons, is incorrect. The coverage of the field of

diffraction analysis, whether by X-rays, neutrons or electrons, is inadequate; but with few exceptions the quality of the articles and definitions is good at an intermediate level of knowledge. Most of the articles are less than 1,000 words in length although a few are much longer and some consist of brief definitions; but 89 pages out of a total of 886 (that is, 10 per cent of the volume) are devoted to a list of isotopes and their properties. This list is presented on successive pages and gives somewhat more information than the usual tabular form permits. I am not sure, however, that the middle of an encyclopædia is the right place for it: there might be quite a demand for a separate booklet containing the information presented.

The *Encyclopaedia* has no index, and this at once raises the problem of how easily information is to be found. This has been tackled by entering terms in more than one word order with appropriate references, and by cross-references to related articles; but only partial success has been achieved, and I think that an orthodox index might well be included in any further edition.

I did not notice many misprints—"Van der Waal's" for "Van der Waals" on p. 82, and "Zircalloy" for "Zircaloy" on p. 108 are all I spotted—but I did notice in the symbolism a lack of conformity with the usually accepted conventions. Thus we find such symbols as $_{34}Se^{70}$ instead of $^{70}_{34}Se$ throughout, and n/cm²/sec instead of n/cm² sec.

The volume is well produced, easy to read and easy to handle. It should prove to be extremely useful to those who wish to look up a point of interest without having to consult more weighty books of reference, and would seem to justify Dr. Kronberger's remark in his foreword that "its place is on the desk not on the shelf".

J. Thewlis

ASPECTS OF CHILD PSYCHOLOGY

Readings on the Exceptional Child

Research and Theory. Edited by E. P. Trapp and P. Himelstein. Pp. xii + 674. (The Century Psychology Series). (London: Published by Methuen and Co., Ltd., 1962.) 55*s*.

THIS is an excellent hand-book edited by Trapp and Himelstein, both of Arkansas University. It contains forty-eight chapters divided into sections, and covers a wider field than the title leads one to expect.

The first section is concerned with studies of children of exceptional intellectual processes and discusses not only the mentally deficient but also the intellectually gifted. This is followed by a section on exceptional sensory and motor processes, which includes the aurally and visually handicapped, the speech handicapped, the brain damaged, and the physically handicapped. A final section is devoted to emotionally disturbed children. These sections are preceded by three introductory chapters which discuss, in turn, the incidence of exceptional children (in the United States), the functional classification of exceptional children, and the meaning of research. There are also two excellent indexes, one of names and one of subjects. In addition, each chapter has its own list of references, and although each is written by a different author, the book is well compiled with a sense of continuity throughout, and reveals careful editing.

The book's sub-title *Research and Theory* probably gives a fair picture of its contents. Most of the chapters are written by well-established authorities, but there are also accounts of original research not previously published. The editors state in the preface that this is a novel feature of the book, and that their aim throughout has been to include studies which will stimulate research. The main emphasis, in consequence, is on the contemporary scene, although occasionally a subject may be treated historically because from this angle have branched many fruitful lines of investigation.

The mentally deficient child has received most attention, and seventeen chapters in all appear in this part of the book. One chapter of interest is that devoted to the concept of pseudo-feeblemindedness in which the work of the Clarkes is mentioned, one of the few references to British psychologists. The author of this chapter distinguishes two meanings of pseudo-feeblemindedness which are mutually exclusive. One occurs when a mistaken diagnosis has been made, the other is described as a condition of mental deficiency which can be ascribed to factors other than those customarily held to be the conditions of mental deficiency. The writer complains of the indiscriminate usage of this term, and the failure to separate these two meanings. Even in the same article or report they may be confused.

Other chapters in this section include a historical survey, the concept and classification of mental deficiency, and the measurement of rigidity. The latter by Zigler is a well-written and valuable study with a critical survey of the work of Lewin, Kounin and Goldstein. There are also chapters on different aspects of learning and of reasoning in the mentally deficient. The final chapter in this section considers the effects of a severely mentally retarded child on the family, an important consideration. These titles may serve to indicate the scope of this portion of the book.

Terman's longitudinal studies of the gifted child form the focal point of the next section, and a useful summary is given of this research. It contains an account of the data obtained at school age, and traces the careers of the children from school to their subsequent life-work and status at middle-age. The contributions provide supplementary interesting material on the educational and personal problems centring round the highly intelligent child. The attitude of teachers of gifted children is described as negative in some instances.

The deaf and the blind are adequately presented and point the way to further research. Writings on the brain damaged and on the physically handicapped occupy eleven chapters, all of them are absorbingly interesting and yield valuable information of recent studies. These include studies of disorders of conceptual thinking in the brain-injured child, and the use of certain perceptual measures of brain injury with mentally retarded children. Other relevant discussions, to mention a few of them, are concerned with the effect of distraction on the performance of brain-injured; concrete and abstract thinking in organic and non-organic mentally retarded children; motivation, adjustment and anxiety of cerebral-palsied children.

The final section (Part 4) of the hand-book gives some indication of the problems of emotional difficulties, and although there are only six chapters in this section, they provide interesting reading. School phobia is discussed as is also the use of tranquilizers. The present trends in schizophrenic research and its implications for childhood schizophrenia form the material of one contributor, followed by a separate discussion by a different author on the parental attitudes of mothers of schizophrenic, brain-injured, retarded and normal children. Other chapters in this section are equally important.

Considered as an entirety, this is a valuable book. The title is misleading because it is delimiting. The contents are more than readings; they are sources of information and incentives for research. The term 'exceptional children' has a wider connotation in the text than the generally accepted definition. This book can be recommended to all interested in children, and to professional workers of different disciplines. It will also serve as a guide to students in the fields covered in the text, and certainly will be a valuable addition to many libraries. It is also an excellent supplementary book to students of child psychology.

Mary Collins

RUTHERFORD AND THE ATOM

POETIC PORTRAYAL OF A SCIENTIFIC DISCOVERY

By Prof. JOHN FRANCIS

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THE whole structure of our everyday world is dependent on scientific knowledge, and this knowledge has profoundly influenced philosophy. It is therefore surprising that the romance of scientific discovery has received such slight attention from the poets[1].

A notable exception is the poem on Rutherford by Douglas Stewart[2] of some thirty-six verses. One of these tells of the young New Zealander's reaction when he learned of his appointment to Cambridge:

"I'll dig no more potatoes!" so he had vowed
That day the telegram from Cambridge came,
And dug no more indeed, nor milked nor ploughed
Except in the great seas of thought and fame;
So he stood now, Lord Rutherford of Nelson.

Another verse describes him engaged in his research:

Mostly too busy to think—too busy thinking.
But thinking was doing; there was such satisfaction
Watching those tiny comets darting and winking
It really left no time for speculation.
Thought would go outwards, expansion; his was a shrinking,
How to get mind and hand so small, that was the problem,
That in one final thrust of concentration
They would be able to move inside an atom.

Other verses link his scientific work with his father's craftsmanship and the New Zealand countryside:

That craftsman's hands still moving inside his own.
It was a haunted place, this tower of knowledge,
Calm with old books but wild with thoughts unknown,
All dark except for lamps like lights of courage
Where lonely scholars sought for truth in stone.
It shut the whole world out from a man and his work;
But while the white stars glittered above the college
A wheel moved somewhere far away in the dark—

That waterwheel of his father's lifted up
Water and sunlight in its wooden hands . . .
In long-lost Nelson wheels that his father had made,
And good wheels too, were serving the people still . . .

The universe turned and moved above him so vast,
Full of black space, the huge wheel slowly spinning,
He knew he stood with his specks of radiant dust
Not at the end of things but at the beginning.
What was he now but that small lump of a boy
Who made his own miniature wheel to splash in the water
Such ages ago; working all day in the joy
Of pure and bubbling creation, copying his father:

The social implications of atomic energy are described, together with the great fascination of the quest for discovery and achievement:

Take off his trappings and, naked, hungry and fierce,
All over the earth, in jungle or civilized city,
Men were but savages yet; God help the poor brutes,
For this new power, appalling to love and pity,
Was force that no savage yet had dreamed of wielding.
Then you could turn away, pack up and go home,
Dismantling the apparatus. He'd have his herd of Jerseys,
And up in the dawn to milk then, hitch up the cart,
Off to the factory to yarn with others of his type
(Looked like a farmer, always a farmer at heart,
Corpulent, bushy-moustached, smoking his pipe) . . .

But he had grown to like
This life of power where scientists met together
And felt they were priests and rulers. He liked to talk
With his great peers that language wrapped in mystery—
But he'd be plain if he could. No, it was rather
He liked the thought that what he touched was history.

Then the triumph of discovery and Rutherford's final belief that the essential goodness of the world would prevail:

He was so clumsy and blind, beyond all patience:
But out of the dark, from nowhere, flashed the conception
Like force in the atom and filled him with its radiance;
And steadily, patiently, always in the right direction
Despite his stumblings it moved in him in silence
Until at last what it wanted to do was done.
All things, it seemed, moved through time to perfection,
Through earth and wood and flesh, through the mind of man . . .

And when the old passions were roused and blood was spilt
What else could man do but stand against the marauder?
That was the thing that must change, this pattern of savaging.
For never had there been such a weapon since time began
And men who would stop at nothing might stop at fear . . .

And yet as he looked at the sky so dark with warning
Vast over earth and its towers, the night heaved over
Close and familiar as a waterwheel turning
And shed its stars like drops of crystal water
And radiant over the world lay the clear morning.
Men moved in darkness truly, but also in the sun
And on that huge bright wheel that turned for ever
He left his thought, for there was work to be done.

These extracts may give some idea of the poem, which provides a unique impression both of Rutherford and of scientific discovery.

Fitzgerald[3] has appraised the diverse literary work of Douglas Stewart, and his radio play, *Fire on the Snow*, which describes Scott's epic journey to the South Pole, is well known. Many of Stewart's poems do not have the same quality as *Rutherford*, but *Elegy for an Airman* (1940) shows the same feeling for the New Zealand countryside. Then, after arriving in Australia, Stewart achieved the feat of displaying the same powers of observation and visualization to capture the spirit of a harsh, dry landscape in his *Birdsville Track*[4].

Obviously, however, *Rutherford* goes far beyond these poems, as it combines Stewart's feeling for the countryside, his father's craftsmanship and the life of a peaceful agricultural community, with a dramatic portrayal of the greatest scientific discovery of the age. He also shows a fine appreciation of the social implications and finally expresses his faith in human survival.

Fitzgerald describes the poem as "a towering concept . . . as a work of art it is a masterly piece of design and integration".

Rutherford obviously has a special appeal to scientists, and I feel sure that it will remain memorable as a major attempt at the poetic portrayal of a scientific discovery.

[1] *Times Literary Supplement*, 153 (March 1, 1963).
[2] *Rutherford and Other Poems* (Angus and Robertson, Sydney, 1962). *Rutherford* was first published in *Quadrant*, **14**, 30 (Sydney, 1960).
[3] Fitzgerald, R. D., *The Elements of Poetry; Motif in the Work of Douglas Stewart* (Univ. Queensland Press, 1963).
[4] *The Birdsville Track, and other Poems*, 29 (Angus and Robertson, Sydney, 1955). *The Birdsville Track* was first published in *The Bulletin*, 18 (October 22, 1952).

Although many will agree with Prof. Francis that scientific discovery has received but slight attention from the poets, on the other hand there have been, and still are, men of science who were or are also good poets. One need only mention the late Sir Ronald Ross and the late Sir Charles Sherrington and Prof. E. N. da C. Andrade

and also refer to the poem "Death of a Laboratory" recently published in *Nature* (194, 711; 1962), which was acclaimed by many readers. This poem was written by D. Bolton, who is a chemist.

Attention should also be directed to the book on the poetic relations of science and technology by W. Eastwood, reviewed in *Nature* (192, 98; 1961) by T. R. Henn, lecturer in poetry and drama in the University of Cambridge, whose own Hatfield Technical College lecture on "Science and Poetry" was published in *Nature* (191, 534; 1961).

Here are the 'two cultures' meeting on a common ground—that of poetry; but so far it would seem that the scientists have moved more than half-way. Poets such as Douglas Stewart are helping the other side to gather momentum. To-day science is presenting a great canvas to the world; perhaps there would be fewer disparaging remarks among non-scientists concerning science and scientists if artists of all types, especially painters, poets and novelists, began to cover that canvas with material which might capture the imagination and perhaps even the admiration of the scientific and non-scientific world.

Even in technology there is beauty; but it needs the right man to reveal it. The day must come when science is accepted for what it is—the revelation of the truth. Then there will be no need to argue about 'two cultures' (or indeed any number of cultures); for, with the help of the arts, literature, music and science the entire world will become civilized and cultured. Sir Thomas More's perfect social and political system will no longer be imaginary or unpractical, it will be real. [EDITOR.]

THE INTERPLANETARY MAGNETIC FIELD

By PROF. V. A. BAILEY
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(1)

ALTHOUGH the magnetometers of the space probes *Pioneer* 5, *Explorer* 10 and *Mariner* 2 have now provided much information on the interplanetary field $\boldsymbol{F}_i$, the published discussions of this information have left many serious obscurities in our conception of this field and no reliable idea of its origins. (This opinion is expressed clearly in the comment (*a*) of Dr. E. J. Smith (Jet Propulsion Laboratory, Pasadena) quoted in Section 4 of this article.) For this reason it is here proposed to subject the magnetic data and the concurrent plasma data to a new fundamental analysis.

It will then be seen that this new analysis has justified itself by revealing a new and important aspect of the data which seems to have been completely overlooked in the publications on the three space probes.

(2) Data from *Pioneer* 5 during March 11–April 30, 1960

Magnetic data obtained from *Pioneer* 5 are given in publications by P. J. Coleman, C. P. Sonett, D. L. Judge and E. J. Smith[1] and by P. J. Coleman, Leverett Davis and C. P. Sonett[2]. These authors reported "the apparent existence of a quiet-time field perpendicular to the ecliptic"[1].

The magnetometer on *Pioneer* 5 measured only the component $B_\perp$ which was transverse to the spin axis of the vehicle. But since there was no obvious change of quiet-time $B_\perp$ as this spin axis changed its inclination to the Earth–Sun line from 27° (on March 11) to 2° (on April 8) and then back to 22° (on April 30) it was concluded that $B_\perp$ was approximately perpendicular to the plane of the orbit and therefore also to the ecliptic.

During the period March 31–April 3 the magnetometer also recorded the occurrence of a magnetic storm field $\boldsymbol{B}_s$ at the same time as a similarly varying storm field $\boldsymbol{F}_s$ was observed at the Earth.

All these data were used later by me[3] to infer the sense of the perpendicular quiet-time magnetic vector $\boldsymbol{B}_q$, and it was found that very probably:

$\boldsymbol{B}_q$ was directed from north to south of the ecliptic plane (1)

(3) Data from *Explorer* 10 during March 25–27, 1961

Explorer 10 was launched for the purpose of obtaining vector magnetic field and plasma flux measurements along a trajectory which extended into the interplanetary medium up to a distance of 42·6 Earth radii from the Earth.

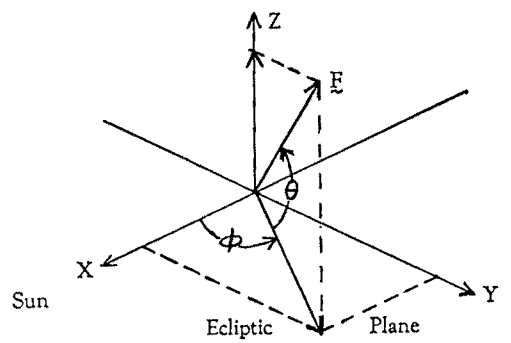


Fig. 1

Magnetic data from this probe are given in a publication by J. P. Heppner, N. F. Ness, C. S. Scearce and T. L. Skillman[4], and are there discussed and interpreted with the help of the corresponding plasma data published by the "MIT group". The magnitude and direction of the field vector $\boldsymbol{F}$ are there expressed mainly by means of the ecliptic co-ordinates F, θ, φ shown in Fig. 1.

The summary of this publication is set out in eight paragraphs in their Section 17.

Various conclusions are to be found in this summary, the most interesting of which relate to a hypothetical "large-scale cavity".

For example, paragraph 3 states that at distances > 22 Earth radii and before a particular sudden commencement, the field is typically oriented in one of three directions, designated regions A, B and C, which correspond to three distinctly different levels of average field intensity. Region A alone correlates with the absence of plasma and is interpreted as lying within the cavity. Regions B and C are observed only at the same time as plasma and lie more than 30 and 23 Earth radii respectively from the Earth.

Paragraph 6 states, "The cavity characteristics indicated by the measurements do not agree in detail with the theoretical cavity models".

Paragraph 7 states "the fields measured external to the geomagnetic cavity are interpreted as fields of solar-interplanetary origin".

Lastly, paragraph 8 states that the tendency of given field conditions to persist indicates that the solar wind possesses structure with dimensions of the order of 0·01 A.U.

Nowhere in this summary or in the rest of the paper is there any reference to the fact, shown by Figs. 25–27,

that in all the regions, A, B and C, the average magnetic field vectors slope downwards, that is:

they have components which are perpendicular to the ecliptic plane and are directed from north to south (2)

This fact is confirmed by the curves in Figs. 12–17, which represent the values of the inclination θ of the total field vector ($\boldsymbol{F}_t$) to the ecliptic plane, before the sudden commencement (*SC*) indicated in Fig. 17; for the values of θ are there negative during 98 per cent of the journey between the distances 12 and 41 Earth radii from the Earth. It is also confirmed by the statistical distribution of values of θ shown in Figs. 23*a* and *b*.

The absence of mention of this fact by the authors suggests that they were concentrating on behaviour of the magnetic field in accordance with certain orthodox ideas and principles in physics and astronomy.

The vectors in Figs. 25–27 and the curves in Figs. 12–17, which represent the azimuthal angle φ (defined in Fig. 1), show that the components F_x, F_y of $\boldsymbol{F}_t$, which lie in the ecliptic plane, frequently change their directions.

In this regard they differ profoundly from F_z, the component perpendicular to the ecliptic, which, about 98 per cent of the time, has only one direction, namely, from north to south. These facts are also illustrated by the vectors shown in Fig. 31.

These facts seem to point to the conclusion that the observed interplanetary field vector $\boldsymbol{F}_t$ is the resultant of at least the following two kinds of magnetic vectors $\boldsymbol{A}$ and $\boldsymbol{B}$:

$\boldsymbol{A}$ has all its three components A_x, A_y, A_z variable in direction;
$\boldsymbol{B}$ is perpendicular to the ecliptic and always directed from north to south (3)

Thus:

$$\boldsymbol{F}_t = \boldsymbol{A} + \boldsymbol{B} \qquad (4)$$

(4) Data from *Mariner* 2 during late August and early September, 1962

The most recent available scientific report on the magnetic data from *Mariner* 2 in interplanetary space is that published on December 7, 1962, by P. J. Coleman, jun., Leverett Davis, E. J. Smith and C. P. Sonett[5]. For convenience their abstract may be quoted here in full:

"Preliminary analysis of *Mariner II* magnetometer data indicates a persistent interplanetary field varying between at least 2 and 10 gamma. The interplanetary field appears to lie mainly in the ecliptic plane, although there is a substantial, fluctuating, transverse component. The *Mariner II* data agree reasonably well with the prior *Pioneer V* observations. Typically, variations as large as 5 to 10 gamma in the field component radial from the sun are measured. Correlations with the *Mariner II* plasma measurements have been observed".

Some care is needed here in comparing their data with those of *Explorer* 10 because their report used a notation for the co-ordinate axes which differs from that used in my Fig. 1. It appears that after *Mariner* 2 was stabilized (on September 3) their co-ordinates X, Y, Z were related to the co-ordinates X, Y, Z in Fig. 1 as thus shown:

	Perpendicular to—	In—	the ecliptic plane
Mariner 2	X	Y	Z
Fig. 1	Z	Y	$-X$ (X is sunwards)

Thus their symbols B_x, B_y, B_z represent respectively the components along the Z and Y axes in Fig. 1 and the component against the X axis in Fig. 1, and so positive B_x is directed northwards perpendicularly to the ecliptic plane and positive B_z is directed radially away from the Sun.

Since only the variations ΔB_z of the radial component were then known we shall here consider the components B_x and B_y alone. The values of B_x and B_y during the period 06·00–06·50 G.M.T. on September 3 are alone given in this report, namely, by means of the top right-hand curves in its Fig. 2 (p. 1100).

The curve for B_x shows that in that period B_x was always negative and so we conclude that then:

there was always a component of the field perpendicular to the ecliptic and directed from north to south (5)

This is in exact agreement with the conclusions (1) and (3) derived from the data of the other two space probes.

The curve for B_y shows that in this period $\boldsymbol{B}_y$ did not change its direction.

The authors also state, "Our earlier speculation [in *Phys. Rev. Letters*, 5, 43 (1960)] that in very quiet periods the transverse component might be mainly perpendicular to the ecliptic plane is inconsistent with the *Mariner II* data for this period". Reference back to their paper of 1960 shows that in fact there was no change in $B_\perp$ as the spin axis of *Pioneer* 5 changed its inclination to the Earth–Sun line by about 23°, and so their "earlier speculation" was a necessary inference and now shows that at that part of the solar cycle the two components in the ecliptic plane were relatively much smaller in value than the same components were in August–September 1962.

In the *NASA News Release* of 10.00 a.m., October 10, 1962, one of the aforementioned authors, Dr. E. J. Smith, made the following statements about the *Pioneer* 5 and *Mariner* 2 magnetic data:

"(*a*) we don't really understand where interplanetary fields come from.

"(*b*) The present theoretical concept is that these fields are drawn fron the surface of the sun and into interplanetary space.

"(*c*) But it was observed during *Pioneer V* measurement that at certain times the field obtained a very steady value. For long periods of time, the order of an hour or two, the variations were essentially negligible. This, which was a surprising result to many scientists, has been observed also on the *Mariner II* magnetometer.

"(*d*) It was anticipated, on the basis of certain theoretical models, that this transverse component would lie in the plane of the ecliptic. The *Mariner II* results, however, indicate that there is typically a substantial component which is perpendicular to the plane of the ecliptic".

To the last statement (*d*) the remark may now be added that this substantial component is most probably directed from north to south.

(5) General Discussion

The most important general conclusion which now emerges from the combined data obtained from *Pioneer* 5, *Explorer* 10 and *Mariner* 2 is that which includes the special conclusions (1), (2) and (5), namely, the statement (4) that the interplanetary field vector $\boldsymbol{F}_t$ is normally composed of two vectors $\boldsymbol{A}$ and $\boldsymbol{B}$ which are characterized under (3).

The vector $\boldsymbol{A}$ can be provisionally analysed into the radial and transverse components of E. N. Parker's well-known field drawn out of the rotating Sun by the solar wind[6] and also a component representing generalized magneto-ionic waves propagated in the magnetized plasma and originating at the Sun.

The vector $\boldsymbol{B}$ was a surprising discovery for the great majority of physicists, except possibly F. Hoyle (1958) and J. W. Dungey (1960), as may be seen from the discussion by Coleman *et al.*[2] of the quiet-time field recorded by *Pioneer* 5; for practically every known model, except one, for producing interplanetary fields was there considered and then dismissed.

The exception was first given in an article by me[7] published on May 14, 1960. This particular article did in fact predict that a negatively charged Sun could set up

in a frame of reference accompanying the Earth a magnetic field H_r which is perpendicular to the ecliptic and directed from north to south. On the last page of this article it was also pointed out that a test of this model could be made "by means of satellite-borne magnetometers", and this is what is incidentally being considered here. The same model and test were more fully discussed in three later publications[8–10].

A more recent development of this model will be published elsewhere. This, by taking account of the polarization of the plasma constituting the solar wind, can account for the observed perpendicular fields $\boldsymbol{B}$ in magnitude as well as in direction and sense, and also can account for the larger changes in $\boldsymbol{B}$ produced by solar disturbances.

As implied in Dr. E. J. Smith's statement (*a*) in Section 4, apparently no other model has been successfully proposed which accounts for a field like $\boldsymbol{B}$.

Summarizing, we may now regard the observed interplanetary field $\boldsymbol{F}_t$ as due to motion of the magnetometer relative to a negatively charged rotating Sun from which magnetic lines of force are drawn out into space by a flow of plasma clouds and from which solar disturbances are propagated as generalized magneto-ionic waves.

This hypothesis promises to cover all the facts discovered hitherto. Accordingly it may be used to make testable predictions about the magnetic data from *Mariner* 2 which have not yet been published, as well as predictions about the magnetic data from the future space-probe which it is planned to launch towards Mars.

These predictions will include a possible variation of the magnitude of $\boldsymbol{B}$ inversely as the cube of the distance of the probe from the centre of the Sun[10]. Thus, as obtained from *Mariner* 2, $\boldsymbol{B}$ will perhaps be found to have increased by the factor $(1{\cdot}5 \times 10^8/1{\cdot}1 \times 10^8)^3 = 2{\cdot}05$ when in the neighbourhood of Venus.

These predictions depend on the assumption that when the Sun is quiet the screening effects due to interplanetary polarized clouds of plasma may be nearly constant in the region between the Earth and Venus.

Many other simple and testable predictions follow from this hypothesis.

(6) Conclusion

It is important to enquire why the fact that the quiet-time field component perpendicular to the ecliptic is almost always directed from north to south has not been pointed out in the publications of the eight principal physicists concerned.

That it is a crucial fact cannot be doubted since, as we have seen, no orthodox theory exists which has explained it satisfactorily. It emerged only because I was looking for evidence bearing on the unorthodox hypothesis that the Sun carries a large negative charge. This demonstrates clearly the heuristic value of the hypothesis.

Certainly this hypothesis appears to contradict terrestrially ascertained laws of electromagnetism and electronics. But that is not necessarily fatal since we have no reliable knowledge about the magnetic fields inside a star like the Sun, which often throws up to its surface such immense and intense fields as those existing at and near sunspots, and therefore (for all we know) even more immense and intense magnetic fields in the solar interior may exist at some places and trap an excess of electrons. Alternatively the hypothesis may result from some unorthodox process in the solar interior like the apparent continual creation of electricity which has been postulated in earlier publications[7,8]. Consequently this hypothesis cannot be dismissed until it has first been thoroughly tested by means of instrumented satellites. The tests by means of *Pioneer* 5, *Explorer* 10 and *Mariner* 2 at present seem to support it.

The gap between the orthodox and unorthodox views is fortunately now being reduced by a tendency to admit the possibility that different space charges can exist in the inner and outer regions of the radiation belts[11] and that large net electric charges exist on the Moon, Venus and Mercury[12,13], which enable these bodies to influence certain terrestrial events such as the occurrence of precipitation, ice-nuclei and micrometeorites in the atmosphere and of geomagnetic storms.

We can now see that it has become essential to have some future space probes carry devices which can determine the concentration of the protons and electrons separately or else the concentration of the protons and of the excess charges per unit volume of the plasma. It would also be of great value to have them carry a device for measuring ambient electrostatic fields ranging from 10 to about 1,000 V/cm; a plate attached to an electrometer and periodically screened by 'a 'grounded' rotating sector might serve for this purpose.

Note added in proof. A report by L. J. Cahill and P. C. Amazeen (*J. Geophys. Res.*, 68, 1835; 1963), on the magnetic data from *Explorer* 12, likewise verifies the predictions from my hypothesis about the Sun.

[1] Coleman, P. J., Sonett, C. P., Judge, D. L., and Smith, E. J., *J. Geophys. Res.*, **65**, 1856 (1960).
[2] Coleman, P. J., Leverett Davis, and Sonett, C. P., *Phys. Rev. Letters*, **5**, 46 (1960).
[3] Bailey, V. A., *Nature*, **189**, 44 (1961).
[4] Heppner, J. P., Ness, N. F., Scearce, C. S., and Skillman, T. L., *J. Geophys. Res.*, **68**, 1 (1963).
[5] Coleman, P. J., Leverett Davis, Smith, E. J., and Sonett, C. P., *Science*, **138**, 1099 (1962).
[6] Parker, E. N., in *Science in Space*, by Berkner and Odishaw, 235 (McGraw-Hill, 1961).
[7] Bailey, V. A., *Nature*, **186**, 508 (1960).
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BIOLOGICAL BASIS OF PERSONALITY*

By Prof. H. J. EYSENCK

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LIKE other sciences, psychology has the dual task of: (*a*) describing accurately, and preferably quantitatively, the material which forms its subject-matter, that is, behaviour; (*b*) of producing a causal theory which deduces the observed behaviour patterns from more fundamental causes. As human beings are biological organisms, it seems reasonable to expect that ultimately these causes should be looked for in the constitutional endowment of the person, that is, in certain physiological, neurological, biochemical and endocrinological properties of his body. Genotypic variations along these lines will, of course, interact with the environmental influences to produce the phenotypic behaviour patterns which we can observe and describe, and it is not part of our thesis to deny the importance of environmental differences. However, social psychologists, clinical psychologists and others have, in recent years, stressed environmental influences to the almost complete exclusion of biological factors, and it seemed desirable to redress the balance to some extent by stressing certain facts supporting the view that bio-

* Substance of the Thomas Young Lecture delivered at St. George's Hospital Medical School on June 17.

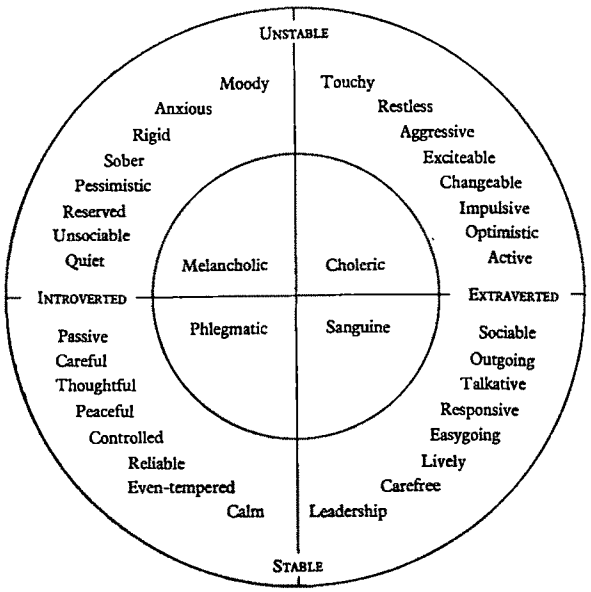


Fig. 1. Diagram showing medieval typology (inner circle) and modern personality description in terms of correlational analysis of trait measurements (outer circle) (ref. 7)

logical factors were of considerable importance in the genesis of individual differences and personality.

Descriptively, there is now considerable agreement among experts that a large part of personality can be reduced to the interplay of two dimensions, one of which is usually labelled extroversion–introversion, the other emotionality, neuroticism, or instability as opposed to stability. This theory goes back at least as far as the great medieval physician Galen, and his doctrine of the four temperaments, and the relation between these temperaments and our two dimensions is shown in Fig. 1; the outer ring of this figure shows the results of large numbers of factor analytic studies of ratings and self-ratings, carried out by such workers as Cattell, Guilford and myself. There is considerable agreement at this descriptive level between medieval theory and modern discovery[1].

My main reason for assuming that these personality dimensions do in fact have a constitutional basis lies in the results from large numbers of experimental investigations carried out on identical and fraternal twins, showing that with respect to both extroversion and neuroticism, identical twins are very much more alike than are fraternal twins. This, of course, is also true with respect to intelligence, and in line with modern genetic theories it is commonly interpreted as providing strong evidence for the hereditary nature of these traits and abilities. The criticism has sometimes been made that possibly identical twins, being so alike, are also treated more alike by parents, teachers, etc, than are fraternal twins who resemble each other less closely. If this were true then the general argument would be weakened, and one would be forced to put a great deal of weight on an interaction effect between heredity and environment. The recent work of Shields[2], however, suggests that this criticism is not, in fact, valid. In addition to fraternal twins, he examined identical twins brought up separately, as well as identical twins brought up together, and with respect to intelligence, extroversion and neuroticism, he found that identical twins brought up separately were, if anything, more alike than identical twins brought up together. The actual figures of the inter-class correlations were as follows, listing first the value for the twins brought up separately, and secondly, the value for the twins brought up together: intelligence, 0·77 and 0·76; extroversion, 0·61 and 0·42; neuroticism, 0·53 and 0·38; for fraternal twins the values were: 0·51, −0·17 and 0·11. These results leave very little doubt about the importance of the part which heredity plays in the genesis of these personality dimensions.

The genetic argument can be extended in various ways. Recent work in Germany, for example, has shown that investigations of familial similarity with respect to extroversion and neuroticism show a degree of relationship to consanguinity, which also strongly supports the hypothesis of inheritance. Another line of argument is derived from the fact that criminals, psychopaths and children suffering from behaviour disorders tend to lie in the 'choleric' quarter so far as personality is concerned; in other words, they tend to be extroverted and unstable. Following the work of Lange in Germany on "Crime as Destiny" much effort has gone into the task of locating criminals with twins and checking whether these twins were concordant or discordant with respect to criminality. So far as adults are concerned, out of 225 twin pairs 71 per cent were concordant among identical twins, but only 34 per cent among fraternal twins. So far as childhood behaviour disorders are concerned, the proportions are 87 per cent and 43 per cent, respectively. For juvenile delinquents the proportions are 85 per cent and 75 per cent. Altogether these figures again strongly support the hypothesis that genetic factors are of considerable importance in the genesis of these particular behaviour patterns.

When we come to look for a biological basis for our two dimensions of personality, we find relatively little difficulty in relation to neuroticism, emotionality or instability. Persons high on this dimension are characterized by very strong emotional reactions to all classes of stimuli, and there is a well-recognized relationship between emotional reaction and the activity of the autonomic nervous system, particularly its sympathetic branch. There is also evidence, again relating largely to twin studies, strongly reinforced by breeding experiments in animals, that the lability of the autonomic nervous system is, to a considerable extent, determined by hereditary factors. There seems little reason to doubt that the biological basis of individual differences in emotionality is closely connected with the activity of the autonomic nervous system[1].

This conclusion needs certain qualifications, particularly in view of the recent findings in the United States of considerable response specificity in the reaction of the autonomic nervous system. Thus it is found that the autonomic nervous system does not act as a whole, but that certain sub-systems may be activated much more strongly than others. It has been found that this specific innovation is characteristic of a given person even though many different types of stimuli may be utilized, and it has been found that this response specificity is preserved over periods of time extending to several years at least. It is likely that this specificity itself is inherited; it represents, as it were, specific areas of strength and weakness of the autonomic nervous system, with the latter acting as a kind of fuse which may be blown in the case of a neurotic breakdown.

When we turn to extroversion–introversion no obvious identification with any structure in the nervous system can be made, but we hope to show that even so it is not impossible to formulate certain hypotheses which have some limited support in the experimental evidence. The beginning of a theory resulting in such a link-up may be found in the postulation by modern learning theories, following Pavlov, of the importance of excitatory and inhibitory potentials in accounting for a large number of laboratory phenomena. In relation to inhibition, for example, it is postulated that massed practice on any task produces inhibitory potentials which depress the quality of the performance which is being practised, so that subjects working under conditions of spaced practice will show superior performance. It is also maintained that this inhibition, being a kind of cortical fatigue state, will

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dissipate during rest, thus giving rise to the so-called 'reminiscence' phenomenon, that is, an improvement of performance during rest, as indexed by subtracting performance at the end of the pre-rest period from that at the beginning of the post-rest period. Thirdly, it is deduced from the theory that the growth of inhibition should ultimately lead to the cessation of activity, that is, the occurrence of certain blocks or involuntary rest pauses. All these consequences have frequently been observed, as have others more in the perceptual field such as the so-called 'figural after-effect'.

I have suggested a link between personality and the theory of excitation and inhibition by postulating that extroverts are characterized by the particularly rapid rise of cortical inhibition, its slow dissipation, and its relatively high level. Conversely, introverts are characterized by the slow growth, rapid dissipation and generally low level of cortical inhibition. The opposite prediction is made with respect to excitation, that is, introverts show a high degree of excitation, extroverts a low degree. (It will, of course, be realized that we are talking about cortical inhibition and excitation, not about inhibited or excited behaviour. Cortical inhibition, by depressing the activity of the highest centres, may give rise to uninhibited behaviour[3].)

Proof for a theory of this kind would, of course, be sought in examining the behaviour of behaviourally extroverted and introverted subjects on laboratory tests of massed and spaced practice, reminiscence, amount of 'blocking', length of figural after-effects, etc. Much evidence has been accumulated in recent years to show that the predicted effects do, indeed, differentiate extroverts and introverts in the predicted direction. Technically much of this work is quite complex, as it is often difficult to rule out irrelevant factors which interfere with the pure measurement of the effects in question. Nevertheless, there seems to be little doubt at the moment that in its broad outlines the theory is supported by the experimental facts amassed so far.

Fig. 2 gives a systematic picture of the hypothetical relations between the genotypic level we have been discussing hitherto, and the phenotypic level of extroverted and introverted behaviour. It will be seen that we postu-

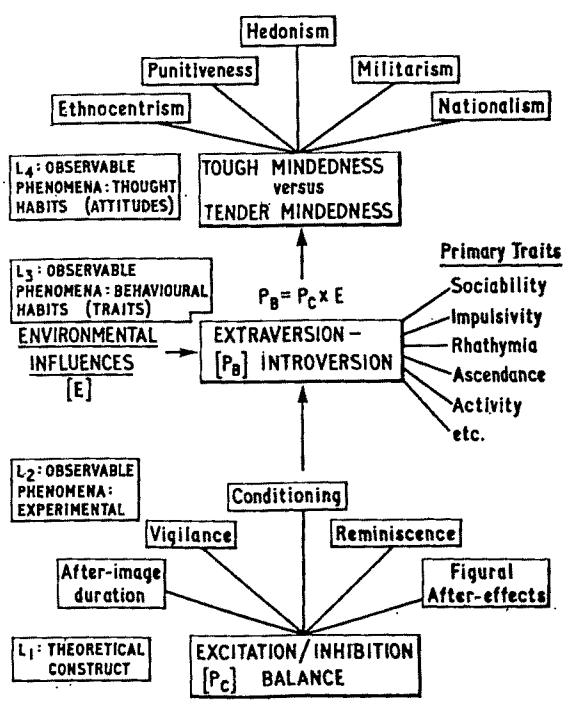


Fig. 2. Diagram illustrating relationship between genotype and phenotype in the personality field (ref. 8)

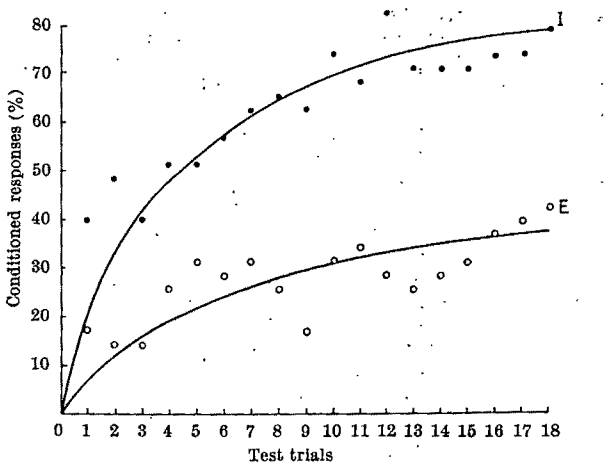


Fig. 3. Rate of eyeblink conditioning in groups of introverted and extroverted subjects (ref. 9)

late several different levels, going from the hypothetical excitation–inhibition balance at the bottom, which we considered to be the physiological basis of our constitutional personality (P_C), to the higher levels of behavioural personality (P_B), which is a product of constitution and environment, and which manifests itself in the form of behavioural habits or traits such as sociability, impulsivity, ascendance, etc., or in terms of thought habits or attitudes. (There is an empirical relationship between tough-minded attitudes and extroverted behaviour patterns.)

The relationship between levels 2 and 3, that is, observable, experimental phenomena, and the observable, behavioural phenomena, is a very complex one which will undoubtedly repay intensive study. We can only discuss here very briefly one particular link as an illustration of the type of argument and empirical demonstration which requires to be adduced. It will be noted that among the laboratory phenomena at the second level I have listed Pavlovian conditioning; the hypothesis states that the process of conditioning produces inhibitory potentials, that it does so more strongly in the extrovert than in the introvert, and that consequently introverts should condition better than extroverts. Fig. 3 shows the results of some experiments in which introverted and extroverted subjects were given the eyeblink conditioning test in which a sound delivered over earphones constitutes the conditioned stimulus and a puff of air to the cornea of the eye the unconditioned stimulus, producing closure of the eyelid. It will be seen that the hypothesis is supported by the data, introverts showing about twice as high a level of conditioning as do extroverts.

Modern learning theorists have hypothesized that neurotic symptoms, such as phobias, anxieties, obsessional and compulsive behaviour patterns, etc., are nothing more than conditioned autonomic and skeletal responses, and that they can be extinguished according to the rules which govern the extinction of such conditioned responses in the laboratory; there is much empirical support for such a contention. Now it will be clear that such conditioned responses are more likely to occur in individuals predisposed to the development of conditioned responses by the possession of a central nervous system which conditions easily. Thus we would expect, on the basis of these theories, that neurotics of this type would be introverted from the descriptive point of view, and would also condition extremely well. There is much impressive evidence to support both these deductions[4,5].

It has also been argued that the process of socialization, by means of which the young child learns the mores of his society, is mediated by Pavlovian conditioning, and it would seem to follow that psychopaths ('moral imbeciles'), and criminals generally, should be found largely

among persons who are extroverted and who condition poorly. (The argument is, of course, exactly the opposite to that which applies to the neurotics discussed in the previous paragraph.) The evidence is indeed very strong that persons in these groups are extroverted and there are several investigations which show that they condition very poorly, if at all. Pavlovian conditioning, therefore, seems to provide an efficient bridge between our level 1, that of the theoretical construct of the excitation–inhibition balance, and level 3, that of observable behavioural phenomena.

Is it possible to locate any structure within the nervous system which may be responsible for individual differences in these mysterious processes of excitation and inhibition ? I would suggest that the ascending reticular formation may fit this prescription reasonably well. This formation, which is located on the spinal cord from the brain stem through the mid-brain to the diencephalon, provides an alternative pathway for ascending impulses proceeding from the periphery to the cortex, impulses travelling along the classical afferent pathways entering the reticular formation via collaterals from the afferent, and giving rise to impulses that are not only directed to the primary cortical projection area but may also be projected profusely over a wide area of the cerebral cortex. Parts of the formation (the so-called ascending reticular activating system) are known to be involved in cortical facilitation; they thus fit in with the psychological concept of 'excitatory potential', while the so-called 'recruiting system' acts as a suppressor and thus has affinities with the psychological concept, 'inhibitory potential'.

Evidence for this identification is largely indirect, the strongest argument in favour being perhaps that deriving from the action of stimulant and depressant drugs. These are known to shift a person's position on the extroversion–introversion dimension in the direction of greater introversion (stimulant drugs) or in that of greater extroversion (depressant drugs), and it is known that these drugs on the whole tend to act through the ascending reticular formation[6]. This hypothesis linking psychological concepts with physiological structures is offered only very hesitantly; it will undoubtedly require much modification before being found acceptable. However, it does serve to unify several quite distinct lines of research and may, in turn, give rise to experimental studies likely to clarify the issue and to reveal to us more directly some of the biological roots of personality.

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A THEORY OF CROSSING-OVER BY MEANS OF HYBRID DEOXYRIBONUCLEIC ACID

By Dr. H. L. K. WHITEHOUSE
Botany School, University of Cambridge

THE term 'crossing-over' was introduced into genetical literature by Morgan in 1912 to describe the phenomenon he had discovered whereby new combinations of linked characters arose. Several theories to explain the process have been put forward in the intervening half-century. Ideas suffered a radical change in 1955 when the classical theory of reciprocal recombination was overthrown. This followed acceptance of the notion that, within small segments of the hereditary material, the products of recombination are not always complementary. Lindegren[1] had first suggested this on the basis of his work with *Saccharomyces cerevisiae*; but the idea did not gain wide support until Mitchell[2] demonstrated the phenomenon in *Neurospora crassa*. One of the first examples of non-reciprocal recombination which she found was remarkable in that conversion (as the non-reciprocal process is often called) had taken place in opposite directions at allelic sites.

In attempting to formulate a general theory of the mechanism of recombination, a difficulty has been the diversity in the results obtained with different organisms. In *Aspergillus nidulans*, where in ordinary linkage studies there appears (unlike *Neurospora*) to be no interference between cross-overs, Pritchard[3] found it necessary to postulate the frequent occurrence of more than one cross-over in short segments, which he called regions of 'effective' pairing, while non-reciprocal recombination has been somewhat rarely recorded[4]. In *Ascobolus immersus*, on the other hand, Lissouba and Rizet[5] and Lissouba *et al.*[6] have shown that non-reciprocal phenomena occur regularly at loci concerned with spore pigmentation, and moreover that a polarity is evident in the data. Within a group of apparently linearly sited alleles, if one is found to show conversion more frequently than another placed to the left of it, then the right-hand member of any other pair of them is found to show a similar difference from the left-hand member. Such polarity has not been demonstrated in *Neurospora*.

Variability is also shown within species. In *Neurospora*, Lindegren and Lindegren[7] found a significant excess of two-strand double cross-overs across the centromere of one of the linkage groups, while others (for example, Stadler[8]) have found no such chromatid interference. In *Ascobolus*, Lissouba *et al.*[6] have found that within a series of multiple alleles different pairs may show diversity in the relative frequencies of occurrence of reciprocal and non-reciprocal recombination.

Copy-choice Hypothesis

The hypothesis that has most frequently been used to explain non-reciprocal recombination is the theory of 'switch synthesis' or 'copy-choice', which relates recombination to replication of the hereditary material. This hypothesis requires that homologous chromosomes shall be paired, at least at certain points along their length, at the time of replication and that the new strands may be replicas of one parental strand in one region and of the other one in another region (Fig. 1). This idea provides a neat explanation of how within a tetrad one site may show a 2 : 2 ratio and a neighbouring one a 3 : 1 ratio.

The theory has been extended by Lissouba *et al.*[6] to take account of their observations on polarity, by postulating that the direction in which replication occurs is from left to right, that is, towards sites with the higher conversion frequency, and, furthermore, that once a copying 'error' has been started it continues to the end of the group of sites. They have coined the term 'polaron' for such a group of sites within which crossing-over appears to be absent.

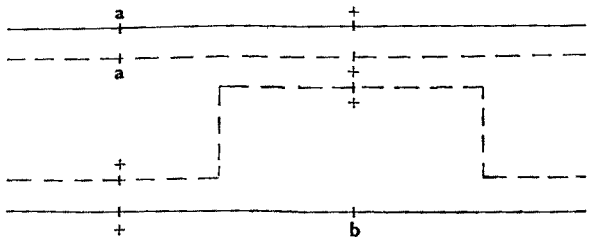


Fig. 1. Diagram to illustrate the copy-choice hypothesis. Old chromatids are shown by unbroken lines, and newly synthesized chromatids by broken lines; *a* and *b* represent the sites of allelic mutations, and the + signs their normal counterparts

The copy-choice theory, and its extension in the polaron concept, are open to a number of serious criticisms:

(1) The theory of the polaron, as proposed by Lissouba *et al.*[6], demands that few or none of the copying errors should continue through the intervening so-called 'linkage structure' to the next polaron, yet Stadler[9] has shown that, according to Lissouba's own data, a majority do so.

(2) Watson and Crick[10] postulated that the method of replication of deoxyribonucleic acid (DNA) was such that each new DNA molecule contained one of the nucleotide chains from the parent molecule and one new one. The work of Meselson and Stahl[11] showed that DNA replication in *Escherichia coli* followed the Watson and Crick method, and these observations were extended to a chromosomal organism, *Chlamydomonas reinhardi*, by Sueoka[12]. Taylor[13] showed that the chromosomes in root-tips of various flowering plants replicated by the same method, so far as their DNA was concerned. These observations suggest that the terms 'old' and 'new' must be applied, not to the two daughter-chromatids from one chromosome, but rather to half of each DNA molecule within them. On the other hand, the idea of copy-choice implies the contrary, namely, that replication is at the chromatid and not the sub-chromatid level.

(3) Meselson and Weigle[14] and Kellenberger *et al.*[15] showed that in lamba phage of *Escherichia coli*, genetic recombination can occur in the absence of replication of DNA.

(4) The necessity, on the copy-choice theory, to associate conversion with replication of DNA presents difficulties, since replication appears to occur at the end of the resting-stage prior to meiosis, before homologous chromosomes are thought to have associated[16]. Furthermore, if reciprocal recombination (crossing-over) also occurs at the time of DNA replication, then it is presumably necessary to postulate that chiasmata must already exist at the earliest stages of meiosis, though not observed. Alternatively, if crossing-over is thought to occur at the pachytene stage, as generally supposed, then a mechanism is required to associate the places of occurrence of reciprocal and non-reciprocal recombination in each meiosis. For it is evident from tetrad analyses in *Neurospora crassa*, such as those by Case and Giles[17], that about half the conversions were associated with crossing-over of 'marker' genes on either side of the pair of alleles. Yet the marker genes were so closely linked that they would have been expected to remain in their parental combinations in more than 90 per cent of instances if the two phenomena had been independent of one another. Earlier work[18,19] in which prototrophs at a nutritional locus were selected among random ascospores from crosses between alleles, had pointed to the same conclusion. Thus, if non-reciprocal and reciprocal recombination are thought to occur at different times, it is necessary to associate them, for example, by postulating that they occur in the same effective pairing segments.

In view of these difficulties and contrary evidence, and above all on account of the fundamental dilemma presented by the occurrence of DNA replication at the sub-chromatid level, the copy-choice theory must in my opinion be discarded as an explanation of genetic recombination between chromatids at meiosis.

Search for an Alternative Theory

In looking for an alternative to the copy-choice theory, one feature of conversion which appears highly significant is the association between it and crossing-over. Not only are the two phenomena often associated in position, as indicated here, but they also appear to be associated as regards the strands involved. Data from the analysis of tetrads in *Neurospora crassa* are given in Table 1. The early results obtained by Mitchell[2] and by Case and Giles[17] had indicated that, when reciprocal and non-reciprocal recombination occur in proximity, two strands might be participating more often than three, although the difference was scarcely significant. However, additional data obtained in this laboratory by Threlkeld[21] and by Cooke[22] make it appear certain that this excess is real. This conclusion is confirmed by the extensive data, not yet published, of Stadler and Towe[23]. Indeed, on the basis of the total data, three chromatids appear to participate so rarely (6 instances out of 42) as to suggest that a third strand may be involved only when the cross-over concerned is not in fact in the immediate vicinity of the non-reciprocal event.

The data of Kitani *et al.*[24] on the segregation of marker genes on either side of the locus for grey *versus* normal black spores in *Sordaria fimicola* point to the same conclusion. Out of 11 asci showing a 6 : 2 ratio of spore colours, and also showing recombination of marker genes on either side, the frequencies of those involving 2 and 3 strands were 9 and 2, respectively. Similarly, out of 52 asci showing 5 : 3 ratios and marker recombination, the 2- and 3-strand frequencies were 48 and 4, respectively. The markers spanned about 4 units of the linkage map.

In searching for an alternative to copy-choice as an explanation of non-reciprocal recombination, it was also evident that some replication of DNA must be postulated, in addition to the doubling which occurs just before meiosis. The occurrence within a tetrad of 3 : 1 ratios at a mutational site demands that such additional replication shall have taken place. Since there is clearly some connexion, from the evidence given here, between crossing-over and conversion, it would be reasonable to suggest that short segments of the DNA of a chromatid may undergo an extra replication at about the same time as crossing-over occurs. This would presumably be at the zygotene or pachytene stage. Was it possible that such further replication could occur in the process of crossing-over itself ?

Theory of Crossing-Over by Molecular Hybridization

It has been customary to regard crossing-over as a process of breakage of two chromatids at corresponding points, followed by rejoining the other way round. As applied to two DNA molecules, this would mean breakage of internucleotidic links in each pair of nucleotide chains

Table 1. THE FREQUENCIES WITH WHICH TWO AND THREE CHROMATIDS TAKE PART WHEN RECIPROCAL AND NON-RECIPROCAL RECOMBINATION OCCUR IN PROXIMITY IN *Neurospora crassa*

Author	No. of tetrads		Distance apart in map units of marker genes
	Two chromatids	Three chromatids	
Mitchell (ref. 2)	2	0	6
Case and Giles (ref. 17)	7	2	10
Suyama *et al.* (ref. 20)	1	0	17
Threlkeld (ref. 21)	6	1	15
Cooke (ref. 22)	2	0	17
Stadler and Towe (ref. 23)	18	3	15
Total	36	6	—

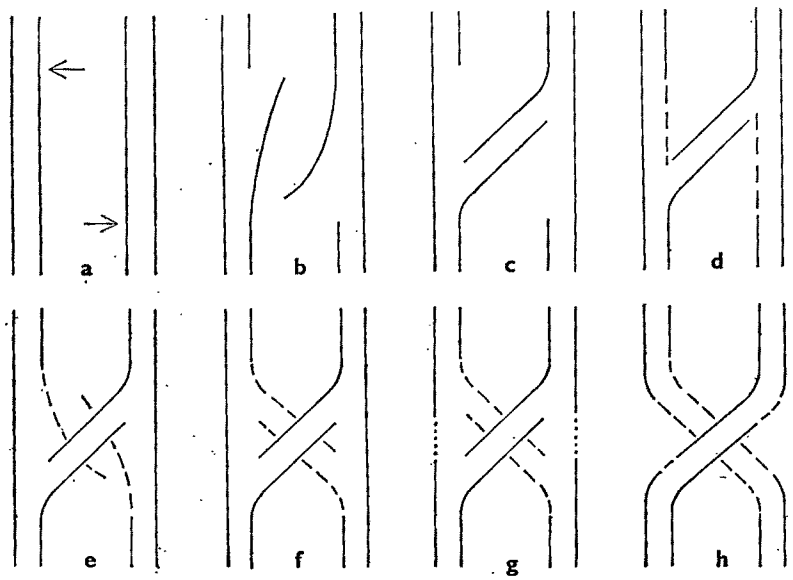


Fig. 2. Hypothetical stages in the process of crossing-over between two DNA molecules, according to the first model, in which the initial points of breakage (arrows) are non-homologous. Old nucleotide chains are shown by unbroken lines, newly synthesized chains by broken lines, and chains which are breaking down by dotted lines. No attempt has been made to show the spiral coiling

in each molecule, followed by re-establishment of the links with new partners. Such behaviour is out of keeping with the known peculiarities of the molecule, of which the most remarkable is the complementary base-pairing between the two nucleotide chains. Was it possible to explain crossing-over by means of this base pairing ?

If both chromatids were broken at precisely corresponding places, crossing-over by base-pairing would appear to be impossible (but see following). On the other hand, if the breaks were at not quite identical places, and involved one nucleotide chain from one molecule and the complementary chain from the other, then the two longer broken ends would have terminal portions which overlapped and hence were complementary to one another. Base-pairing would thus be possible between them, giving rise to one of the cross-over DNA molecules. The reciprocal cross-over molecule could not, however, be formed in the same way, because the other two broken ends would necessarily both be deficient for the segment which the longer broken ends had in common. It was evident that to construct the reciprocal cross-over molecule some replication of DNA was required. Such replication was just what was needed to explain non-reciprocal recombination, and so the possibility was opened of explaining both kinds of recombination by a common mechanism.

On the hypothesis now proposed, broken ends of nucleotide chains of DNA do not join terminally, but instead all reconstruction is by lateral association of chains through complementary base-pairing. Two ways of bringing about crossing-over then appear possible.

First Model

The steps in crossing-over would be as follows:

(1) Complementary nucleotide chains, one from each DNA molecule, break at nearly but not exactly homologous points (Fig. 2*a*).

(2) The parts of the two broken chains between the two breakage points uncoil from their complementary chains (Fig. 2*b*), and then coil round each other so that complementary base-pairing occurs between their terminal portions to form a cross-over molecule (Fig. 2*c*).

(3) New nucleotide chains are synthesized alongside the old chains that have not crossed over (Fig. 2*d*).

(4) The new chains uncoil from their complements, peel back (Fig. 2*e*) and unite by complementary base-pairing to form the other cross-over molecule (Fig. 2*f*).

(5) The old nucleotide chains that have not crossed over break down (Fig. 2*g*) and complementary nucleotides fill the gaps in the two cross-over molecules (Fig. 2*h*).

The detailed consequences of crossing-over, if it occurred by this means, are shown in Fig. 3, where the individual nucleotides are indicated by the letters of the alphabet, capitals (*A*, *B*, *C*, *D* . . .) for those contributed by one parent, and lower case (*a*, *b*, *c*, *d* . . .) for those from the other. (There are, of course, only 4 different nucleotides, but the whole alphabet is required in order to define a sufficient length of the unique sequence of them.) The situation in Fig. 3 (i) corresponds to that in Fig. 2*e*, where the first cross-over molecule has formed and the second one is about to do so. The end-products of the cross-over are shown in Fig. 3 (ii). Both cross-over molecules necessarily have a segment of the DNA in which one nucleotide chain is derived from each parent. The question of whether such DNA is heterozygous or homozygous is discussed later. The expression 'hybrid DNA' as used here is intended merely to imply that it is of dual parentage. The ends of the first-formed hybrid segment (*G–T* in the diagram) are determined by the positions of the initial breakages, while those of the second one (*H–Q* in the diagram) are determined by the points where the formation of the two new chains ceased. Thus, as in the example illustrated, the hybrid regions in the two molecules need not correspond and, moreover, might be expected to vary in length and relative position from one cross-over to another. An important consequence of the theory is evident, namely, that a cross-over does not occur between specific nucleotides of the two DNA molecules,

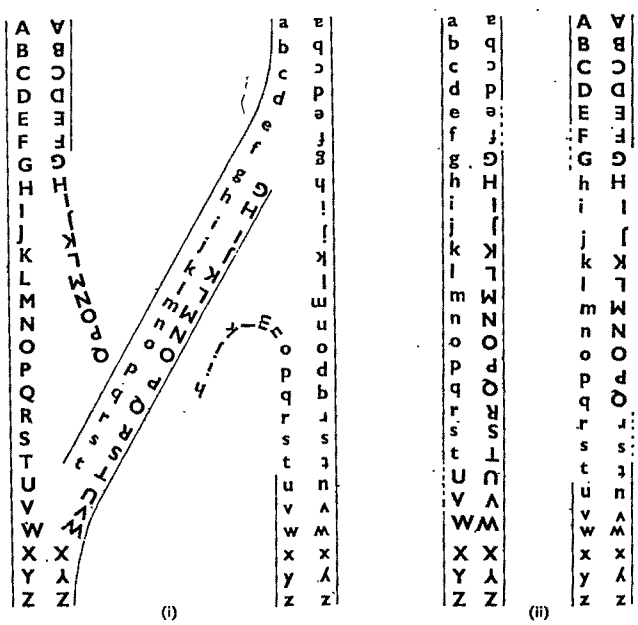


Fig. 3. Stages in the process of crossing-over, according to the first model. The unique sequence of nucleotides is indicated by the letters of the alphabet (although there are, of course, only four different nucleotides), and the parentage by capitals and lower case, respectively. Inverted polarity in the chains is shown by inverting the letters. An unbroken line alongside the lettering indicates old nucleotide chains, the absence of a line implies a new chain synthesized during crossing-over, and broken lines show chains which might be either old or new. No attempt has been made to show the spiral coiling. (i) Stage of crossing-over shown in Fig. 2 (*e*); (ii) end-products of the cross-over

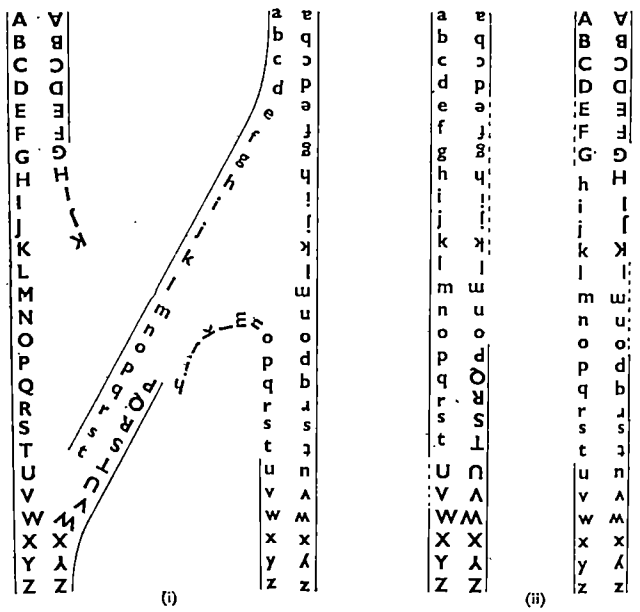


Fig. 4. Stages in the process of crossing-over, according to the first model, corresponding to those in Fig. 3, to show how loss of primary segment *G–O* and secondary segment *L–Q* will cause non-reciprocal recombination (conversion) at sites *L–O*

but instead occupies a significant length of the nucleotide chains, and therefore has a number of mutational sites lying within it.

The account of crossing-over just given does not explain non-reciprocal recombination, but the occurrence of additional replication of DNA gives to the mechanism the potentiality of doing so. A slight modification to the steps outlined here will suffice to bring it about. This alteration is illustrated in Fig. 4, where stages are shown corresponding to those in Fig. 3. The essential difference is that certain nucleotides are missing from the contribution to the cross-over by one parent. Such loss could no doubt readily occur as a result of breakage of one or other or both of the segments of the chain when necessarily unpaired prior to formation of the cross-over molecules. In Fig. 4 (i), as compared with Fig. 3 (i), losses are shown in both segments contributed by one parent, *G–O* from the primary chain and *L–Q* from the secondary one, giving a deficiency for nucleotides *L–O*. After formation of the cross-over molecules, the gaps caused by this deletion can only be filled by nucleotides complementary to those in the other chain, with the result that both cross-over chromatids will have this segment derived from the same parent (Fig. 4 (ii)). This implies that non-reciprocal recombination has taken place over this region. As can be seen from the diagram, the converted segment (*l–o*) lies between the two regions of hybrid DNA (*P–T* in the primary cross-over molecule and *H–K* in the secondary one). The direction in which conversion takes place will depend on which parental molecule suffers the deletion of a segment from the chain that is about to cross over.

Second Model

It was suggested earlier that if the initial breaks were in corresponding positions instead of being staggered, there would be no opportunity for complementary base-pairing, and hence for crossing-over. However, if, following such breakage, the nucleotide chains separated in one direction from the points of breakage, as in Fig. 5 (*b* or *n*), chain synthesis could occur alongside the unbroken chains (Fig. 5. *c* or *o*), and cross-over molecules could arise by base-pairing between the complementary old and new broken chains (Fig. 5, *d* and *e*, or *p* and *q*). Breakdown of the parental previously unbroken chains would complete the process of crossing-over (Fig. 5, *f* and *g*, or *r* and *s*).

In the first model, one cross-over molecule was constructed from the two old chains and the other from the two new ones, and so their formation was likely to be sequential. In the present scheme, the formation of the cross-over molecules could be simultaneous, as each has one old and one new chain. As with the first model, loss of segments from the two free ends of the chains belonging to one parent, such as to leave this parent's contribution to the cross-over deficient for one or more nucleotides, will automatically lead to a corresponding converted segment in the middle of the cross-over.

On this second model, crossing-over could occur in the manner outlined, provided the nucleotide chains unwound in one direction only from the point of breakage, that is, either upwards (Figs. 5, *b–g*) or downwards (Fig. 5, *n–s*). If they separated in both directions, a remarkable situation would develop, illustrated in Fig. 5, *h–m*. Chain synthesis could occur alongside the unbroken chains (Fig. 5, *i*), but the new chains could not become attached at either end because of the old ones. If they separated from their unbroken complements, they could pair with the broken chains, provided they changed sides as in Fig. 5, *j*, *k*. The consequence of this, if the process followed a similar course to that outlined here for crossing-over, would be to produce hybrid DNA in both molecules, but no cross-over (Fig. 5 *l*, *m*).

The detailed effects of this process of nucleotide exchange are shown in Fig. 6, where diagram (i) corresponds to the stage reached in Fig. 5 *k*. The secondary chains have changed sides, and the pairing with their complementary broken primary chains is almost complete. In Fig. 6 (ii), the end-products of the process are shown, the molecules being hybrid from *E* to *R* and from *I* to *V*, respectively. The process that has taken place is effectively one of exchange of a segment between one nucleotide chain of each of two DNA molecules, but with the provision that the exchanged parts need not be exactly the same as regards their length and position in the molecules.

The effects will now be considered of loss of terminal segments from one or more of the four primary free ends in the configuration under discussion. If the lost part of a chain is long enough to give a deficiency in that parent's contribution to the process of exchange, conversion will result. In Fig. 6 (iii) it has been supposed that primary segment *G–M* of Fig. 6 (i) had been lost, thereby shortening this arm to a point beyond the end of the secondary chain (*I–V*) of the same parentage. In consequence, sites *G* and *H* show conversion to the other parent. In this diagram it has also been imagined that primary segment *n–t* has been lost, and since the secondary chain of this parentage ends at site *r*, conversion to the other parent will occur at sites *s* and *t*. It is evident that losses of the kind indicated, that is, from a primary chain and extending beyond the end of the secondary chain of the same parentage, will produce conversion without crossing-over. In general, they may cause conversion in either direction in either or both of two nearly adjacent regions. In any particular instance, the interval between these regions will be determined by the distance between the appropriate ends of the secondary chains (*I* to *r* in the example given).

Fig. 6 (iii) also illustrates an even more remarkable possibility. It is supposed that primary segment *k–m* has been lost. In conjunction with the loss of the homologous primary segment derived from the other parent (that is, *K–M*), this will give rise to reciprocal conversion, that is, a two-strand double cross-over in a short interval, sites *K–M* being exchanged between the two parents. Since this end-result merely requires loss of homologous segments, however short, from the primary chains, it can occur in the absence of conversion, which, as indicated

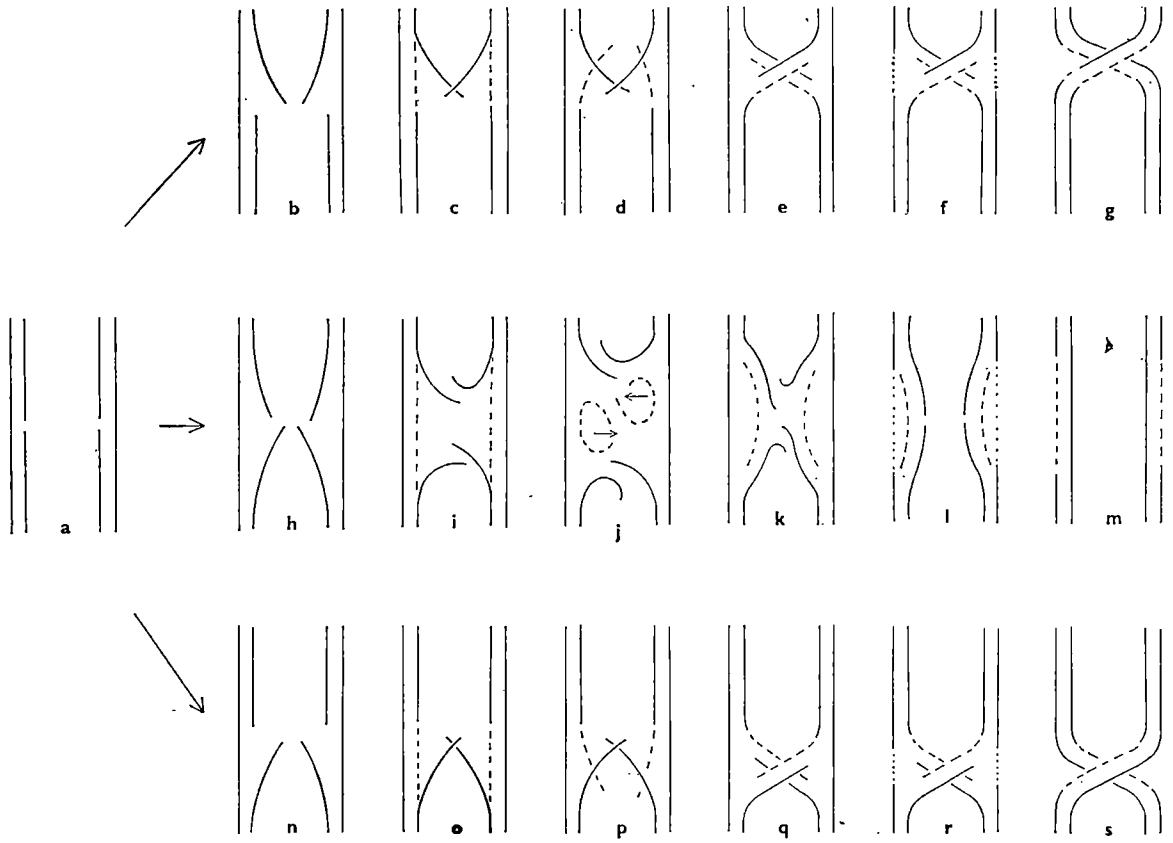


Fig. 5. Hypothetical stages in the processes of crossing-over and of nucleotide-chain exchange, according to the second model in which the initial points of breakage are homologous. Old nucleotide chains are shown by unbroken lines, newly synthesized chains by broken lines, and chains which are breaking down by dotted lines. No attempt has been made to show the spiral coiling. Diagram (*a*) shows the initial breakage; diagrams (*b*)–(*g*) show stages of crossing-over when the upper chains separate from their complementary chains; (*h*)–(*m*) the stages of nucleotide chain exchange when both upper and lower chains separate; and (*n*)–(*s*) the stages of crossing-over when only the lower ones separate

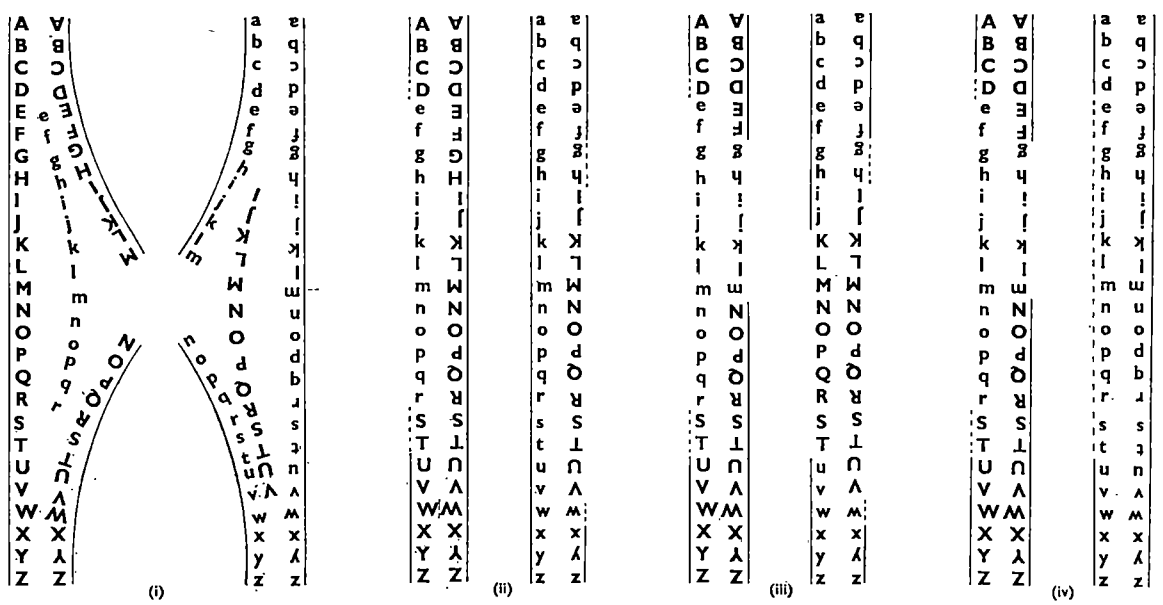


Fig. 6. Diagrams to illustrate the process of nucleotide chain exchange. The significance of the lettering and lines is the same as in Figs. 3 and 4. (i) Stage of nucleotide exchange shown in Fig. 5 (*k*); (ii) end-products of the exchange process; (iii) end-products if primary segments *G*–*M*, *k*–*m* and *n*–*t* have been lost; sites *G* and *H* show conversion in one direction, and *S* and *T* in the opposite direction; sites *K*–*M* show a reciprocal conversion, that is, they are spanned by a two-strand double cross-over; (iv) end-products if primary segment *G*–*M* and the entire secondary chain (*I*–*V*) have been lost; sites *G*–*M* show conversion

here, requires the lost segments to be long enough to extend beyond the end of the secondary segments.

Another variation which might be expected to occur, arising from this configuration with the four primary free ends, is the result of a failure of one of the secondary chains to be formed, or having formed, to pair with its complementary broken primary chain from the other molecule. Such failure would lead to hybrid DNA in one molecule, but to parental DNA in the other. Loss of a segment, however small, from either of the primary broken ends belonging to the parent which failed to produce a secondary chain will then cause conversion. This is illustrated in Fig. 6 (iv), where it has been supposed that one of the secondary chains (*I–V* in Fig. 6 (i)) failed to develop. It has further been supposed that primary segment *G–M* has been lost, with the result that these sites show conversion.

If the initial breakage-points were at homologous positions in similar instead of complementary nucleotide chains of the two DNA molecules, no exchange of material would be possible, unless segments from one molecule broke off and became directly incorporated, without replication, in the other. On the other hand, if the breakage-points were non-homologous, as in the first model of crossing-over, and if the chains unwound in both directions from the points of breakage, then conversion without crossing-over would be possible. It would be necessary for a secondary chain to form against one of the longer broken arms, and then for this chain to become detached and pair with the broken chain of the other molecule, and to span the gap between its two broken ends. This would give hybrid DNA in one molecule, and if the tip of one of the broken chains had been lost, conversion would occur. The reciprocal process could also take place, thereby giving rise to conversion in opposite directions in neighbouring but not quite adjacent regions. On the other hand, reciprocal conversion (a two-strand double cross-over) could be generated only by making the improbable assumption that both the longer arms lost segments of particular lengths while they were exchanging secondary chains.

To sum up, the first model (with non-homologous points of breakage) appears able to account for crossing-over with and without conversion, but in order to explain conversion without crossing-over, it is necessary to postulate that similar instead of complementary chains were broken in the two DNA molecules. On the other hand, the second model (with homologous breakage-points) will account for all three possibilities as the result of complementary strand breakage. This model also predicts the occurrence of two-strand double cross-overs in short intervals, which are unlikely on the first model.

Hybrid DNA

A fundamental feature of the theory of crossing-over that has been proposed here is the occurrence of hybrid DNA in the region of cross-overs or potential cross-overs. Heterozygous DNA would imply segregation of genetic differences at the first mitoses after meiosis, and such an occurrence, although known, is quite rare. It is presumed, therefore, that the hybrid DNA, although often potentially heterozygous, usually becomes homozygous in the process of formation of the hybrid molecules. This could be achieved by the removal from one chain at each heterozygous site or region of those nucleotides which did not conform to those in the other chain, followed by the insertion of nucleotides complementary to those in the first chain. At any particular site of heterozygosity it is to be expected that the two ways of making it homozygous (such as changing *Aa* to *AA* or to *aa*) will not necessarily occur with equal frequency. Indeed, the relative frequencies of the two alternatives might be expected to depend on the nature of the heterozygosity itself, that is, on whether the mutation is a nucleotide substitution or a minute structural change such as a deficiency or a duplication.

This process of replacement of the nucleotide sequence peculiar to one parent by that of the other may lead to non-reciprocal recombination, in the same way as does loss of segments from the primary broken chains. Whether or not conversion is manifest will depend on the direction of the change from the heterozygous to the homozygous condition, and on what has happened at this site in the other molecule. If both molecules contain hybrid DNA, there are four possibilities: if nucleotide replacement occurs in the same direction in both molecules, conversion will also take place in that direction (to *A* or to *a*). If the replacements are in opposite directions, either the parental condition will be restored or a reciprocal conversion (two-strand double cross-over) will have been produced spanning the site.

There would thus appear to be two ways in which conversion can take place: first, by a failure of one parent to contribute to the formation of potentially heterozygous DNA, and secondly, by nucleotide replacement in such DNA so as to make it homozygous. The frequency of occurrence of conversion by the first method will depend on the frequency of loss of the appropriate segments, and by the second method on the frequencies of nucleotide substitution in the two directions. The relative importance of the two methods can be established only from experimental data. It may be that the nucleotide substitution, whereby the DNA is rendered homozygous, is the method by which conversion is usually brought about, even though the alternative, namely, loss of particular segments of the primary broken chains, would automatically cause conversion.

Discussion

Detailed examination of the data on aberrant tetrads in *Neurospora crassa*, *Aspergillus nidulans*, *Sordaria fimicola* and *Ascobolus immersus*, in the light of the present theory, has not yet been attempted. It should assist discrimination between the two models of crossing-over, and provide information about the behaviour of potentially heterozygous DNA. Thus, the observation by Kitani *et al.*[24] that, in *Sordaria* heterozygous at the grey spore site, asci with 6 : 2 ratios and others with 5 : 3 ratios for spore colour may occur in the same cross, is of particular interest and implies on the present theory that when the DNA is hybrid at this site, its heterozygosity is sometimes maintained through meiosis. It is evident that, on either model, the theory will explain the association, both in position and in strands, between conversion and crossing-over, and also the occurrence of either phenomenon in the absence of the other. It will also explain how two conversions can take place in proximity but in opposite directions, as first found by Mitchell[2] in *Neurospora*.

The observation that reciprocal and non-reciprocal recombination are affected differently by heat treatment[19] or ultra-violet light[25] is not unexpected on the present theory, since the two kinds of recombination, although related, are caused in different ways. On the postulated mechanism of crossing-over, although there is no net change in DNA content, inhibition of DNA synthesis at the appropriate time will prevent crossing-over. This suggests that the theory might be more readily tested by looking for suppression of crossing-over or chiasma formation, as a result of such inhibition, than by attempting to detect the DNA synthesis directly.

Explanation of the polarity of the *Ascobolus* conversion data of Lissouba *et al.*[6] would be facilitated if marker genes were available on either side of the sites of the spore pigment mutations. However, Hastings[26] has pointed out that the polarity could be explained by making the following two postulates:

(1) In any particular region of the chromosome, when crossing-over or potential crossing-over is about to occur,

either the process of homologous pairing of chromatids or the formation of hybrid DNA takes place progressively along the chromosome in a fixed direction, say, from right to left.

(2) With certain mutations, the formation of hybrid DNA often ceases immediately beyond (to the left) of their sites, presumably as a consequence of the heterozygosity, which is perhaps structural.

Conversion would thus occur less frequently at sites with heterozygosity to the right of them than when this region was homozygous, since the heterozygous site would shield a region to its left (which would often include the other site) by preventing hybridization of DNA, either directly or through inhibition of pairing of chromatids.

The second model described here, where two free ends give rise to crossing-over, and four to conversion without crossing-over, suggests an explanation for the phenomenon of interference in the position of neighbouring cross-overs. As is evident from Fig. 5, both the crossing-over and the conversion have a common starting-point in the breakage at corresponding positions of complementary nucleotide chains from the two DNA molecules. It follows from this that the initial points of breakage might be randomly distributed, but the cross-overs show interference, if the effect of a cross-over was to divert neighbouring potential cross-overs into the other course leading to conversion.

Hastings[26] has suggested that the coiling of complementary nucleotide chains round one another, which is an essential part of the process of constructing the cross-over molecules, might itself lead to the uncoiling of the same chains at the next points of breakage and thereby favour diverting these cross-overs into the conversion path. If transmission of spiral torsion were the correct explanation of interference, the lack of it between cross-overs in opposite arms of a chromosome would imply that the DNA was not continuous from one arm to the other, or at least that the spiral torsion was not transmitted (cf. Darlington[27]). Conversely, the occurrence of interference throughout a chromosome arm would suggest that the DNA was continuous, in agreement with Taylor's observations[13]. That interference should exist within a chromosome arm, even when the cross-overs involve different chromatids, would presumably be a consequence of the pairing behaviour of chromosomes, such that only two chromatids participate in crossing-over in any particular region.

It is of interest that the occurrence of crossing-over and conversion in proximity and involving three strands altogether (Table 1) appears to happen more often than would two cross-overs in the same interval, implying that there is less interference (or perhaps none) between the places of occurrence of the two phenomena (cf. Stadler[28]). Moreover, in *Aspergillus*, where interference is absent, Strickland[4] found conversion to be comparatively rare, and his data are consistent with the view that conversion takes place only in conjunction with crossing-over. On the theory of interference suggested above, this would mean that, in *Aspergillus*, coiling of nucleotide chains round each other at one point fails to lead to their uncoiling at a neighbouring point.

The second model for crossing-over also offers an explanation for the excess of two-strand double cross-overs which Lindegren and Lindegren[7] found between genes placed on either side of the centromere of a *Neurospora* chromosome. Their results have sometimes been attributed to overlapping of the spindles at the second division of meiosis and not to crossing-over. However, analysis of their data (Whitehouse[29]) has shown that the cross-over phenomenon is real, and it has, moreover, been confirmed in this laboratory (Rifaat[30], Scott-Emuakpor[31]). A curious feature of these two-strand double cross-overs spanning the centromere is that they are frequently associated with an additional cross-over in the proximal region of either or both chromosome arms. On the present hypothesis of interference, if crossing-over is rare near the centromere, as is usual, conversion and its associated variant, reciprocal conversion, would be correspondingly frequent. If two mutant sites on either side of the centromere both fell in regions of reciprocal conversion in the same chromatids, provided there was no intervening cross-over, the result would appear as a two-strand double cross-over between the mutants, together with further cross-overs on either side, in agreement with observation.

Summary and Conclusions

It is suggested that crossing-over occurs by base-pairing between complementary nucleotide chains from two homologous DNA molecules. Two possibilities are discussed, depending on whether the initial breakage-points are homologous or not. Either model will account for non-reciprocal recombination (conversion), for its frequent association with crossing-over, and for such an apparent anomaly as double conversions in proximity but in opposite directions. An explanation for polarity in conversion frequencies is also offered. The second model (with homologous breakage) will explain, in addition, the phenomenon of interference between cross-overs, both as regards their position and the strands involved.

If the theory were accepted, the inference would be that the chromosome is single-stranded, in the sense that each gene is represented only once, and that the DNA is arranged longitudinally within it. The gulf that has existed hitherto between the study of heredity at the molecular and the chromosomal levels would then have been bridged.

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NEWS and VIEWS

Mathematics at Royal Holloway College: Prof. F. R. Keogh

Dr. F. R. Keogh, senior lecturer in pure mathematics at the University College of Swansea, has been appointed to the newly created second chair of mathematics at Royal Holloway College, University of London. Dr. Keogh, who is forty years of age, worked at the Royal Aircraft Establishment, Farnborough, for six years before entering the University of Manchester as a student in 1947. He graduated in Manchester in 1950 and then spent three years at Emmanuel College, Cambridge, where he carried out research in analysis under the supervision of Dr. M. L. Cartwright, and later Prof. J. E. Littlewood. At Cambridge he gained a Ph.D. in 1954 and an Sc.D. in 1963. He has been a member of the staff of the Department of Pure Mathematics at Swansea since 1953 and was appointed senior lecturer in that Department in 1958. Dr. Keogh has published numerous articles on a wide field of classical mathematical analysis: univalent functions, Fourier series, and summability of sequences. His work is well known both from these publications and from the talks he has given at various colloquia and seminars. Analysts everywhere will welcome his appointment to this new chair.

Research at Unilever Ltd.: Dr. A. J. Kieran

Dr. A. J. Kieran retired on September 1 from his post in connexion with the administration of research in Unilever Ltd. After graduating at the University of Liverpool, Dr. Kieran joined Gossages, Widnes, an associate company of Unilever, as a research chemist in 1924. Five years later he became works manager of Gossages, and three years afterwards he was appointed to the position of works manager of Crosfields, Warrington. In 1938 he joined the headquarters staff of Unilever in London. During the Second World War, in addition to his work with Unilever, he was engaged on various Government assignments in the United Kingdom and North Africa. In 1945 he became a member of the South American Group Management which had just been established by Unilever. He returned to the United Kingdom, after a brief spell in the Philippines, in 1951, and took part with Dr. E. G. Woodroofe (now a vice-chairman, Unilever Ltd.) in the establishment of the newly created Unilever Research Division. Dr. Kieran is succeeded by Mr. A. C. Smith, who was previously information officer, Unilever Research Laboratory, Port Sunlight, Cheshire.

U.K. Advisory Council on Scientific Policy

The following four new members of the Advisory Council on Scientific Policy have been appointed for the period ending August 31, 1966: Prof. H. G. Callan, professor of natural history at St. Salvator's College, University of St. Andrews; Prof. A. H. Cottrell, Goldsmith's professor of metallurgy at the University of Cambridge and part-time member of the United Kingdom Atomic Energy Authority; Sir Ewart Jones, Waynflete professor of chemistry at the University of Oxford, member of the Council for Scientific and Industrial Research and Chairman of the latter Council's Research Grants Committee; Sir Patrick Linstead, rector of the Imperial College of Science and Technology and foreign secretary of the Royal Society. All four are Fellows of the Royal Society.

The Committee on Social Studies

It has been announced that the fields of interest of the Committee on Social Studies, set up under the chairmanship of Lord Heyworth (*Nature*, 199, 231; 1963), will include: economics, economic and social statistics, sociology, social psychology, social anthropology, and politics. In addition, the following fields include branches of social study which will concern the Committee: law—crime, organization of justice, etc.; medicine—social medicine, social psychiatry, etc.; geography—social and economic geography. Historical and international studies will present a special problem for the Committee. In general, it will concern itself with such research as is directly relevant to contemporary social and economic questions involving the United Kingdom. In considering the applications of research, the Committee will look for evidence in (*inter alia*) the following: government; administration (public, industrial, social and military); employment and industry; education; and the social services. Mr. A. B. Cherns (Department of Scientific and Industrial Research) has been appointed secretary to the Committee, and Mr. R. P. S. Hughes (Ministry of Health) assistant secretary.

Harwell Postgraduate Education Centre

The United Kingdom Atomic Energy Authority has decided to establish a Postgraduate Education Centre at the Atomic Energy Research Establishment, Harwell. This Centre will organize courses, outside the specialist area of reactor science, intended to further the education of the Authority's graduate staff. Some of these courses will be given by staff of the Atomic Energy Authority and may interest workers in allied fields outside the Authority. From time to time, therefore, such lecture courses as are arranged will be announced in *Atom*. The Reactor School, which has for many years given formal courses at graduate-level on reactor technologies, is now incorporated in the Postgraduate Education Centre. Its programme will continue along the same lines as before. Some of the Harwell research facilities have been made available to university Ph.D. students in the past and it is hoped that the Postgraduate Education Centre will be able to help in organizing such activities in future. Further information can be obtained from Mr. J. F. Hill, head of the Postgraduate Education Centre, Atomic Energy Research Establishment, Harwell, Didcot, Berks.

U.S. Naval Observatory 60-in. Astrometric Reflector

It is perhaps a little unusual nowadays to find a 60-in. telescope being constructed for astrometric purposes. However, it is very important that very faint stars should have their distances accurately known. A star may be faint for several reasons, two being that either the star is intrinsically bright but far away or intrinsically faint and nearby. It is to investigate this latter category of stars that this new telescope has been designed. Intrinsically faint stars—white dwarfs, red dwarfs and sub-dwarfs—are of fundamental importance for theories of stellar evolution. For such investigations it is necessary to know precisely how much energy is actually being radiated by the star and in order to determine this the distance of the star must be known. The 60-in. telescope has been designed for the measurement of the parallaxes of those stars in the group mentioned here which lie within 30 parsec of the Sun. The mirror is made of silica because of its low coefficient of thermal expansion and is paraboloidal of 15-m nominal focal length. The telescope will be mounted in a fork. The support of the mirror uses a compressed air system in place of the more usual system of levers. The telescope will be housed at the Flagstaff station of the U.S. Naval Observatory. The station is

2,300 m above sea-level and has excellent observing conditions. The new Observatory building has been designed to minimize the effects of high daytime temperatures, the whole building being insulated against the daytime rise in temperature (*App. Optics*, 2, 1; 1963).

Institute of Tropical Meteorology at Poona

An agreement has recently been made under which the United Nations, in collaboration with the World Meteorological Organization, will provide assistance for the establishment of an Institute of Tropical Meteorology at Poona. The object is to establish an Institute, as part of the Indian Meteorological Department, for carrying out investigations in applied meteorology. During the period of the International Indian Ocean Expedition, the Department will also establish a Special Division at Bombay to function as an International Meteorological Centre for co-ordinating and supporting the meteorological activities of the International Indian Ocean Expedition. At the conclusion of the activities of the Centre, the equipment and the relevant personnel will be transferred to Poona. The assistance of the United Nations Special Fund, through the World Meteorological Organization as the executing agency for the project, will continue for a period of three years. Seven experts will be provided for a total period of 15 man-years in the fields of forecasting techniques, tropical cyclone analysis and prognosis, use of machine methods in meteorological analysis and prognosis, climatology, hydrologic forecasting and meteorological research. There will be a chief expert for the project, appointed for a period of three years, who will also be the chief research forecaster in the International Meteorological Centre. On the side of the Government of India the Director-General of Observatories will have the overall responsibility for the execution of the project. Seven fellowships of one-year duration each will be awarded to technical personnel of the Indian Meteorological Department for advanced training abroad. Meteorological equipment, cloud and storm investigations equipment and an IBM 1620 electronic computor for data processing, calculating machines and photographic equipment and accessories will also be provided. In all, for this project, the Special Fund will contribute 873,500 dollars (U.S.) and the Indian Government about Rs. 50 lakhs.

Scientific Documentation in South and South-East Asia

Under the title *Scientific Documentation in South and South-East Asia*, the Unesco South Asia Science Co-operation Office, New Delhi, and the Indian National Scientific Documentation Centre, New Delhi, have published a survey of a regional seminar held at New Delhi during March 7–16, 1961, under the auspices of Unesco and the Government of India (Pp. iii+47. New Delhi: Unesco South Asia Science Co-operation Office; and Indian National Scientific Documentation Centre, 1963). It includes the inaugural addresses, a brief account of the organization of the seminar, a report on the position of scientific documentation in Asian countries (covering Burma, Ceylon, India, Indonesia, Japan, Malaya, Nepal, Pakistan, the Philippines, Singapore, Taiwan and Thailand), summaries of the technical papers presented, a note on co-operation, and a list of the proposals formulated for consideration. The last-mentioned include a request to be submitted by Unesco to the International Postal Union for reduced air-mail rates for scientific periodicals, expansion of the existing exchange between documentation centres of publications and photo-reproductions, establishment of an adequate network of microfilming units, and encouragement of the manufacture within the region of the simpler types of equipment for scientific documentation. The attention of governments is directed to the importance of developing, in each country, libraries with large collections of scientific literature as an essential basis for documentation services, and to the importance of Unesco Coupons for operating documentation services. The governments are also urged to make use of the facilities offered by the Unesco Technical Assistance and Participation Programmes and other aid programmes. Acceleration of the publication within the region of Union Catalogues, national lists of periodicals, and current National scientific bibliographies is also urged, and the importance of the exchange of scientific publications in developing scientific documentation generally is emphasized.

Recommendations for Letter Symbols, Signs and Abbreviations

Part 6 of the British Standards Institution's *Recommendations for Letter Symbols, Signs and Abbreviations* deals with *Electrical Science and Engineering* (*B.S.* 1991: Part 6: 1963. Pp. 52. London: British Standards Institution, 1963. 12*s.* 6*d.* net). The recommendations are listed under three sections which are entitled: "General Principles", "Specific Usages" and "Indexes". The first outlines the principles for use in scientific and technological work. These are intended, in particular, "for use in printed papers but they should be observed as far as possible in manuscript and typescript (with suitable instructions to the printer when necessary), and also by instructors in this work". This is all very well, but the section gives no indication to potential authors of how to signify to the printer details of case, type or font when writing by hand or using a normal typewriter—an essential aspect for consistency of style. "Specific Usages" lists quantities and their symbols, units and their symbols, and abbreviations: a Greek alphabet is appended. Section 3 is an alphabetical list of recommended symbols for physical quantities. An "Index of Words" is provided, also a short statement concerning the background, aims, objectives and organization of the British Standards Institution.

Inaugural Lectures at the Imperial College of Science and Technology

The acceptance of a chair in a university in Great Britain usually carries with it to-day the duty of the preparation and delivery of a public inaugural lecture at which the professor is formally introduced to the university and the wider public. Originally the inaugural lecture was merely the first lecture given by the professor in the ordinary course of his teaching duties. The text of the inaugural lecture is now printed and published. In addition, since the inaugural lectures at the Imperial College of Science and Technology, London, are all confined to science and technology, they can be combined in groups to be published in book form. The fourth volume in the series of *Inaugural Lectures* at Imperial College has recently been issued. It contains nine lectures covering the period of the two academic sessions 1960–61 and 1961–62. They are: K. G. Denbigh (chemical engineering science) on "The Science of Continuously Operating Systems"; H. Elliott (physics) on "Cosmic Rays and the Exploration of Space"; J. S. Rowlinson (chemical engineering) on "The Impact of Chemical Physics on Technology"; B. G. Neal (applied science with special reference to engineering) on "Some Problems in Plasticity"; B. J. Mason (cloud physics) on "The Physics of Clouds, Rain and Lightning"; M. H. Fleming (mineral technology) on "Mineral Technology—Progress and Problems"; J. H. Westcott (electrical engineering) on "A Prospect of Automatic Control"; W. D. Gill (oil technology) on "Geology in the Search of Oil"; and Sir Willis Jackson (electrical engineering) on "The Partnership between Science and Electrical Engineering".

Man the Tool Maker

Dr. Oakley is well known for his extensive research on Quaternary geology and early prehistory. His work lies

in the anthropological section of the British Museum (Natural History), South Kensington. *Man the Tool Maker* is a classic and first appeared as long ago as 1949. It is one of the best introductions to the subject of early prehistory yet published. Recently, a reprint has become available of the fifth edition which has been brought completely up to date (Pp. v+98+2 plates. London: British Museum (Natural History), 1963. 4*s*.). Many spectacular finds of earliest man have been made in East and South Africa and Dr. Oakley has visited not a few of the sites and checked the results claimed. Not only has it been necessary to be sure that the new finds are really *in situ*, but also their date, both in the geological sequence and their absolute age as determined by the carbon-14 method, has had to be settled beyond cavil. The first appearance of man has been pushed back into a far more remote past than had formerly been realized. But the book is, of course, not merely concerned with the first appearance of man on Earth. The whole subject of Old Stone Age man comes under review, and not only in Western Europe. Naturally, in the small compass of some 98 pages, the subject has to be treated in a summary fashion, but great skill in selection has been shown and anyone wanting to know something authoritative about palæolithic man would be well advised to start with *Man the Tool Maker*.

Canadian Arctic Geology

In 1955 the Geological Survey of Canada undertook a major project, appropriately named "Operation Franklin", to map the rocks of the Canadian Arctic Archipelago. Over an area of some 200,000 square miles, field parties were set down from the air at points selected from aerial photographs as the most suitable for detailed stratigraphical and structural studies, and were then serviced throughout the season by two heavy helicopters. A full account of this pioneer campaign has now become available (*Geology of the North-Central Part of the Arctic Archipelago, North-West Territories*. By Y. O. Fortier and others. Pp. 671+6 maps. Mem. 320, Geological Survey of Canada, Ottawa, 1963. Price 6.00 dollars). The principal geological formations discovered include the deposits of a miogeosyncline of Lower Palæozoic age, maybe more than 35,000 ft. thick, largely marine except for predominantly non-marine Upper Devonian; and those of a younger geosyncline (the Sverdrup Basin) comprising more than 40,000 ft. of marine and non-marine Pennsylvanian to Cretaceous strata with minor volcanic Cretaceous rocks and wholly non-marine Tertiary sediments. The longitudinal axis of this basin coincides with a zone of gypsum piercement domes and diapir folds. The survey has provided substantial indirect evidence that petroleum may occur in commercial quantity, although no live seepages were found. Coal is common in four formations—Upper Devonian, Upper Triassic or Lower Jurassic, Lower Cretaceous, and Upper Cretaceous or Tertiary, the last with seams measuring up to 30 ft. These important investigations have been continued annually since 1955 and in the current 1963 field season fifteen geologists of the Geological Survey of Canada, with nineteen ancillary staff, have been operating in the arctic District of Franklin.

Soils and Vegetation of the Congo

The considerable scientific work on tropical soils and crops carried out by the Belgians was largely responsible for the fifth International Congress of Soil Science being held in 1954 in the Congo. There have been numerous publications from this field, and this is one of a series on natural resources from L'Institut National pour l'Étude Agronomique du Congo (*Carte des Sols et de la Vegetation du Congo, du Rwanda et du Burundi*. 18: *Bassin de la Karuzi (Burundi)*. *A*: Sols. *B*: Vegetation. Notice Explicative de la Carte des Sols et de Vegetation. Par P. Pahaut et D. van der Ben. Pp. 48+5 planches. Brussels: Institut National pour l'Étude Agronomique du Congo, 1962). It consists of a memoir describing the general features of Rwanda and Burundi with a population of four million—75 per km²—entirely dependent on agriculture on the better soils of the hill slopes. This obviously presents a serious problem for the future of an undeveloped country. The Karuzi Basin was selected for detailed study. It is a region of 900 km² at an elevation of roughly 1,250–2,000 m lying some 130 km north-east of Lake Tanganyika. An outline of the geology and climate is followed by a discussion of the interactions of parent material, soil and vegetation. There is a description of the main physical and chemical properties of the soils and of their classification, and a chapter on the vegetation associated with the main soil groups. The vegetation types include aquatic, semi-aquatic or marsh associations, forest, the *Acacia abyssinica* valleys, the wooded and grass-shrub savannas and post-cultural natural growth. There are several photographs illustrating the nature of the ground and soil profiles and two maps of soils and vegetation on the 1 : 50,000 scale.

The British Institution of Radio Engineers: Awards

The following awards have been made by the British Institute of Radio Engineers for papers (given in brackets) published in the Institution's *Journal* during 1962: *The Clerk Maxwell Premium*, to M. W. Gough ("Propagation Influences in Microwave Link Operation"–July issue of the *Journal*); *The Associated Rediffusion Premium*, to I. J. P. James and W. A. Karwowski ("A Constant Luminance Colour Television System"–April); *The Vladimir K. Zworykin Premium*, to Dr. L. Kay ("Auditory Perception and its Relation to Ultrasonic Blind Guidance Aids"–October); *The A. F. Bulgin Premium*, to I. A. Harris ("Electron Transit Time and other Effects in a Valve Voltmeter Operating at Extremely Low Current"–November); *The Leslie McMichael Premium*, to W. L. Wright and S. A. W. Jolliffe ("Optimum System Engineering for Satellite Communication Links with Special Reference to the Choice of Modulation Method"–May); *The Charles Babbage Award*, to I. Aleksander and Dr. R. W. Scarr ("Tunnel Devices as Switching Elements"–March); *The Lord Rutherford Award*, to Dr. G. Dearnaley ("Semi-conductor Nuclear Radiation Detectors"–August); *The Marconi Award*, to A. Ciuciura ("Switchable 405/625 Line Time-Bases"–October); *The Arthur Gay Premium*, to D. Hinchcliffe, J. R. W. Smith and G. H. King ("Automatic Component Assembly in the Telephone Industry"–September); *The Dr. Norman Partridge Memorial Premium*, to G. D. Browne ("A Pulse Time Multiplex System for Stereophonic Broadcasting"–February); *The Sir Jagadis Chandra Bose Premium*, to Dr. B. R. Nag ("A Two-State Device with Two Inductively Coupled Colpitts Oscillators"–July).

The Institution of Electrical Engineers

Sir Albert Mumford, engineer-in-chief of the Post Office, has been elected president of the Institution of Electrical Engineers for the session 1963–64. The Council for next session will include: *Vice-Presidents*, B. Donkin, L. Drucquer, O. W. Humphreys, J. A. Ratcliffe, Dr. R. L. Smith-Rose; *Honorary Treasurer*, C. O. Boyse; *Members of Council*, S. S. Carlisle, Sir Robert Cockburn, A. R. Cooper, A. T. Crawford, D. Edmundson, G. Lyon, Dr. J. S. McPetrie, J. H. H. Merriman, Prof. C. W. Oatley, G. F. Peirson, M. J. L. Pulling, Dr. N. H. Searby, H. West, Dr. R. C. G. Williams. New chairmen of the three Divisions of the Institution have also been elected, as follows: Dr. R. C. G. Williams, chief engineer, Philips Electrical Ltd. (Electronics Division); Mr. C. D. Wilkinson, formerly chief engineer (reconstruction), National Coal Board (Power Division); Dr. J. R. Mortlock, assistant chief engineer, Associated Electrical Industries Power Group (Science and General Division).

The Corday-Morgan Memorial Fund Executive

The Corday-Morgan Memorial Fund Executive is to sponsor two tours to Africa during the winter 1963–64. Prof. R. A. Raphael, Regius professor of chemistry in the University of Glasgow, is to visit Commonwealth territories in East Africa, including Uganda, Tanganyika and Kenya, and Prof. G. Kenner, Heath Harrison professor of chemistry in the University of Liverpool, will tour Commonwealth countries in West Africa. The Corday-Morgan Memorial Fund Executive is derived from a bequest to the Chemical Society by Sir Gilbert Morgan, a past-president who died in February 1940. The Executive consists of the presidents and immediate past-presidents of the Chemical Society, the Royal Institute of Chemistry, and the Society of Chemical Industry, and its purpose is to promote the unification of the chemical profession within the Commonwealth. It seeks to achieve this object by the appointment from time to time of a lecturer to visit some designated area of the Commonwealth overseas. In general, the visitor will tour the main academic and, where appropriate, industrial centres to give informal colloquia as well as formal lectures, and to discuss problems of mutual interest in the countries visited. Further information can be obtained from the Secretary, Corday-Morgan Memorial Fund Executive, c/o The Chemical Society, Burlington House, London, W.1.

The Field Survey Association Annual Prize, 1963

The Field Survey Association is offering a prize of £50 for the best article in English on any subject that falls within the Association's scope and is published during 1963 in a professional or trade journal of any organization which carries out any of the activities included within those of the Association. The prize is open to any person under the age of forty on the date of submission of his article for publication. It is not necessary to be a member of the Association to enter for the prize. The rules of the prize, the history and objects of the Association and the conditions of membership can be obtained from the honorary secretary of the Award Committee, Commander R. Bill, Vickers House, Millbank Tower, London, S.W.1.

The Night Sky in October

Full moon occurs on Oct. 3d 04h 44m U.T. and new moon on Oct. 17d 12h 43m. The following conjunctions with the Moon occur: Oct. 3d 12h, Jupiter 4°N.; Oct. 20d 04h, Mars 4° S.; Oct. 26d 20h, Saturn 2° N.; Oct. 30d 17h, Jupiter 4° N. In addition to these conjunctions with the Moon, Venus is in conjunction with Spica on Oct. 6d 04h, Venus being 3° N. Mercury is a morning star, rising about 1h 40m before the Sun on October 8, and well placed for observation. Venus and Mars are too close to the Sun for observation. Jupiter is above the horizon most of the night, setting about two hours before sunrise on October 31. It is in Pisces, its stellar magnitude is −2·5 and its distance from the Earth on October 15 is 370 million miles. Opposition occurs on October 8. Saturn sets at 1h 15m, 0h 15m and 23h 15m at the beginning, middle and end of the month, respectively. It is in Capricornus, becoming stationary on October 21 and thereafter resuming its eastward motion among the stars. Its stellar magnitude is +0·8, and its distance from the Earth on October 15 is 875 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Oct. 6d 23h 12·4m, δ Tau. (*D*); Oct. 7d 0h 14·2m, δ Tau. (*R*); Oct. 7d 0h 29·5m, 64 Tau. (*R*); Oct. 7d 0h 35·9m, 68 Tau. (*D*); Oct. 7d 1h 35·0m, 68 Tau. (*R*); Oct. 10d. 1h 44·4m, 63 Gem. (*R*); Oct. 13d 4h 22·4m, 37 Leo. (*R*); Oct. 29d 20h 28·5m, 30 Psc. (*D*); Oct. 29d 22h 34·9m, 33 Psc. (*D*). *D* and *R* refer to disappearance and reappearance, respectively. The Giacobinid meteors are active during October 9–10, but conditions for observation are rather unfavourable. The Orionid meteors are active during October 15–25, with a maximum on October 21; the radiant is near R.A. 6h 24m, Dec. +15°, and conditions for observation are very favourable.

University News:

Cork

Dr. M. J. T. Fitzgerald has been appointed reader in anatomy in University College, Cork (National University of Ireland). This is the first readership to be conferred since the foundation of the University.

London

The title of professor of computing science has been conferred on Dr. R. A. Buckingham, in respect of his post at the Computer Unit. The title of reader has been conferred on the following: Dr. W. C. Chaloner (botany), in respect of his post at University College; Mr. H. Billet (mechanical engineering), in respect of his post at University College; Dr. D. H. Martin (physics), in respect of his post at Queen Mary College; Dr. D. V. W. Parke (biochemistry), in respect of his post at St. Mary's Hospital Medical School.

Sussex

Dr. Z. Barbu, at present senior lecturer in the Department of Political and Social Theory at the University of Glasgow, has been appointed senior lecturer in social studies. Dr. R. R. Birss, at present lecturer in physics in the Department of Electrical Engineering, Imperial College of Science and Technology, London, has been appointed senior lecturer in experimental physics. Dr. S. Swierczkowski and Dr. G. L. Pratt have been appointed to lectureships in mathematics and chemistry, respectively.

Announcements

The eighteenth Calorimetry Conference will be held at the Petroleum Research Center, Bartlesville, Oklahoma, during October 16–18. Further information can be obtained from Mr. John P. McCullough, Bartlesville Petroleum Research Center, P.O. Box 1321, Bartlesville, Oklahoma.

The fourth international plastics industry fair will be held at Düsseldorf during October 12–20. The range of exhibits will cover main and ancillary materials, products, machinery and tools. Further information can be obtained from Nordwestdeutsche Ausstellungs- und Messe-Gesellschaft m.b.H.–NOWEA, 4 Düsseldorf 10, Postfach 10203.

An ordinary meeting of the Midlands Section of the Society for Analytical Chemistry will be held in Nottingham on October 15. A paper on "Sugars in Foodstuffs—some Newer Methods" will be presented and discussed at the meeting. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

The laboratories of the Chalk Lime and Allied Industries Research Association will be open to visitors from industry during October 9–10. The Association is concerned with research and with providing technical information for the lime, sand-lime bricks and the ready-mixed mortar industries. Further information can be obtained from the Secretary, Chalk Lime and Allied Industries Research Association, Church Street, Welwyn, Herts.

A course in "Ultrasonic Testing", arranged by the South Wales Branch of the Non-Destructive Testing Society of Great Britain, will be held in Swansea on five consecutive Tuesday evenings, commencing October 1. The course will include: basic principles; methods and application; examination of castings; examination of forgings and plant in service; modern developments. Further information can be obtained from the Publicity Officer, the Non-Destructive Testing Society of Great Britain, 36 Willow Green, Ingatestone, Essex.

EDUCATIONAL POLICY IN BRITAIN

THE debate on educational policy in the House of Lords on July 24, which was opened by the Earl of Longford, brought a spirited defence of Government policy from Lord Newton, but added substantially little to the present debate. The Earl of Longford had pointed out that the cost of education had risen from £414 million in 1951–52 to nearly £1,250 million in 1963–64, and in the same period the percentage of the national income spent on education had increased from about 3·1 to 4·9. He agreed with Sir Edward Boyle's assumption that the reserve of potential ability among young people was not tapped to anything like the full extent. Lord Newton quoted also the view expressed by Sir Edward at Belfast on July 5 that if children were separated at any stage it should be done in their interests, and not because it pleased their parents or because of any social, class or colour distinction. We should work towards a situation in which every child had an equal chance of developing its interests and personality to the full. Defending the Government's impartiality regarding any particular pattern of secondary school organization, Lord Newton thought also that the achievements in school building had been considerable in terms alike of quantity and of economy and quality. This was due particularly to the work of the development group in the Ministry of Education, whose efforts now provided new schools at a cost, at constant prices, little more than half that in 1949. Lord Newton also referred to the need to re-examine many traditional assumptions and practices about the organization of schools in Britain. Sir Edward Boyle at Belfast had suggested that there might be room for more flexibility in the size of the groups to be taught, that we should ask what were the limits of an acceptable balance between men and women in the different stages of the schools system, what were the true functions of a highly trained teacher and whether their skills and experience were at present being dissipated in tasks which might well be delegated to other hands. All these questions, Lord Newton observed, required discussion, and, while in the recruitment of part-time teachers much of the initiative must rest with the schools and the local education authorities, the Government could help, particularly by commissioning research into the obstacles to be overcome and into the most fruitful lines of development. To reduce all classes to 30 or less would require a further 110,000 teachers, while the decision taken in January to increase the capacity of the training colleges to 80,000 students by 1970 would only give a net addition of 5,000 teachers during the decade.

In a maiden speech, Viscount Samuel, directing attention to the possibility of technological unemployment as the result of automation in industry, stressed the urgency of raising the minimum school-leaving age and referred also to its bearing on the wise use of leisure. Apart from Lord Taylor, speakers in the debate made little reference to the universities, but Lord James of Rusholme stressed the urgency of expansion and thought that the target of 170,000 students by the early 1970's was neither large enough nor likely to be reached without a new sense of urgency. He thought that the universities should see themselves as one end of a spectrum of higher education, distinguished from the others by the relative difficulty and originality of the ideas discussed and evaluated within their walls. He had few misgivings about standards, but some about the possible loss of liberty. While he himself regarded the Ministry of Education as a friend and an enlightened counsellor, it was vital that whatever administrative changes were made the essential liberties of institutions of higher learning should be defined by those who value them, and respected by those who administer them. The liberties to think, to speak, to publish the truth as one sees it; to decide what are the standards to be maintained and the studies to be pursued; to follow the argument where it leads—these are the essentials of teaching and research at the highest level, and on their preservation depends the quality of higher education.

In replying on the debate for the Government, the Earl of Dundee made no comment on Lord Tayler's suggestion that the Ministry of Education should take over from the Treasury its responsibilities for university education, but pointed out that by 1966–67 besides 150,000 students at universities there would be 137,000 students at teacher training colleges, colleges of advanced technology and other advanced technical colleges, or 10 per cent of the relevant age group, and a good deal higher in 1970.

RESEARCH INTO STRATEGIC TRANSPORT PLANNING

IN replying for the Government in a debate on the needs of transport for research in the House of Commons on July 30, which was opened by Mr. W. T. Rodgers, who asked specifically about economic studies, the encouragement given to postgraduate training, and research into strategic transport planning, the Parliamentary Secretary to the Ministry of Transport, Mr. T. G. D. Galbraith, said that the aim in the Ministry should be to keep a balance between action and research. Sometimes only when dealing with a problem in practice could one see where the difficulties lay, what additional information would be helpful, and where more research was needed. In transport, research was best concentrated, therefore, so as to help the solution of actual problems of policy and planning. This was recognized in the Hall Report, which gave the general outlook for traffic in 1981, and began with a statement of the fundamental problems confronting the Ministry before discussing possible lines of research. In transport in cities, for example, the difficulty was to obtain a solution which gave the right balance between conflicting interests. To do so it was necessary to discover the future pattern of personal travel, the strength of preferences for different patterns of travel, the future needs for the movement of goods, the pattern of transport facilities to-day, and the costs of adding to them in various ways, etc. Even with all the facts from such a survey, techniques of handling this knowledge were required and over the whole range of transport there were in fact two stages: research to discover the facts, and research to enable the right lessons to be drawn from them.

The Hall Report and the Beeching Report (see *Nature*, 198, 1233; 1963) were two examples of research which had been proceeding to obtain the facts; on the urban side, the Buchanan Report would soon be available, with its wide implications for city life, and the Ministry and the London County Council had jointly commissioned the London Traffic Survey, which would give detailed information of future traffic needs to enable these to be related to changes in employment, residential patterns, etc. The

survey was being undertaken jointly by British and American consultants working together. The Merseyside Survey should be ready this year and others would follow, while the City of Glasgow had requested a comprehensive survey to cover the Clyde Valley. Parallel with these special studies was a continuing programme of research to find out more about long-term trends in transport, examples of which were the large-scale sample survey of road–goods transport and continuing series of surveys of the ownership and use of private cars recently undertaken.

Mr. Galbraith pointed out that many of the skills required were those of economists, and for this interpretation it was necessary to rely largely on outside sources such as the universities, where transport research was developing in two ways: the first was in the growth of traffic engineering from engineering proper into the wider questions of the economics of transport, pricing, and so on. Secondly, there was increasing interest in transport as a subject of its own with the overall field of economics. The Government was endeavouring to increase the demand for traffic engineers by emphasizing to local authorities the value of such engineers, and the Ministry had been strengthening its links with the universities, and a useful two-way traffic in ideas was developing. It was proposed to use the token sum specially voted for research to stimulate more research and where appropriate to commission specific studies which would help in making policy decisions. An example was the cost–benefit study of investment in a railway electrification scheme just commissioned with British Railways, and the Road Research Laboratory had done a good deal of work on this technique in relation to roads.

ROTHAMSTED EXPERIMENTAL STATION

REPORT FOR 1962

THE size of a modern research station and the range of its activities is such that to keep in touch with its activities by reading the annual report is a major undertaking. Yet to have compressed a report on the work of Rothamsted into little more than 300 pages is in itself a substantial achievement*.

The place of Rothamsted in agricultural science is evident in the notes on personalities and events. Three members of the staff were elected to fellowships of the Royal Society, and invitations to staff members to visit other countries were more than could be accepted without detriment to their work.

The name of Rothamsted is particularly associated with work on soils, and on the conditions that determine their fertility. As Mr. F. C. Bawden, the director, remarks, "There are many reasons for needing to increase crop yields, but no minor one is that when agriculture is inefficient, it is profligate in its use of land". A great part of the work reported is directed towards the more economical use of this basic agricultural resource. The Soil Survey of England and Wales is based at Rothamsted, and Rothamsted provides a base for the Commonwealth Bureau of Soils. Through these two organizations its influence extends both over Britain and to the world at large.

The high level of fertility characteristic of the agricultural soils of England has been brought about very largely as a result of the work on manures and fertilizers at Rothamsted. Progress has been such that in recent years water has increasingly become the limiting factor in yields. Rainfall is barely adequate for unrestricted crop growth in eastern England, and even where the annual total is sufficient, irregularities in distribution give rise to shortages over periods long enough to depress yields. Under Penman's leadership, the Physics Department has opened up a most productive field of work on water supply in the soil and the consumptive use of water by crops. A few years ago it might have been argued that water, though the most important, was the least understood of the major plant nutrients. To-day, water ranks with the classic mineral elements in the fertility investigations at Rothamsted.

It was at Rothamsted that R. A. Fisher, whose sad death is recorded in this report, carried out the studies that established new standards of precision in biological experimentation. The enterprise that started with one man on a temporary basis is now a large and world-famous department, making extensive use of computers, and providing advice and guidance as well as computation service both in Britain and to Commonwealth countries overseas.

A report is primarily a record of work done, and this report is worth all its three hundred pages. In his wise and critical discussion of "The Use and Abuse of Toxic Chemicals" the director contributes to the assessment of the impact of research on agriculture and society. If it is permissible, after so comprehensive a report, to ask for a little more, one might hope that the director will in future use the general report more extensively for this kind of evaluation.

J. B. Hutchinson

* Lawes Agricultural Trust. Rothamsted Experimental Station—Report for 1962. Pp. 316. (Harpenden: Rothamsted Experimental Station, 1963.)

RADIO RESEARCH 1962

THE report of the Radio Research Board*, under its new chairman, Dr. E. Eastwood, has recently been published. It states that 1962 was an eventful year in the life of the Radio Research Station in so far as its work is now making use of rockets and satellites for exploring the Earth's atmosphere out to more than 1,000 km, and obtaining a better understanding of the dependence of its physical characteristics on the Sun's radiation.

Two successful *Skylark* rocket firings have been made at Woomera, carrying experiments devised at the Station in collaboration with the University of Sheffield, and these are being continued during the present year. By co-operation with the Canadian and American authorities, the ionosphere is now being explored from above as well as from the older established ground observatories. In assisting other universities and organizations in the conduct of experiments in satellites which are relevant to the Station's research programme, the network of tracking stations under its control has accumulated extensive telemetry records on magnetic tapes. So far these records have been sent to the U.S. National Aeronautics and Space Administration for processing, but arrangements are now in hand for the first stage of the processing to be carried out at the Radio Research Station. Liaison with other workers in the United Kingdom is maintained through the British National Committee for Space Research under the auspices of the Royal Society.

In its programme of research on the ionosphere, considerable attention has been directed to exploring the

* Department of Scientific and Industrial Research Radio Research 1962: The Report of the Radio Research Board and the Report of the Director of Radio Research. Pp. iv+26+4 figs.+2 plates. (London: H.M.S.O., 1963.) 3s. net.

D-region (below 90 km), and the lower portion of the *E*-region up to about 100 km, since it is at these heights that the absorption of radio waves of all frequencies mainly occurs, and that the phase and polarization of the lower frequencies is determined. For this exploration of the lower atmosphere, a quasi-stationary pattern is set up by a vertical beam of waves at 200 kc/s, which are reflected back from the ionosphere. A rocket fired up through this pattern measures the characteristics—strength and polarization—of the field, and these depend on the height distribution of the electrons and on the frequency with which they collide with neutral molecules. The field pattern, as recorded on three mutually perpendicular aerials carried in the rocket, is complicated, and attempts are being made to explain the results on the basis of a 'model ionosphere' suggested by theoretical workers in the subject.

The staff of the Radio Research Station have taken full advantage of the opportunity provided by the Canadian 'Top-side Sounder' project to explore the characteristics of the ionosphere from above the height of maximum ionization. The satellite travelling at a height of about 1,000 km carries a complete ionosphere sounding station, and telemetry records have been obtained when the satellite passes over the Station at Slough and the out-stations at the Falkland Islands and Singapore. Some typical results are illustrated in the report, and these, together with others obtained by their Canadian and United States colleagues, have been discussed previously in *Nature* (197, 636; 1963).

For the past two years, oblique incidence sounding of the ionosphere has been carried out over a long-distance path between New Delhi and Slough. As the frequency is raised, the value at which the signal becomes weak or fades out is termed the maximum usable frequency. In this way, the highest useful frequency for a practical communication circuit over this path can be found at any time, and used to adjust the long-term average values which are provided from the prediction charts issued by the Station. Trials have been conducted over a number of paths in co-operation with the various civil and military authorities operating the related circuits. The technique should be found of advantage during the coming period of minimum sunspot activity when the usable frequencies will be severely compressed into a narrow part of the spectrum, and when any information which enables the operator to work outside it will be of great value.

To provide the facilities for the expansion of the research programme on the lines described here, some curtailment has been necessary on a few of the items which have received much attention in the past. The short-term ionospheric forecasting has been reduced to the provision of an ionospheric index for use with the long-term world charts which are now available for each of the 24 h. The information in these charts is based on the knowledge and experience obtained at the Radio Research Station in studying the characteristics of the ionosphere over more than two sunspot periods. The main programme of radio noise measurements, which was in progress for some ten years before the International Geophysical Year of 1957–58 and has continued since, has now been terminated. Finally, the preparation for publication of the *Abstracts and References* to radio literature, which has been the responsibility of the radio staff of the Department of Scientific and Industrial Research since 1926, was discontinued at the end of 1962, in the knowledge that the field surveyed was becoming increasingly covered by other abstracting services. Acknowledgment from users of the *Abstracts and References* is paid to the small section of the staff who, over the period of thirty-six years, devoted themselves to this work with unusual enthusiasm and energy.

Other features of the director's report show that he and his staff are maintaining and improving the high traditions of the Radio Research Station in advancing knowledge in the field of radio science in step with the contemporary progress being accomplished by the launching of satellites for space research. R. L. SMITH-ROSE

TELEMETRY APPLIED TO ENGINEERING AND MEDICINE

A MEETING was held at the Institution of Electrical Engineers on August 8 in connexion with the first International Telemetering Conference, which will be held at the Institution during September 23–27.

The chairman of the U.K. Organizing Committee, Dr. J. S. McPetrie, outlined the rapid growth of telemetry, the science/technology of measurement at a distance. He explained that the term telemetry applied to all cases in which the information from any form of measurement was transferred to some point other than that at which the measurement itself was made. This means that the measurement has to be made by a special type of equipment, known as a transducer, which transforms the result of the measurement into a form, usually electrical, suitable for transmission to the remote receiving point where the reading is required. Telemetry assumed great importance towards the end of the Second World War with the emergence of guided weapons and later satellites and space vehicles. In the development of such complex equipment, in which reliability of performance is of paramount importance, it is essential to know at all times how the various component parts are operating, and for this purpose some form of telemetry is essential. Telemetry, of course, is not limited to the fields of weapons and satellites but has many civil applications in which British scientists have played a leading part. All these fields will be covered by papers to be presented at the Conference.

The chairman then introduced Mr. Pruss, a member of the North American Organizing Committee. Mr. Pruss gave a brief account of the various conferences on telemetry which have been held annually in the United States since 1951. The committee responsible for these conferences, realizing that much work on the subject had been carried out in countries other than the United States, considered that the time had arrived when a conference of a more international character would be extremely useful. It was suggested, therefore, that such a conference should be held in Europe, preferably London. The Institution of Electrical Engineers and the British Institution of Radio Engineers agreed jointly to sponsor this conference. The international character of the forthcoming conference is indicated by the fact that the 500–600 delegates expected will come from some seventeen different countries, including the U.S.S.R., Poland, Australia, Japan and South Africa, while 59 papers will be presented by authors from the United States, the United Kingdom, Canada, Belgium, France, Australia and Japan.

Dr. R. H. Barker, who is chairman of the Programme Committee, next gave a brief summary of the scope of the papers to be presented at the Conference. It opens with an introductory session, at which three papers will be discussed. Two of these deal in a general way with the present state of the art of telemetry, while the third paper outlines the history of the extensive use of telemetry

in the United Kingdom by the electricity supply authorities.

In the second session, on "Industrial Systems", eight papers deal with such topics as the part telemetry has played in the control of a 2,300-mile pipeline for natural gas as well as in an investigation of the vibration of blades in a large turbine. The third session is on "Transducers" of different types, while the fourth session is devoted to "Geophysics and Biomedicine". This latter session should be of particular interest to the layman, as one of the papers describes experiments made using minute radio transmitters, so-called radio pills, in the human body for the diagnosis of disease. Prof. Stewart Mackay (United States), the author of this paper, was present at the meeting and gave an interesting summary of his research work.

The next three sessions, on "Recording", "Data Processing" and "Error Detection", are for the specialist, and with more than twenty papers on these subjects lively discussions are to be expected. The last session, the eighth, is on "Aerospace Systems". As this is a subject of the greatest topical interest, a whole day has been allowed for presentation and discussion.

One of the main functions of such a conference is to bring together for discussion and interchange of information in the laboratory workers in the particular field under review. For this reason, a number of visits of a technical nature have been arranged, and reference to them was made by Mr. J. Langham Thompson, who is chairman of the Visits Committee. Telemetry, he said, plays an important part in the remote control of electric power stations. Delegates will be able to see this on a visit to the main grid control centre of the Central Electricity Generating Board at Redbourn, near St. Albans. They will also be able to visit the Research Laboratories of the Central Electricity Generating Board, Leatherhead. For those interested in the medical aspects of telemetry, visits have been arranged to the National Institute of Medical Research at Mill Hill and the Medical Research Council Bioengineering Laboratories at Hampstead. Oceanographers will be able to see the work at the National Institute of Oceanography, Godalming, Surrey. Telemetry techniques are of great importance in coal mining and train signalling, so visits have also been arranged to the Mining Research Establishment, Isleworth, and the British Railways Track Control Centre, Hackney Downs. The various methods for tracking satellites and the procedures used for analysing the complex data from world-wide observations of orbiting satellites will be seen on a visit to the Radio Research Station, Datchet.

Immediately before the meeting, a cable was received from the United States offering to make available from the Pacific Missile Range in California a 'Constellation' aircraft fully instrumented as a flying laboratory and including telemetry equipment. Dr. McPetrie expressed the hope that the aircraft could be accommodated at the Royal Aircraft Establishment, Bedford, where a visit has already been arranged for those delegates interested in the flight testing of aircraft, one of the most important fields in the application of telemetry.

In answer to a question, Dr. McPetrie explained that the papers presented at each session would not be read by their individual authors but summarized as a group by a rapporteur who would be a specialist in the subject. With this procedure, it was hoped that more time would be available for discussion in which each author would have an opportunity to participate.

During the period of the Conference, an exhibition of telemetry equipment will be held at the Hilton Hotel. A shuttle coach service will be run between the Conference at the Institution of Electrical Engineers and the Hilton Hotel for the convenience of the delegates. A smaller exhibition illustrating particular points in the papers presented at the Conference will be arranged at the Institution.

J. S. McPetrie

THE MUSEUMS ASSOCIATION

THE sixty-ninth annual conference of the Museums Association was held at Newcastle upon Tyne during July 22–26 by invitation of the City and King's College and under the presidency of Sir Trenchard Cox, director of the Victoria and Albert Museum, London.

The conference opened with a welcome from the Lord Mayor and the Rector of King's College, followed by the presidential address. In this, Sir Trenchard naturally referred to the outstanding *Survey of Provincial Museums and Galleries**, recently issued by the Standing Commission on Museums and Galleries. He also stressed the importance of a definite policy for purchases and referred to the several building projects which are now engaging the activities of architects and directors.

The Rt. Hon. Sir Edward Boyle, Minister of Education, also addressed the delegates and especially referred to the importance of the various museum services for schools which now form such a prominent part of the museum movement in Britain. On many occasions he had witnessed the appreciation of the teaching profession for the services rendered. Referring to the *Survey*, he hoped that it would be implemented when Britain's economic position made this possible. He felt that teacher training colleges should include courses on the use that the profession could make of their local museums.

The main part of the conference naturally directed attention on the *Survey*, and delegates were favoured with the presence of the Earl of Rosse, chairman of the Standing Commission. The Hon. Nicholas Ridley and Mr. Kenneth Robinson also spoke and gave support for the main recommendations of the *Survey*, though the former was in favour of admission charges to museums. An informal vote emphatically rejected this suggestion. Mr. Robinson felt that poor museums discredited the movement and hoped that the Standing Commission would have made definite proposals on this subject.

In general, it can definitely be stated that the Conference was in general agreement with the recommendations of the Standing Commission, especially as regards the emphasis it placed on the future part to be played by the Area Museum Councils.

Members welcomed the information, given by the Countess of Albemarle, that the Carnegie Trustees hoped to continue their support for museums during the next quinquennium (1965–70).

At the annual general meeting, the conference agreed to new regulations regarding the award of the Diploma of the Museums Association whereby graduates will be exempted from the intermediate examination. The final examination will be taken by all candidates and include a thesis and a practical examination. It is hoped that revised regulations for the Technical Certificate will be ready for next year's conference, for which Dr. D. Dilwyn John, director of the National Museum of Wales, was elected president.

Social functions included receptions by the Lord Mayor and the Rector of King's College, visits to local museums and tours to the Roman Wall and Farne Islands.

F. S. Wallis

* Standing Commission on Museums and Galleries. *Survey of Provincial Museums and Galleries*. Pp. 302. (London: H.M.S.O., 1963.) 30*s*.

WIND EFFECTS ON BUILDINGS AND STRUCTURES

SOME three hundred scientists and engineers representing twenty-one different countries gathered recently at the National Physical Laboratory, Teddington, for an International Conference on Wind Effects on Buildings and Structures which had been organized by the Laboratory in collaboration with the Building Research Station, the Institution of Civil Engineers and the Institution of Structural Engineers.

In view of the extent to which the safe and economic design of all forms of building and structure is dependent on a proper understanding of the effects of wind, it is perhaps surprising to learn that the Conference at Teddington represented the first major attempt in this field to bring together the research worker and the practising engineer. Most of the twenty-four papers presented at the conference were concerned with the wide range of research at present in progress at various establishments throughout the world and, naturally enough, much reference was made to the use of the wind tunnel as the chief research tool. However, it was encouraging to hear that with the development of new techniques and instrumentation it is proving less difficult than in the past to obtain measurements of wind loading on full-scale buildings.

The Conference was organized into six sessions each under the chairmanship of a distinguished engineer or scientist and each covering a different aspect of the problem. The first session, at which Sir Gordon Sutherland, director of the National Physical Laboratory, took the chair was restricted to the official opening and to an introductory paper by Mr. C. Scruton of the National Physical Laboratory. In his paper, Mr. Scruton reviewed the present position with regard to wind effects on buildings and structures. He directed attention to the fact that the terms of reference of the conference were restricted to the static and dynamic stability of structures and of structural members in wind and excluded such effects as the generation of noise, the promotion of fire and the penetration of rain. With the foregoing exceptions, the action of wind was classified into the static and dynamic effects; the former referring to the steady (time average) forces and pressures tending to give the structure or its component members a steady displacement and the latter to the tendency to set the structure oscillating. Mention was made of the Tay Bridge disaster of 1879 which prompted the engineers to make allowance for the wind in the design of the Forth Railway Bridge. He went on to consider the subjects of design wind speed, determination of the shape and pressure coefficients and the oscillatory effects of wind. Under each heading he stated the problems involved and demonstrated the part played by the individual contributions of the various authors to the understanding of the subject as a whole. In conclusion, a plea was made that the conference would stimulate a movement towards better liaison between the interested organizations in the various countries so that research could be co-ordinated and the results disseminated on a wider and more systematic basis than had been possible hitherto.

In session 2 the first two papers were both concerned with the structure of the wind. Mr. H. C. Shellard of the Meteorological Office (Britain) indicated some of the problems involved in making wind measurements and discussed the factors influencing the application of such measured data to the design of buildings and structures where the site conditions might be very different from those at the meteorological station where the measurements are actually made. Prof. A. G. Davenport (Canada) developed this theme further by introducing the concept of referring design wind speeds to the gradient height and relating the speed at lesser heights by factors depending on the terrain at the site of the structure. The statistical properties of turbulent wind were also discussed both in terms of the spectra and of the return period for the maximum wind speed. The probability theory used in his analysis of wind data yielded increased wind speeds as the life period for the structure is increased.

Papers by Mr. C. W. Newberry (Britain) and Dr. R. Pris (France) were both concerned with the measurement of wind pressures on full-scale buildings. The authors discussed the problems of instrumentation which had hitherto prevented comprehensive full-scale studies.

Session 3 was devoted to a discussion of the wind-tunnel techniques used for the investigation of wind effects on buildings and structures. The shape and pressure coefficient data in common use have been derived almost entirely from wind-tunnel experiments so that it is essential that such experiments should reproduce as closely as possible the behaviour of full-scale buildings and structures in the natural wind. A technique for reproducing the vertical gradient of the speed of the natural wind was described by Mr. N. Franck (Denmark). This was a practical interpretation of Jensen's model law and consisted of the application of coatings to the wind-tunnel floor ahead of the model with a variety of roughnesses designed to reproduce the gradient and turbulence properties of wind approaching over different types of terrain. Prof. W. D. Baines (Canada) reported on his work on the effects of wind gradient on tall skyscraper buildings and showed how, in the presence of a wind gradient, air currents can be induced to flow down the front faces of such buildings to give strong winds at street level. The wind-tunnel studies at present being undertaken in France and Belgium were presented in papers by Dr. R. Pris (France) and M. P. E. Colin and R. D'Have (Belgium) respectively. Both papers described work carried out in open-jet wind-tunnels using models mounted on ground plates. The last paper in this session, entitled "Model Simulation of Wind Effects on Structures" and presented by Mr. R. E. Whitbread (Britain), summarized the approach to wind-tunnel testing of models which has been adopted at the National Physical Laboratory. As well as discussing the effects of wind gradient, the paper introduced the concept of representing the dynamic properties of structures on models of reduced linear scale so that both the static and dynamic behaviour of a structure could be examined in the wind tunnel. The methods were illustrated by reference to an exhibition of models and techniques used at the National Physical Laboratory. The models included one of the General Post Office Tower for Central London and another of a 400-ft. office block. A further consideration introduced into the paper was that of the errors introduced into force and pressure measurements made on wind-tunnel models by an interference effect known as wake blockage. It was pointed out that this effect was of particular significance for tests on building models owing to the wide wakes generated by such models.

From meteorological observations and wind-tunnel investigations come most of the data used to formulate the wind loading clauses in the codes of engineering practice for building. With the previous discussions of both these aspects it was appropriate that papers on codes of practice should follow. These were provided by the papers of M. N. Esquillan (France) and Dr. M. Jensen and Mr. N. Franck (Denmark), who described the proposed revisions of the codes of practice for their respective countries.

The second half of this session introduced a different note into the proceedings which was to persist throughout the remaining papers, namely, the dynamic response of

structures to wind. The present codes of practice have been based on wind-tunnel test data obtained under steady wind conditions; whereas the data so obtained are applied to structures subject to the natural wind which can be both highly turbulent and continually varying in speed and direction. The assumption of a steady wind for design purposes implies that the fluctuations in speed and direction that do occur in the natural wind are so irregular and random that the structure does not respond to them any differently from its behaviour in a steady wind of the same average speed. This assumption was, however, challenged by Prof. A. G. Davenport (Canada). His paper on the buffeting of structures by gusts presented the results of an analysis of the response of a flexible structure to horizontal turbulence and showed, at least for structures of low natural frequency, that the deflexions produced can sometimes exceed the effect of the equivalent steady wind of the same average speed. In a second paper on the same subject, Mr. R. I. Harris, of the Electrical Research Association (Britain), described work with a similar approach which formed part of an extensive but not yet complete study of the special problems of gust effects on very tall transmission masts.

Both sessions on the final day of the Conference were devoted to problems of the oscillation of structures due to the action of steady winds. Since the collapse of the first Tacoma Narrows Bridge in 1940 designs for long-span suspension bridges have been subjected to extensive wind-tunnel testing prior to their erection. Even to-day, 23 years after the disaster, it is still desirable to carry out tests on models to ensure the stability of a particular design. Mr. D. E. J. Walshe (Britain) described the techniques which have been developed at the National Physical Laboratory for this type of testing. He discussed the use of spring-mounted models of typical sections of the suspended roadways and the requirements for such models to be representative of the complete bridge. Papers from the United States, Norway and France discussed the techniques used in these countries and demonstrated the international aspect of this particular problem.

Aerodynamic oscillation featured yet again in session 6, the final session of the Conference, when another problem of international interest, namely that of the galloping of electric power transmission lines, was the subject of four successive papers. The term 'galloping' has been used to describe the large amplitude oscillations which can be set up in transmission lines by wind. The cause can usually be attributed to certain de-stabilizing characteristics of the steady wind forces which often prevail when the transmission lines become coated with ice. In this session papers from Canada and the United States predominated. However, a paper presented by Mr. D. J. W. Richards of the Central Electricity Research Laboratories (Britain) described a case of galloping which occurred in non-icing conditions on the 5,310-ft. span of the 275-kV power crossing of the Rivers Severn and Wye. Wind-tunnel tests on sections of the conductor showed that the instability arose from peculiar aerodynamic effects associated with the lay of the outer layer of the conductor strands. Wrapping the conductor with polyvinyl chloride tape to produce a smooth profile was found to be an effective method of introducing stability to the crossing.

Slender structures with low natural frequencies such as stacks, towers and masts have a tendency to oscillate in response to the shedding of vortices at the natural frequency of the structure. That the vast majority of such structures do not oscillate can be attributed to the fact that they possess sufficient inherent structural damping to cancel out the aerodynamic excitation effect.

Modern methods of construction, although perhaps more economical and structurally efficient, tend to decrease the amount of structural damping and hence increase the liability to wind-excited oscillation. In his paper on this subject Mr. C. Scruton (Britain) mentioned these effects, and others, which influence the aerodynamic stability of stacks, towers and masts. The use of strakes with sharp-edged rectangular section wound as helices around the surface of cylindrical sections had proved to be a most effective device for the prevention of vortex-excited oscillations. A similar method was described in a paper by Professor Nakagawa (Japan). He had adopted a system of wire ropes as strakes, for application to tall stacks of welded steel construction in Japan. However, his advocation of the use of wire ropes appeared to be in disagreement with the statement made by Mr. Scruton in his paper that rounded strakes had been shown to be less effective than those of rectangular section.

R. E. Whitbread

INFORMATION *PER SE*

By Dr. PAUL S. HENSHAW

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RECEIPT and use of information is a feature in all living systems.

The information used by living systems is of three types: inherited, acquired and generated. Inherited information is that brought into organisms at the moment of conception; acquired is that brought in continuously during life by means of the sensory shield; and generated is that formed inside the system by interaction.

The means by which acquired information is received and developed varies widely among species.

Systems in organisms for processing acquired information have undergone evolutionary change comparable with that which has occurred in systems having to do with other functions.

Along with ability to detect and transduce stimuli, and along with ability to transmit information laden impulses, has come an increased ability to comprehend, to understand and to know, also an increased ability to imagine, feel, evaluate and make judgments. Acquisition of the more specialized information processing capabilities, and of the greater emotional capacities, have coincided with elaboration of the cerebral cortex.

In the human species, that is, the species considered to have the most advanced cerebral cortex, the ability to develop and utilize information greatly transcends that of other species. The human mind—the means by which we think—can be regarded as the most highly elaborated product of evolution.

Information is an entity apart from the means by which it is processed, the symbols by which it is represented, or the responses made to it. Messages contain intelligence (dispositional, organized, usable and available information), but as such do not themselves constitute intelligence.

Information can be transmitted and stored by means of impulses and symbols, but it exerts an influence only after removal from the encoded message and interaction with other information, as can happen in a cell, a brain or a computer.

Information can be regarded as existing in pieces or concepts, but probably not as units. Information, as such, is not amenable to quantitation in a manner comparable with that which applies to entities having force, mass or colour.

In last analysis, information consists of data, especially unrelated facts and statistics. With organization of data, intelligence is created and with further organization of organized data, intelligence is developed and enhanced.

Intelligence, like life, is a manifestation of sustained interaction. Each is associated with matter and fully dependent on it. Each, nevertheless, occupies a place apart from or beyond matter. Remove intelligence or life, or both, and the residue of constituent elements remains unchanged.

Life and intelligence are dispositional manifestations. Each consists of information processing carried along by energy systems utilized as vehicles. Life, as we tend to define it, is dependent on wet (protoplasmic) systems, whereas intelligence can exist and build in either wet or dry (hardware) systems.

Life and intelligence involve problem-solving processes with survival of organisms, or of ideas, as automatic and inherent goals. Both life and intelligence consist of self-organizing activities realizable only by means of the vehicles. Moreover, the thoughts evolved and the conclusions reached, if implemented, are implemented by means of the same vehicles in each instance. In final analysis, life and intelligence become reduced to interaction at the molecular level—interaction which provides opportunity not only for channellized energy utilization, but also for information storage and retrieval (memory), and for information and idea interaction (thought).

Early in the course of evolutionary development, as sustained energy processes acquired characteristics of life, there existed in the chemical sense a means and opportunity for receiving and utilizing information. With incorporation of particular molecular configurations in such systems, genetic information was acquired and particular classes of responses to the environment were ensured.

With development of DNA, a means was provided for replication and transmission of genetic information—and thereby opportunity for deterministic cycling of life, as done generally in plants.

With addition of neurones and neuronal networks, opportunity was provided for probabilistic information processing, that involving relevancy screening, judgments and decision-making, leading to purposive responses.

Elaboration of the body, of course, provided better vehicles—those which by means of sense organs and nervous systems enabled greater awareness of the environment, and which by means of musculature could make more meaningful responses to the environment.

More recently (in evolutionary time), with elaboration of the cerebral cortex, capabilities for feeling, abstraction, communication and organization have appeared.

It is noteworthy that in Nature, so far as is known, intelligence is associated only with living systems, and that ability to contemplate intelligence is associated mainly, if not altogether, with the human species. Since it is demonstrated that intelligence can generate when matter becomes organized in particular ways, there is little reason for expecting that intelligence is restricted to wet systems, such as known on Earth, or that the intelligence systems on the Earth may not change substantially or new ones form.

Both life and intelligence are modulated by information —inherited, acquired and generated. Beneficially adaptive use of information is an underlying aspect of each.

Intelligence is the substrate out of which ideas and concepts are formed. Ideas, like living things, are born; they undergo selection; and, in time, they may die.

In the physical world, change occurs in accord with natural forces; such change is compatible with reason and, therefore, said to be 'rational'.

In the sub-human living world, change is in accord with organic evolution—struggle for existence and survival of the fittest; this appears 'natural' and likewise rational.

In the world of human beings—that is, in relation to the species presumed to have the greatest capability for acquiring and utilizing information—change, as revealed by interaction, decision and behaviour, very often is not in accord with intelligence, reason or rationality. Honesty, pride, custom, dogma, principle, superstitition, deities, ethics, propriety, integrity, affection, virtue, honour and humour are features associated mainly with human beings; they involve rationality in the 'organic' and 'natural' sense only to a small degree.

In the world of human beings, perversity, morality and emotion are characteristics to be taken into account along with superior abilities in comprehension and understanding. These are attributes which, in particular, make the human species stand out as different from all others.

Perversity, as a characteristic of human life, is so strong that there is question as to whether the human species would long endure in a completely rational and Utopian world—one of perfection, such as is usually held up as the goal of human effort. There is thus the matter of the organizing power of informational elements beyond those regarded as strictly rational.

The fertilized ovum carries with it a legacy of genetic information—a 'blueprint' with a pattern for life to be read off in time. Continuously, the fertilized ovum or the growing organism, as the case may be, receives information from the environment which alters and helps determine the course of life.

Ideas, decisions and temperament, arising as they do from the interacting elements of stored and continuously received information, are largely the consequence of information previously accumulated, that is, of a conditioning derived from training and previous experiences.

There exists the group or mass mind as well as the individual mind. In the group, the same as in the individual, the kinds of opinions formed and the kinds of decisions reached are determined by the character of the available information, including disbeliefs as well as beliefs, including inaccurate, distorted and biased information as well as accurate and, indeed, including all the incorrect and correct elements of information programmed in to mould the ethical standards, to define the personality, and thus to form the id.

It is customary to speak of the 'soul' of a man, or the 'soul' of a people. Without implication of an entity beyond death of the body, such a term has usefulness here. In the same way that Japanese speak of the soul of a sword, meaning the metal as tempered for stamina and ability to cut through, so also can one speak of the soul of a man or of a people, meaning tempering, stamina and ability to cut through. The soul of a man or of a people can be regarded as the combined total personality, capability and emotionality.

The 'soul', it is submitted, is forged and tempered by the conditioning which results from the interaction of available information (genetic, acquired and generated) during the course of life. Additionally, it is submitted that the ability to feel—to experience emotionally—results from the information organizing aspects of the same interactions.

Since the inherited information that comes into the embryo has to do with capacity for learning more than with learning itself, it follows that what a man becomes to delineate him as man, and thereby the society, is determined largely by the soundness of information programmed in during life.

Rearing and training—the general educational conditioning primarily responsible for character determination —are matters over which man himself exercises the power of discretion.

Because of know-how already available enabling control of disease, development of resources, increase in productivity, management of populations, improvement of heredity, and designation of needed ethical standards, the human species could have for itself almost any standard

and level of living it would care to specify. Despite this favourable situation, however, it is evident that feelings of apprehension, fear and insecurity are intense and widespread.

In the period immediately ahead, it seems necessary to predict, man's greatest challenge will be that of how man will himself deal intellectually with his own intellectual achievements.

In our time—the period of the present generation—the emergence of nuclear and space developments have been seen and their impact felt. Now 'information science' is bursting on us, and while information science is not being heralded by events as spectacular as the detonation of bombs or the orbiting of satellites, its influence, it appears likely, will have an impact fully as great, and perhaps much more so.

'Cybernetics' is a term used in referring to the various fields and extensions of information science (or 'mind science') alluded to in the foregoing paragraphs. 'Heuristics' is another, and quite recently cyberculture[1] has been used. Cybernetics is defined as the 'science of control and communication in the animal and the machine'; heuristics involves the same considerations and extends to include problem-solving by means of search and discovery operative within the system itself; and cyberculture purports to apply the principles of cybernetics and heuristics in dealing with the operation of societies.

Despite the different points of emphasis, the theme of information science remains the same. It is how systems, living or non-living, regulate themselves, reproduce themselves, learn and evolve. It is how strategy and tactics are used in decision-making relative to rules and experience. Central is the question of how the various homoeostatic and reflexive mechanisms become goal-directed and self-adapting.

Governance is the point of departure under cybernetics, whereas under heuristics it is self-organization.

From the point of view of heuristics, there is emerging a mathematics and a practical circuitry which show how, with an adequate vehicle, information processing can be generative and beneficially adaptive with respect to specific goals—be they inherent (as in life processes), programmed in for a particular purpose (as in the case of computer analysis), or evolved (as occurs when complex problem-solving is done by the human mind). Heuristics portends a whole new science, and one with a bright and significant future.

With cybernetic and heuristic techniques, it is becoming possible to analyse the problem-solving strategies used by different human and animal brains and to see how these match with the training, personality and physical condition of individuals and species. By means of these techniques and the utilization of newer computer facilities, analysts are able to deal systematically with complex arrays of variables and thus to characterize the organizational and disorganizational forces operative in life processes and in social systems.

Because of prospective information storage and retrieval systems, combined with existent telecommunications facilities, there is early likelihood of bringing total accumulated knowledge (such as housed in the great libraries) to bear on a single problem or concept, also, for a single mind to probe all recorded subject-matter in selected fields, irrespective of location or language, and to consider the results of analyses quite beyond the capabilities of the human mind.

By means of evolving prosthetic devices intimately linked to human nervous systems, it is becoming possible for man to receive sensory stimuli directly from outside sources and to implement thoughts directly through machine devices, continuously and at great distances, also continuously in hostile or otherwise inaccessible environments (space, high-intensity radiation fields, extreme temperatures, microscopic dimensions, etc.).

Because of these various developments, there is the prospect that human minds and intelligence systems of various types can be combined in such a way as to interact as one great thought facility, doing so without the vagaries of language symbols. Indications are, therefore, of entry into realms of information utilization, concept formulation, analytical computation and emotional experience as yet not even imagined.

We are aware of how elaboration of the cerebral cortex provided a capability for intelligence that has set the human species apart, also that this was accomplished by means of organic evolution over a period of almost countless millennia. Now we are witnessing a transition fully as great, it would seem, but one taking place in an interval of time which, by comparison, is but a twinkling.

Leadership and an earned position of international public trust will accrue to the people most successful in meeting the challenge of information *per se*.

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POSSIBLE ORIGIN OF THE LUNAR WALLED PLAIN PTOLEMAEUS

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PTOLEMAEUS, centrally located near the eastern border of Mare Nubium on the Moon, represents the northern member of a meridian alignment of craters or walled plains consisting of Walter, Regimontanus, Purbach, Arzachel and Alphonsus. It is about 159 km in diameter and about 2·4 km deep; the rim is hexagonal, and approximately level with the mean exterior surface. A light grey material on the floor covers numerous craters. These contrast sharply with younger, undamaged ones, many of which are superimposed on submerged features. Evidently, Ptolemaeus is a transition feature displaying characteristics of both the true craters and the circular maria.

The Ptolemaean floor features have been described by Alter[1] and Arthur[2], and regional tectonic analyses have been reported by Spurr[3] and von Bülow[4]. In the work recorded here the tectonic interrelations between the features in Ptolemaeus and the grid lineaments in the adjacent terræ have been investigated. The results favour the hypothesis advanced previously[1,3,5] that Ptolemaeus was formed by the collapse of a large segment of the crust during a degassing of the Moon's interior.

A generalized map of the regional grid systems and the pertinent Ptolemaean floor features (Fig. 1) was constructed from Plates 8, *C5-a*, *C6-a*, *-b*, *-c*, *-d*, *D5-a*, *-b*, *-c* in the *Photographic Lunar Atlas*[6] and from several photographs obtained through the courtesy of Dr. D. Alter. Fig. 2 shows a reproduction of plate *D5-a*. The nature of the lunar grid comprised of the Imbrium radial and the parallel systems has been examined by Spurr[3], von Bülow[4] and Fielder[7]. Arrays of ridges, valleys, rills, faults, and crater chains define the grids which extend along four or possibly five preferred directions. The valleys and ridges oriented S. 15° W. and S. 25° W. represent the Imbrium radial system, and the three lineaments oriented S. 55° W., S. 20° E., and S. 55° E. constitute the parallel system. The walls of Ptolemaeus and many of its interior features conform to these directions

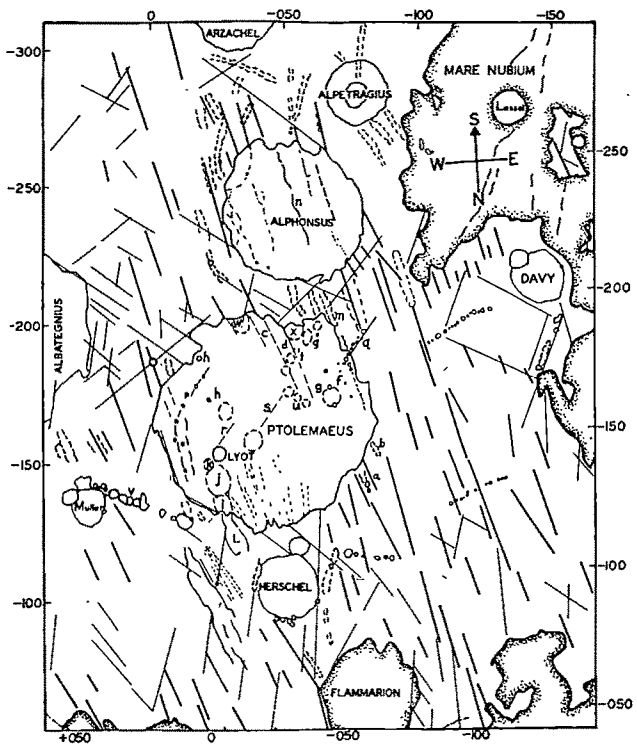


Fig. 1. Generalized tectonic map of Ptolemaeus and vicinity. Selenographic co-ordinates are those shown on Plate *D5–a*, in the *Orthographic Atlas of the Moon*

——, Imbrium radial lineament; ——, parallel grid lineament; ~ ~ ~, mare-type ridge; – – – – –, rill; ○, ghost crater; ○○○, probable coalescing craters

and, in some instances, are continuous with the grid lineaments of the adjacent terræ.

Imbrium Radial System. This system of valleys and ridges radiates from the border of Mare Imbrium for hundreds of kilometres and predominates in the vicinity of Ptolemaeus. The valleys have been identified as either grooves cut by ejecta from an Imbrium collision[8,9] or as grabens which resulted from tectonic processes during the formation of Mare Imbrium[3,4]. The valleys are oriented about two preferred directions which diverge by 15° and hence are not likely to have resulted from projectiles originating at Mare Imbrium. Some of the valleys represent distinct chains of coalescing craters such as those identified by *a* and *b* in Fig. 1. Frequently, the valley walls are scalloped at their intersection with the rims of the large craters. On the southern rims of Ptolemaeus and Alphonsus several scalloped valleys are arcuate at either their northern or southern ends. Although the linear portions of these valleys parallel the radial system, the arcuate segments deviate by 25°–40°. The departure from the radial direction, most pronounced at the northern terminus, suggests that the valleys may have been caused by internal activity along fracture zones.

On the southern floor of Ptolemaeus two shallow, apparently submerged valleys (Fig. 1, *c*, *d*) parallel the radial system and connect with re-entrants in the southern wall. Inside the valleys, which are approximately 50 km long and about 6 km wide, several ghost craters can be distinguished. On the north-western floor two parallel ridges (*e*) appear to outline a submerged valley or a chain of coalescing ghost craters. These shallow valleys may be the submerged equivalents of the adjacent terræ valleys.

A ghost chain (Fig. 1, *i*, *j*, *k*) on the northern floor parallels the radial system and merges with the prominent valley (*L*) which intersects the northern wall. In this area internal stresses may have caused the formation of a rift valley along which the ghost craters originated. The ghost crater (*i*) joining the terræ valley, and the two semicircular ghost rims (*w*, *x*) abutting against the southern wall, appear to have been bisected by faulting along the inner walls of Ptolemaeus. There, subsidence may have displaced the submerged craters to their present low position. The crater Lyot joins the row of ghosts about a point at which its western rim coincides with the eastern wall of the valley. Since the western rim of Lyot is some 180 m lower than the eastern rim[10], renewed activity along this fracture zone may have caused a slumping of the western rim.

A rill paralleling the eastern wall merges into a chain of at least three small craters and one of three large, coalescing craters along the base of the eastern wall. The latter chain aligns with a scalloped valley (Fig. 1, *m*) which intersects the southern wall. The entire group of features parallels the radial system and is-aligned with the central ridge (*n*) of Alphonsus. The interrelationship among the various dissimilar structures indicates that they may have coincided with a major zone of weakness associated with the radial system. Hence, the rill can be identified as a deep-seated fracture rather than as a superficial cooling crack. In addition, the three post-Ptolemaean crater alignments (*f*, *g*, *h*) and the eastern and western walls parallel the radial system.

A set of narrow valleys radiating from the young crater Herschel on to the floor of Ptolemaeus provides further evidence in support of a tectonic development of Ptolemaeus and of the regional Imbrium radial system. These valleys form a fan-like pattern the major axis of which parallels the Imbrium system. They resemble closely the sets of radial grooves near Aristillus and Aristoteles described recently by Fielder and Warner[11]. On the basis of semi-quantitative considerations, they have interpreted the grooves in terms of narrow rift valleys which resulted from the local enhancement of pre-existing, regional tensile stresses during the time of formation of the parent crater. Analogously, regional tensile stresses may have been present in the crust at the time Herschel originated. The regional stresses most likely assumed the same orientations since the central axis of the fan of valleys parallels the Imbrium radial system.

The morphology of the Imbrium radial system near Ptolemaeus and its relation to the diverse Ptolemaean floor features demonstrate that the valleys and ridges resulted from tectonic processes. Whereas the initial development of this system probably preceded the

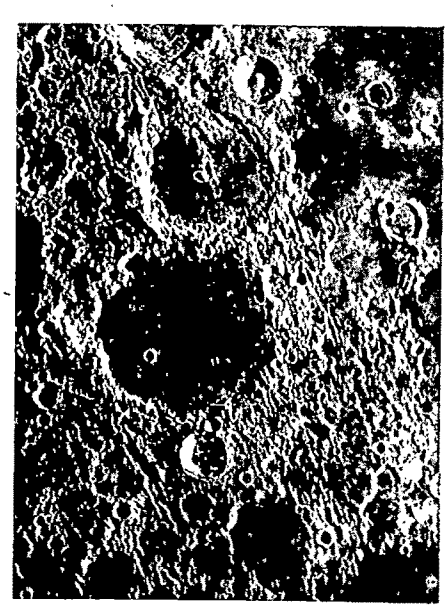


Fig. 2. Nubium N. Mount Wilson Photograph No. 124

inundation of Ptolemaeus, the alignment of the younger post-inundation features with the radial system suggests that the evolution may have continued beyond this stage.

Parallel grid systems. The Ptolemaean features are associated with the parallel grids in a manner analogous with the Imbrium radial system. The directions of the north-eastern, south-western, north-western, and part of the western walls coincide with those of the parallel grids as illustrated in Fig. 1.

On the floor of Ptolemaeus a crater alignment, a crater chain, several ridges, and a ghost chain coincide with the S. 55° E. oriented grid lineaments. The crater chain (Fig. 1, *q*) begins on the floor and continues through a gap in the eastern wall on to the terræ, where it parallels a prominent grid member. It should be noted that the eastern wall is offset at the common intersection of this crater chain with the lineament and the wall. These interrelations are indicative of faulting and possibly of horizontal displacement of the wall. A similarly oriented crater alignment (*p*) occurs on a prominent ridge on the western sector of the floor.

A group of ghost craters (Fig. 1, *t*, *u*) on the south-eastern quadrant of the floor appears to consist of two separate chains related to the north-west–south-east and north-east–south-west parallel grids. The southernmost chain (*t*) aligns with the lineament which forms the north-eastern wall of Alphonsus and with the prominent ridge (*r*), south-east of Lyot, and parallels the ridges (*p*, *s*) and the crater chains (*p*, *q*). The northern ghost chain (*u*) coincides with the north-east–south-west parallel grid in the adjacent terræ and with the north-eastern wall, which is flanked by a prominent crater chain (*v*). These ghosts probably represent submerged crater chains which originated along fracture zones. Alternatively, Dr. Alter has interpreted this group of ghosts as a single, arcuate chain outlining part of a large ghost crater. Both explanations are reconcilable provided the ghosts outline the rim of a large buried crater the north-western and south-western sides of which are bounded by fractures along which the ghost craters originated.

Conclusions. The tectonic relations between the diverse features in Ptolemaeus and the grid systems in its vicinity support the theory of an internal origin for the walled plain. The absence of external walls and the bisected ghost rims joining the inner walls which coincide with the tectonic grid systems, provide cogent evidence that Ptolemaeus collapsed along pre-existing zones of weakness in response to the withdrawal of support during a degassing of the Moon's interior. If Ptolemaeus had resulted from a meteoritic impact, the pre-existing craters would have had to originate after the collision. In a separate study it has been demonstrated, however, that the surface density of the combined ghost and post-lava craters closely resembles that of the craters in the adjacent terræ, and that the formation of many of the ghost craters probably preceded that of Ptolemaeus[12]. Consequently, a large segment of the crust may have been populated by numerous craters before subsidence set in.

Approximately half the number of craters more than 1·5 km in diameter is located on the rims of ghost craters, along the rill and on top of the ridges. This preferential location on structures constituting a mere fraction of the total area disproves a chance occurrence and signifies that many of the post-lava craters may have had an igneous origin.

As stated earlier, Ptolemaeus is one of several aligned, large craters or walled plains located along the western border of Mare Nubium. These aligned structures parallel the major axis of the mare, an admittedly subsided and lava-flooded area. During the subsidence of Mare Nubium, a major zone of weakness may have developed along its western margin together with degassing and collapse of the crust; the Straight Wall attests to renewed tectonic activity along this meridian zone.

The tectonic relationships between the Imbrium radial system and the Ptolemaean ghost features suggest that the formation of Ptolemaeus may have been contemporaneous with that of Mare Imbrium and that its evolution possibly proceeded in stages as follows: (1) subsidence of a segment of crust containing pre-existing structures; (2) formation of craters and related features within the subsided area; (3) inundation of the existing structures by lava and dust; and (4) formation of post-laval craters.

From the results of this investigation it is concluded that the lunar surface features with morphologies resembling those of Ptolemaeus may have had a similar origin.

We thank Dr. D. Alter for several photographs and for advice. This work was supported by a National Aeronautics and Space Administration grant, *NsG*-145-61, with funds administered by the University of California.

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CHEMICAL ETCHING STUDIES AND TRANSMISSION ELECTRON MICROSCOPY OF SILICON CARBIDE

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PREVIOUS investigations have shown that transmission electron microscopy is a powerful tool for the examination of structure and imperfections in metals and semiconductors[1]. For transmission electron microscopy, specimen thicknesses must be of the order of a few hundred Å. In the case of metals and semiconductors, especially germanium and silicon, the reactivity of the materials allows the use of relatively simple polishing techniques to prepare the thin films[2]. No method for the thinning of silicon carbide for transmission electron microscopy has been reported. Some initial investigations performed to develop a chemical polish for silicon carbide are now described. Specimens suitable for transmission electron microscopy were obtained by using molten lead oxide as a chemical polish followed by a final attack by a mixture of chlorine and oxygen. Lead oxide was not

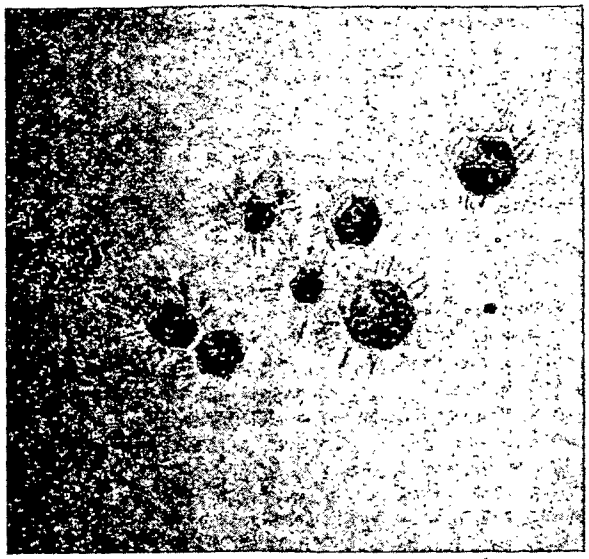


Fig. 1. 'Smooth side' after chemical etching with lead oxide. (Optical micrograph, × 200)



Fig. 3. 'Smooth side' after chemical etching with lead oxide plus lead fluoride. (Optical micrograph, × 500)

only suitable for the preparation of thin films but also had advantages over other etches for the preparation of smooth surfaces and for revealing dislocations.

Chemical polishing and etching. A suitable polishing agent for silicon carbide should have the following characteristics: (1) it should produce a smooth surface; (2) it should give reasonable etch rates at relatively low temperatures; (3) it should allow a controlled, reproducible reaction; (4) it should give volatile and/or soluble reaction products. No etchant was known[3] that would satisfy all these conditions. For example, the decomposition of sodium peroxide, one of the most commonly used etchants for silicon carbide, makes it impossible to obtain strictly reproducible conditions.

In searching for a reactant, lead oxide was found to satisfy all the requirements. The silicon carbide crystals were placed into small pits in a quartz block, and were covered with lead oxide. Etching was carried out at 800° C. Due to the relatively small amount of lead oxide that was used, a high concentration of lead silicates formed. These vitrified on cooling and were difficult to remove. The solubility of the glass could, however, be increased by adding about 5 per cent boric acid or borax to the lead oxide[4]. Hot concentrated potassium hydroxide solution was used to dissolve the reaction products, and this was followed by treatment with hot nitric acid. Alternatively, the bulk of the glass could be transformed into a white powder by boiling in hot concentrated nitric acid.

The rate of etching, calculated as the sum of etching taking place on both faces, was about 100 μ/h. The 'smooth side'[3] of a crystal after etching is shown in Fig. 1, while Fig. 2 shows the 'wormy side'[3] (this surface was obtained on a sample that was sandblasted before etching; see under).

Lead oxide forms a eutectic with lead fluoride[5]. This allowed etching at temperatures where, due to its relatively high melting point, lead oxide in itself could not be used. Thus, etching at 600° C for 1·5 h resulted in no detectable weight change (less than 1·0 per cent weight) although the dislocations were clearly revealed on the 'smooth side' (Fig. 3).

Sample preparation for electron transmission microscopy. Due to the brittleness of silicon carbide, only the centre portion of the specimen was thinned, while the remaining thick outer part served as support. The use of a jet-

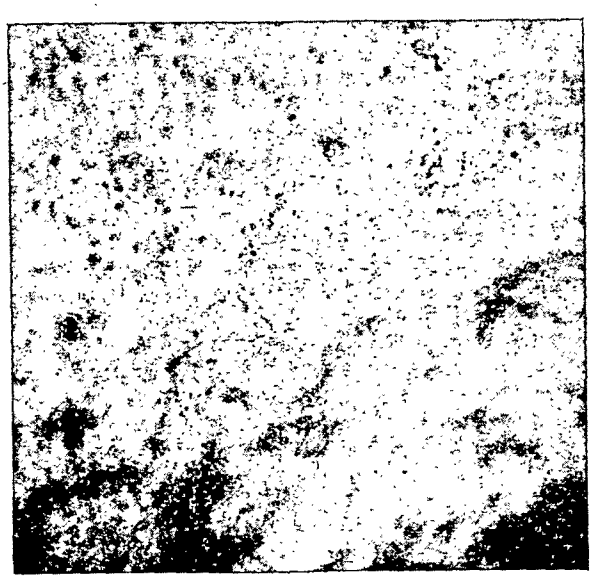


Fig. 2. 'Wormy side' after sandblasting and chemical polishing with lead oxide. (Optical micrograph, × 200)

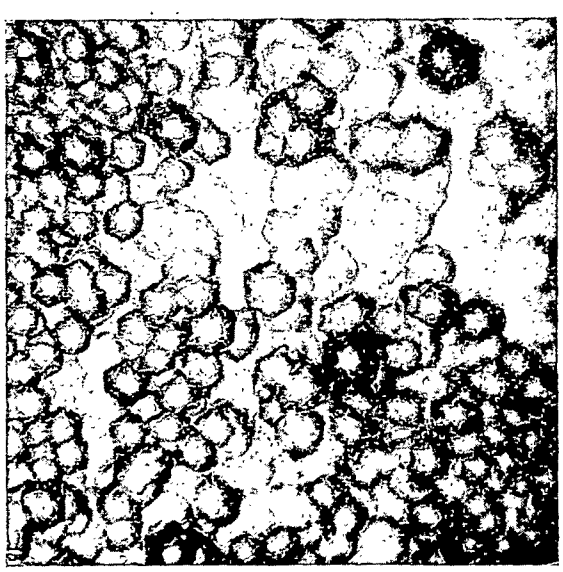


Fig. 4. 'Smooth side' after sandblasting and chemical polishing with lead oxide. (Optical micrograph, × 200)

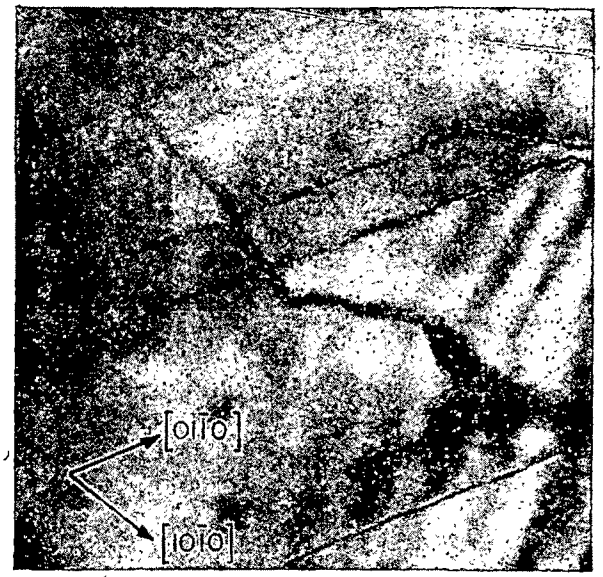


Fig. 5. Grown-in dislocations in silicon carbide film. (Transmission electron micrograph, × *c.* 22,500)

etching technique could not be considered, as no etchant for silicon carbide is known that would be liquid at moderate temperatures[3]. Therefore, an impression was sandblasted into the 'wormy side' of the silicon carbide crystal by using 320 mesh boron nitride powder. The impression was then filled with lead oxide and etching performed at 800° C. It was not possible to obtain a thickness at the bottom of the impression less than about 30μ, since thermal stresses between the lead glass and the silicon carbide film during cooling invariably caused fracturing of the thin silicon carbide portions. Therefore, the glass was removed and the polishing continued with a gaseous reactant.

It has been shown[6] that chlorine containing some oxygen attacks silicon carbide at 1,000° C, the etching rate being on the 'wormy side' approximately 20 times that of the 'smooth side'[6]. Chlorine (10 c.c./min) was passed over the crystals in a quartz tube (25 mm int. diam.) heated to 1,000° C, the outgoing end of the tube being reduced to 10 mm int. diam. along a length of 40 mm. The oxygen was provided by the back-diffusion of air through the open end of the tube. A white film, probably consisting of silicon dioxide, was formed on the silicon carbide crystals. This gave mechanical support to the thin portions of the specimens. After cooling to room temperature, the oxide film could be removed by aqueous hydrofluoric acid. Fig. 2 showed the surface of such a crystal. When sandblasting and etching was performed on the 'smooth side', a pitted surface was obtained (Fig. 4).

A transmission electron micrograph of a silicon carbide single crystal prepared by this method on the 'wormy side' is shown in Fig. 5. Dislocations can be seen as straight dark lines, with their longer portion approximately parallel to a < 0110 > direction in the plane of the film, while the shorter portions are inclined to the basal plane. The irregular broad dark line is due to incomplete removal of the oxide film. It should be noted that during the chlorine treatment no preferential etching attack occurred at places where the dislocations intersect the specimen surface.

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ELECTROLYTICALLY INDUCED CONDUCTIVITY IN RUTILE

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HYDROGEN is known[1] to play an important part in the aqueous corrosion of certain metals. Recently, Leach *et al.*, investigating the aqueous oxidation of titanium and other metals[2–4], have found an apparent increase in the electrical conductivity of the oxide film after the evolution of hydrogen at the oxide surface. They suggest that atomic hydrogen enters the oxide and acts as an ionized donor impurity. This note reports experiments carried out on single-crystal rutile (TiO_2) which confirm the foregoing suggestion and yield more detailed information on the penetration of hydrogen into the oxide.

Single crystal specimens (8 mm × 2 mm × 1 mm) of pure rutile were brought to a suitable conductivity by vacuum reduction and the broad faces ground optically flat and parallel. One of the broad faces of each specimen was coated with gallium and the specimen was then mounted in quick-setting resin, with a copper wire making contact with the gallium-coated surface so as to leave only the opposite face of the specimen exposed. The rutile electrode so formed was immersed in 1 N sodium sulphate solution at room temperature, stirred by a stream of gaseous nitrogen, and a constant cathodic current of 1 m.amp/cm² was passed through it. The field in the sample at no stage exceeded 10 V/cm. The potential of the oxide surface was measured using a Luggin capillary calomel electrode, to ensure that the hydrogen evolution potential was always exceeded. After a given period of hydrogen evolution, the electrode was removed from the solution, the resin dissolved in acetone with the assistance of an ultrasonic agitator, and the gallium contact removed in aqua regia. The specimen was then washed in distilled water, dried, and remounted in quick-setting resin on a hardened steel polishing jig with the cathodically treated face exposed for subsequent conductivity measurements.

Current contacts were made with 'Aquadag' covering the end (2 mm × 1 mm) faces of the specimen and potential probes could be brought into contact with the exposed broad face. After each measurement of conductivity the sample was reduced in thickness by polishing the exposed face with Linde 'A' alumina. The smallest amount of material that could be removed in this way was 1 μ. A conductivity profile was derived from successive measurements of conductivity and thickness. As a check on the homogeneity of the starting material, a conductivity profile was determined with a vacuum-reduced sample which had not been exposed to hydrogen.

Conductivity profiles for several electrolytically treated samples are shown in Fig. 1, the times indicated on the curves representing the time of electrolysis. Anodic oxidation of electrolytically treated samples lowered the

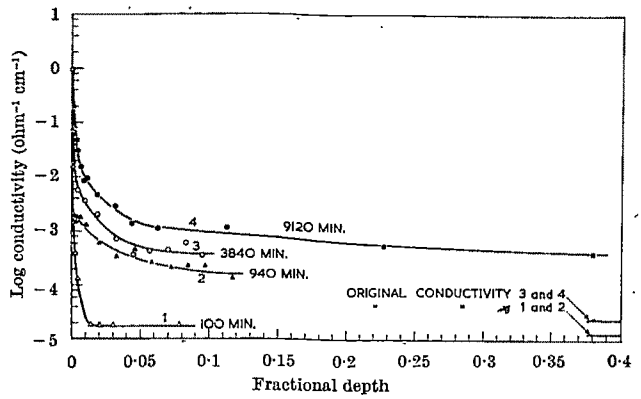


Fig. 1. Conductivity versus depth as a fraction of initial thickness for single crystals of rutile electrolytically treated for the periods indicated. Initial thickness (mm): (1) 1·029; (2) 0·964); (3) 1·081); (4) 0·989

conductivity considerably, but for durations of 1,000 min did not return the bulk conductivity to its original value.

There can be little doubt that the increase in conductivity produced by electrolysis is due to the presence and ionization of atomic hydrogen in the lattice—atomic hydrogen is already known to be a donor in ZnO with an activation energy of 0·04 eV (ref. 5). However, the form of the present results shows that the diffusion is not simple. In the first few microns the concentration falls off rapidly with an apparent diffusion coefficient in the range 10^{-11}–10^{-13} cm²/sec. At greater depths the fall-off is much slower and a considerable and relatively uniform concentration is observed at a depth of 100 μ or greater. In this region the apparent diffusion coefficient is in the range 10^{-7}–10^{-9} cm²/sec.

One interpretation of this behaviour, which may not be confined to rutile, involves a diffusion barrier at the surface resulting from chemical reduction of a thin layer of the oxide by the hydrogen. Surface reduction of this kind has indeed been reported by Thomas and Lander[6] for the case of ZnO in gaseous hydrogen. Such a layer is expected to be disordered, possibly with a high concentration of interstitials[7], and could well have a smaller diffusion coefficient than the bulk. Some evidence for the actual existence of a disordered surface layer was obtained by Prof. M. Blackman and Dr. N. D. Lisgarten of the Imperial College of Science and Technology using grazing incidence electron diffraction on a specimen that had been electrolytically treated for 960 min. The expected spot pattern was observed from the unexposed face while no pattern could be obtained from the treated face. A further point in favour of the surface barrier model is that the concentration deep in the crystal is observed to build up roughly linearly with time.

An alternative interpretation of the results is to regard the long range, fast, diffusion as being along dislocations and the short range diffusion as true bulk diffusion. The dislocation density in these crystals, as revealed by etchpits, is of the order 10^6/cm². Therefore only a small fraction of the evolved hydrogen atoms would be in a position to move directly into dislocations. The vast majority would have to undergo bulk or surface diffusion before reaching a dislocation and many would not reach one in the time-span of the experiment. If bulk and surface diffusion were much slower than diffusion along dislocations this model would account for the form of the present results.

Further experiments are planned to decide between the two interpretations. At this stage it is not possible to say which of the two observed ranges of diffusion coefficient applies to true bulk diffusion. However, it is clear that hydrogen is a very mobile and substantially ionized donor in rutile at room temperature.

It is a pleasure to acknowledge the co-operation of Dr. J. S. L. Leach, whose work on the role of hydrogen in oxidation stimulated this study. Thanks are also due to Mr. A. B. Willott for his experimental assistance. The work was carried out at the Central Electricity Research Laboratory and is published by permission of the Central Electricity Generating Board.

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SOME REACTIONS OF OZONE IN DISCHARGE-FLOW EXPERIMENTS

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THE reaction mechanism of the recombination of oxygen atoms in the presence of molecular oxygen is generally assumed to be[1]:

$$O + O_2 + M = O_3 + M^{-} \quad (1)$$

$$O + O_3 = O_2 + O_2 \quad (2)$$

a small steady-state concentration of ozone being rapidly established. The following kinetic equations can be deduced:

$$-\frac{d[O_3]}{dt} = 2k_1[O][O_2][M] \quad (a)$$

and:

$$[O_3] = \frac{k_1}{k_2}[O_2][M] \quad (b)$$

These equations are not valid if significant amounts of ozone are removed by reactions other than (2). In this communication we report observations using discharge-flow systems which show that such reactions are important under these conditions, and in the chemistry of the upper atmosphere.

In recent experiments, we have studied the reaction between ozone and oxygen atoms, in the absence and presence of molecular oxygen by chemiluminescence methods.

Oxygen atoms free from molecular oxygen were obtained by titrating N atoms with the stoichiometric quantity of carefully dried nitric oxide[2–4]:

$$N + NO = N_2 + O \quad (3)$$

The nitrogen atoms were produced in an 18-Mc/s electrodeless discharge through British Oxygen Company 'white spot' nitrogen which had been purified by passage over heated copper turnings at atmospheric pressure, then through a narrow trap packed with glass wool, maintained at −78° C and, finally, at low pressure, through another packed trap at − 196° C. A discharge through 1 per cent oxygen in an argon carrier was used to produce oxygen

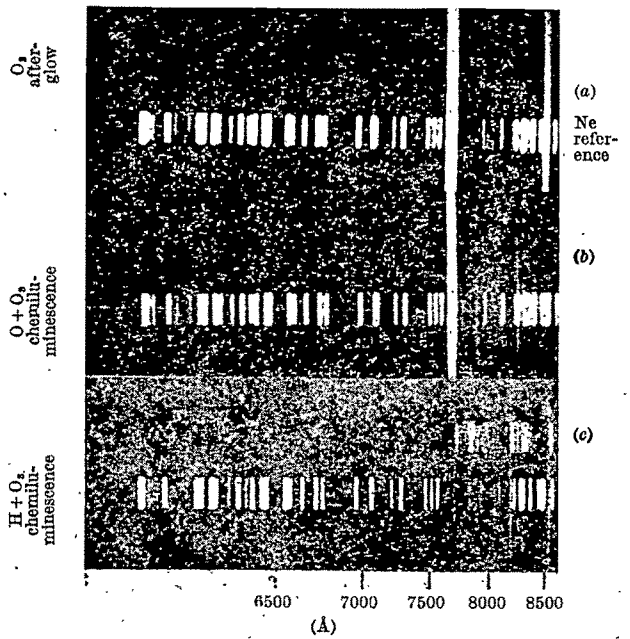


Fig. 1. Spectra of the chemiluminescent reactions of oxygen and hydrogen atoms with ozone

atoms in the presence of relatively small amounts of molecular oxygen.

When dry ozone is added to the oxygen atoms produced by either of these methods, a faint infra-red chemiluminescence is observed. For short reaction times, the intensity of this emission is proportional to the concentration of ozone added. At longer reaction times, the ozone concentration decays quite rapidly downstream, according to a first-order rate law. In these experiments, $[O] \gg [O_3]$, and the rate of ozone removal increased with oxygen atom concentration. Assuming that reaction (2) accounted for all the ozone removal, values of k_2 between 4 and 6×10^{10} cm^3 mole^{-1}s^{-1} were derived at room temperature. These may be compared with values of 2×10^{10} cm^3 mole^{-1}s^{-1} obtained by Leighton *et al.*[5], and $1{\cdot}5 \times 10^{10}$ cm^3 mole^{-1}s^{-1} by Phillips and Schiff[6] also in flow systems. These are all considerably higher than the value of $k_2 = 2{\cdot}3 \times 10^9$ cm^3 mole^{-1}s^{-1} obtained from Benson and Axworthy's[7] data on the thermal decomposition of ozone corrected for the revised dissociation energy of oxygen[8,9].

The infra-red chemiluminescence was photographed on Kodak I–*N* plates, using a Hilger *f*/4 spectrograph. This spectrum is reproduced in Fig. 1 and, apart from the oxygen atmospheric band ($^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$), which appears as a background in experiments where oxygen is passed through the discharge, it shows the Meinel bands[10] of OH (vibration–rotation spectrum of OH from levels up to $v = 9$), which have been shown by McKinley, Garvin and Boudart[11] to be produced in the reaction:

$$H + O_3 = O_2 + OH \qquad (4)$$

A spectrum obtained from the H + O_3 reaction is also shown to illustrate that the bands observed are identical. The observation of the Meinel bands in the experiments with O atoms is somewhat surprising, since the purification methods used imply that the H atom concentration must be much less than the O atom concentration. Reaction (4) is extremely rapid at 300° K. McKinley and Garvin[12] have shown that $k_4 > 2 \times 10^{12}$ cm^3 mole^{-1}s^{-1} and Phillips and Schiff[13] report a value of $k_4 = 1{\cdot}6 \times 10^{13}$ mole^{-1}s^{-1}. In the presence of oxygen atoms, OH radicals produced in reaction (4) will rapidly regenerate H atoms by the reaction:

$$O + OH = H + O_2 \qquad (5)$$

which also has a very high rate constant[14,15]. Reactions (4) and (5) together provide an H atom catalysed removal of ozone, which has the same overall stoichiometry as reaction (2) between O and O_3. Putting $k_4 = 1{\cdot}6 \times 10^{13}$ cm^3 mole^{-1}s^{-1}, the hydrogen atom concentration needed to account for all the ozone removal observed in our experiments is 7×10^{-13} mole cm^{-3}. This corresponds to about 20 parts per million of H atoms in the flow system, or a ratio of O : H of 300 : 1. It is clear that data regarding the rate of removal of ozone and its steady-state concentration in discharge flow experiments must be regarded with great caution until satisfactory methods have been developed for eliminating traces of hydrogen-containing substances from the gases used. We therefore consider that the corrected value[8,9] of Benson and Axworthy's rate constant[7], $k_2 = 3 \times 10^{13} \exp(-5600/RT)$ cm^3 mole^{-1}s^{-1}, is the most reliable at present available as no H atoms would be present in this system; and their expression is consistent with data on the thermal decomposition of ozone in flow systems[16] and in shock tubes[9]. The high intensity of the Meinel bands in night sky spectra is clearly due to the high ratio of $k_4 : k_2$.

Although the reactions of nitrogen dioxide are very similar to those of ozone, yet traces of hydrogen atoms probably do not affect the reaction kinetics in the same way. The process:

$$H + NO_2 = NO + OH \qquad (6)$$

is very rapid[13,17] ($k_6 = 2{\cdot}9 \times 10^{13}$ cm^3 mole^{-1}s^{-1}) (ref. 13), but the reaction of oxygen atoms with nitrogen dioxide:

$$O + NO_2 = NO + O_2 \qquad (7)$$

is very much faster than with ozone[18] ($k_7 = 2 \times 10^{12}$ cm^3 mole^{-1}s^{-1}), and traces of hydrogen will make little difference to the rate of nitrogen dioxide removal.

At higher pressures and higher initial oxygen atom concentrations, emission of the oxygen atmospheric band[19] becomes more intense, and the behaviour of oxygen molecules in the $^1\Sigma_g^+$ state can conveniently be studied downstream from a silver foil, which greatly reduces the concentration of oxygen atoms in the flow line.

In these experiments, the partial pressure of $O_2(^1\Sigma_g^+)$ was found to be $10^{-4} - 10^{-3}$ mm mercury. This was determined from measurements of the absolute emission intensity of the oxygen atmospheric bands, for which the radiative transition probability is known to be 0·14s^{-1} (refs. 20 and 21). Introduction of NO, NO_2 or O_2 to the flow line had no significant effect on the intensity of the atmospheric band emission, but the addition of ozone greatly reduced the strength of these bands, and intensified the air afterglow emission (which is associated with the reaction:

$$O(^3P) + NO + M = NO_2 + M \qquad (8)$$

showing that $O(^3P)$ atoms are formed. We therefore conclude that the reaction:

$$O_2(^1\Sigma_g^+) + O_3(^1A) = 2O_2(^3\Sigma_g^-) + O(^3P) \qquad (9)$$

which is 13 kcal/mole exothermic, occurs to an appreciable extent, although some dissociation of ozone may be produced by other metastable species.

A reaction of this type will regenerate oxygen atoms removed by reaction (1), thereby reducing the apparent value of k_1 if equation (*a*) is used to analyse the experimental data. Such processes may be important in the work of Kaufman and Kelso[19,22], who observed that the apparent value of k_1 in the products of a discharge through oxygen decreased when the power of the discharge through oxygen decreased when the power of the discharge was increased, or when a higher proportion of the oxygen used was passed through the discharge. Both these procedures would be expected to increase the number

of metastable oxygen molecules in the reaction system which could decompose the ozone as it was formed.

To summarize, the presence of metastable excited oxygen in the flow experiments can affect both the kinetic equations (*a*) and (*b*), and may account for the wide range of published values for k_1 (ref. 22). The presence of traces of hydrogen atoms in the discharge products will affect equation (*b*), but not (*a*). It is interesting to note that the presence of hydrogen atoms would minimize the effect of excited molecules by catalysing the ozone removal with the same stoichiometry as reaction (2).

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SEPARATE SYNTHESIS OF FIBRILLAR AND MATRIX PROTEINS IN THE FORMATION OF KERATIN

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THE mechanism by which sulphur is incorporated into the growing hair is not clear. Harkness and Bern[1] interpreted the intense localization of [^{35}S]cyst(e)ine in the keratogenous zone as showing that there is an absolute increase in sulphur in the hair above that in the cells of the bulb, in support of the theory of sulphur-enrichment[2,3] during keratinization. They pictured the formation of keratin as a *de novo* synthesis in the keratogenous zone, with the proteins carried up by cells from the matrix (bulb) serving as partial precursors (amino-acid sources) but with the cyst(e)ine being largely taken up from the blood by the keratogenous zone. However, the autoradiographic evidence does not rule out the possibility that, prior to or simultaneously with keratinization, there is hydrolytic removal of amino-acids which do not contain sulphur, resulting in an apparent enrichment with sulphur[4].

Rothman[5] supported the idea that in keratinizing structures cellular proteins are hydrolysed to free amino-acids which are then converted to keratin in the keratogenous zone. In support of this, De Bersaques and Rothman[6] found that several labelled amino-acids, including cysteine, were simultaneously incorporated into growing hairs in guinea pigs. They concluded that keratins are formed directly from free amino-acids, and that there is no superimposed cystine incorporation into pre-formed proteins.

A possible mechanism suggested by Rudall[3] and favoured by Mercer[7] from electron microscopic evidence is that relatively sulphur-poor microfibrils are synthesized in the lower regions of the follicle and as the cells containing them move through the keratogenous zone an entirely different protein, sulphur-rich and non-fibrous, is synthesized there *de novo*. This protein(s) forms a cementing matrix in which the fibrils become embedded and covalently linked by disulphide bonds. Alternatively, the matrix protein may be synthesized directly on the filaments, establishing an actual peptide linkage with existing polypeptides[7]. Rogers[8] mentioned other possible mechanisms in which only matrix protein might be enriched with sulphur by a reaction of the serine side chains with homocysteine derived from free methionine.

The proteins of fully hardened keratins can be made soluble by either oxidation[9–11] (giving keratoses) or reduction[12] (giving kerateines). After either treatment the proteins can be fractionated into two main groups. One group is poor in sulphur and of high molecular weight: α-keratose and kerateine *A*. The other is rich in sulphur and of low molecular weight: γ-keratose and kerateine B. These two distinct protein types, generally termed the low-sulphur proteins and the high-sulphur proteins, are at present held to be derived respectively from the fibrillar (microfibril) and matrix components of the original keratin[8,13]. The 'prekeratins' of cow's horn[3] and of the wool root[14], which occur completely in the sulphydryl form, can also be isolated and separated into two similar fractions. The rationale behind this relationship is based on extensive but mainly indirect evidence except for an electron microscopic study which indicated that γ-keratose preferentially extracted from oxidized wool originates in the matrix[15].

We have re-investigated the problem of sulphur incorporation during keratinization in the wool follicle based on the assumption of the two-stage synthesis[7]. Thus if enrichment with cystine is a process essentially involving the matrix protein, it follows that, after injecting [^{35}S] cystine into a sheep, the specific activity of the sulphur should be higher in the γ-fraction than in the α-fraction. For such differential labelling to be readily detectable there has to be a reasonable time-lapse in the process of keratinization between initial synthesis of the fibrillar (low-sulphur) protein and the synthesis of the matrix (high-sulphur) protein.

Experimental procedure for [^{35}S]*cystine labelling of follicle tissue.* Each of four sheep *S*1, *S*2, *S*3, *S*4 was given an intravenous dose of DL-[^{35}S]cystine hydrochloride

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Table 1. Specific Activity (c/m at Infinite Thickness) and Sulphur Content* of 'Prekeratin' Proteins

Fraction	*S1* c/m	*S1* % S	*S1* c/m/%S	*S2* c/m	*S2* % S	*S2* c/m/%S	*S3* c/m	*S3* % S	*S3* c/m/%S	*S4* c/m	*S4* % S	*S4* c/m/%S
Whole protein	469, 466 → 467	1·74	268	92, 89 → 91	1·62	56·1	376, 356 → 366	1·96	187	355, 355 → 355	1·81	196
High-sulphur protein	1,117, 1,103 → 1,110	3·53	314	216, 222 → 219	3·17	69·1	863, 883 → 873	3·61	242	752, 760 → 756	3·23	234
Low-sulphur protein	359, 343 → 351	1·48	237	68, 74 → 71	1·42	50·0	232, 228 → 230	1·41	163	242, 248 → 245	1·46	168
Specific activity ratio: low-sulphur/high-sulphur			0·753			0·723			0·674			0·718

* Sulphur analyses by Dr. K. W. Zimmerman, C.S.I.R.O., Microanalytical Service, Melbourne.

(about 100 μc.; 1 mg) diluted with 5 ml. of 0·9 per cent sodium chloride. After intervals of 1·0, 1·1, 4 and 17 h respectively, the sheep were killed and flayed and the wool roots removed from the skin by the wax sheet method[14].

The degree of labelling of sulphur was examined in the low- and high-sulphur proteins from two regions of the wool root, namely, the prekeratin protein developing in the keratogenous zone and isolated by dispersion with urea[14], and secondly, that portion of each wool root not dispersable by urea (wool root residue) which contained the keratin produced in the time interval after the radioactive dose had been given (Fig. 1). The maximum time allowed to elapse after administration of a dose was only 17 h and was insufficient for the labelled zone in the keratinized fibre to reach the skin surface. The wool root residue contained this zone and a quantity of unlabelled fibre grown earlier.

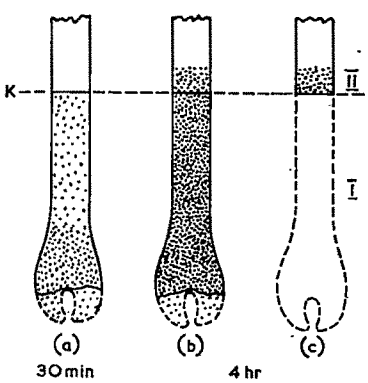


Fig. 1. Diagram of the keratin-forming region (lower third) of wool or hair follicles. The part shown approximates that isolated by the wax sheet method and includes much of the region where the labelling with sulphur-35 takes place. Above level *K* keratinization is complete (*a*) Shows the relative distribution of label (stippling) found 30 min after injection; (*b*) is distribution of label 4 h after injection, and shows that some label is now present in the keratin; (*c*) illustrates situation (*b*) after removal of the prekeratin by urea from the keratogenous zone, I, leaving behind the residue of keratin, II

'*Prekeratin*'. After isolation of the wool roots, a sample was set aside and the remainder was immediately extracted with 8 M urea containing iodoacetic acid at *p*H 8, and the extract fractionated[14] into high- and low-sulphur protein fractions. The extracted roots were washed well with water, ethanol, and ether, and dried.

The soluble fractions prepared from the urea extract and finally freeze-dried were analysed for sulphur, and their specific activities determined by direct counting of infinitely thick samples (1 cm²) with an end-window Geiger–Müller counter. Before they were assayed, the proteins were allowed to stand overnight exposed to the laboratory air to prevent changes occurring in the counting rates due to the uptake of moisture. Since the amount of dry matter was afterwards found to be practically the same for each sample (89·3 ± 1·2 per cent), the counting rates may be presumed to give reliable relative values for the specific activities. The results are shown in Table 1 together with the ratios of the counting rates to the sulphur contents; these ratios are proportional to the specific activities of the sulphur in the respective fractions. In each case the value for the low-sulphur protein was about 30 per cent lower than for the high-sulphur protein.

Samples (100–200 mg) of the high-sulphur and low-sulphur proteins from three of the experiments (*S2*, *S3*, *S4*) were oxidized as described by Myers[16] for wool. Benzidine sulphate was prepared from each and assayed as described here. The relative specific activities obtained by this direct method (0·700 (*S2*); 0·649 (*S3*); 0·831 (*S4*)) agreed reasonably well with those previously calculated (Table 1).

To show that these differences in specific activity were not simply due to the fractions containing different proportions of some non-radioactive sulphur compound, samples of the high-sulphur and low-sulphur fractions from *S3* were hydrolysed and the hydrolysates eluted from an ion-exchange column as described by Gillespie and Springell[17]. In each case the only detectable radioactivity in the effluent was associated with S-carboxymethyl cysteine (SCMC), which accounted for 90 per cent of the radioactivity put on the column. The relative specific activities of the two lots of SCMC were calculated from the counting rates of 0·1 ml. aliquots taken from the appropriate fractions of the effluent and counted on paper by a liquid scintillation method[18] and from the optical densities at 570 mμ obtained by treating other 0·1-ml. aliquots of the same fractions with ninhydrin[19]. The results showed that the specific activity of the SCMC from the low-sulphur protein was 74 per cent of that from the high-sulphur protein.

Labelling in the keratin. 1 g of the wool root residues from each of the experiments *S3* and *S4* was oxidized with performic acid[11] and fractionated into α- and γ-keratose. The sulphur contents were measured by the oxygen flask method, essentially as described by Fritz and Yamamura[20] and Lysyj and Zarembo[21]. The specific activity of each fraction was measured by combustion and liquid scintillation counting, substantially as described by Kalberer and Rutschmann[22]. From the results which are shown in Table 2, it can be calculated that the specific activity of the sulphur in the γ-keratose from *S3* (4 h after injection) was about 11 times that of the sulphur in the α-keratose. The corresponding ratio for *S4* (17 h after injection) was 4·4. The radioactivity in the wool root residues in these experiments includes the fully hardened keratin formed soon after the injection (Fig. 1).

Table 2. Specific Activity and Sulphur Content* of Keratoses Isolated from Wool Root Residues (Keratin)

Sample	*S3* c/m/mg	*S3* % S	*S4* c/m/mg	*S4* % S
Wool root residues	7·27, 5·25 → 6·3	2·65, 2·93 → 2·79	21·9, 23·8 → 22·9	2·51, 2·61 → 2·56
Oxidized wool root residues	5·15, 5·64 → 5·40	—	18·9, 18·2 → 18·5	—
α-Keratose	0·509, 0·543 → 0·53	1·71	4·09, 4·45 → 4·27	1·79, 1·77 → 1·78
γ-Keratose	13·18, 13·11 → 13·1	3·79	43·0, 40·1 → 41·5	4·00, 3·80 → 3·90

* See footnote to Table 1.

General conclusions. In previous work, the specific activity of the sulphur in the α- and γ-fractions from the first labelled wool obtained in successive clippings from sheep dosed with cystine labelled with sulphur-35 has been measured (Downes and Sharry, unpublished work), and although small differences were detected the results were inconclusive. In the present study it was possible to isolate the first-formed radioactive keratin with certainty and large differences were observed in the specific activity of the sulphur in the low-sulphur (fibrillar) and high-sulphur (matrix) proteins.

In the 'prekeratin' the specific activity of the sulphur in the high-sulphur protein is significantly higher than in the low-sulphur protein and this difference is greatly accentuated in the corresponding ratio for the keratoses from the keratin of the wool root residues. The reason for the smaller differences for the 'prekeratin' is probably due to the fact that wool roots as prepared include most of the developing fibre below the keratogenous zone[14] so that the urea extracts contain much of the newly formed fibrillar protein as well as matrix protein. Nevertheless the results support the mechanisms described by Rudall[3] and by Mercer[7] in which the high-sulphur proteins are synthesized at a late stage in the keratogenous zone of the follicle.

In conclusion, it would appear that the results of De Bersaques and Rothman[6] are in fact consistent with the mechanism for sulphur incorporation supported by the present results. Their results only require that some protein, not necessarily all the proteins in keratin, be synthesized in the keratogenous zone.

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OCCURRENCE OF PSEUDO-BARBITURATES IN POST-MORTEM MATERIAL

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ON several occasions during routine toxicological analyses we have obtained unusual ultra-violet absorption spectra. These spectra have been observed on examining solutions of both the strong acid and barbiturate (weak acid) group residues, and have occurred after using direct ether or protein precipitation[1,2] extraction techniques. We have referred to them as 'pseudo-barbiturate' spectra because of their similarity, between wave-lengths 230 to 260 mμ, in dilute ammonia and acid, to the absorption curves of the barbiturates (Fig. 1). These pseudo-barbiturates have been noted in liver, kidney, muscle, blood and urine extracts, obtained from fresh, old and exhumed post-mortem specimens.

Most of these spectra have certain features in common:

Solvent	Maxima
0·5 N NH_4OH	240, 292
Dilute H_2SO_4	275
0·5 N NaOH	240, 292

This basic pattern is shown in Fig. 1, but minor variations are sometimes observed, for example, an additional point of maximum absorption at 328 mμ in 0·5 N ammonium hydroxide and 0·5 N sodium hydroxide solutions is given by some extracts.

Ascending paper chromatography, using the *n*-butanol/ammonia[3] or amyl alcohol/ammonia[4] systems on Whatman No. 1 paper, of residues containing pseudo-barbiturates gave variable results. Both absorbing and fluorescent spots were visible under filtered ultra-violet light (2537 Å). The absorbing spots were not intensified by exposure to ammonia vapour and none of the spots gave a positive reaction with the cobalt chloride reagent[4], thus distinguishing them from genuine barbiturates. The ferric chloride/ferricyanide reagent[5], for phenolic compounds, reacted with most of these spots. Additional spots, not observed with ultra-violet light, were revealed by this reagent on some of the chromatograms. The chromatographic examination of these residues had therefore shown that we were dealing with a complex mixture of interfering chromogens.

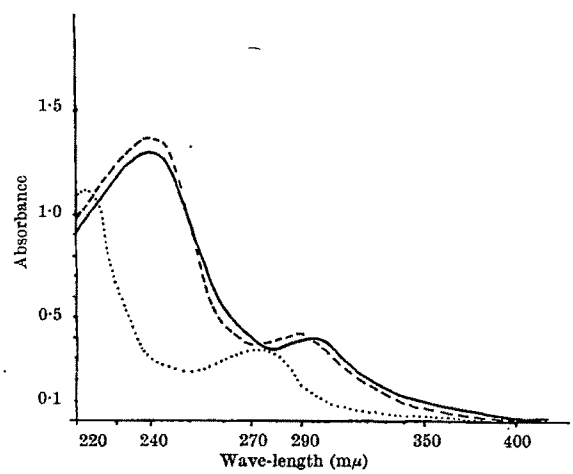
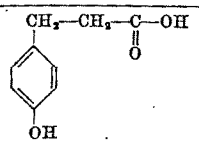
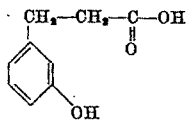
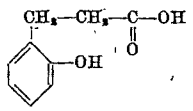
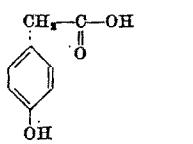
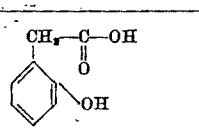
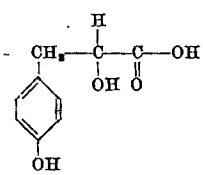
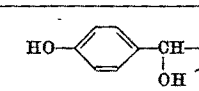


Fig. 1. Ultra-violet absorption spectrum of a 'pseudo-barbiturate' ····· *p*H 2; – – –, *p*H 10; ——, *p*H 13

One of the absorbing spots (R_F value 0·10, using the amyl alcohol/ammonia system) was consistently observed in chromatograms of material from old or exhumed viscera. This spot was eluted and identified by X-ray diffraction (Fig. 2) and infra-red absorption techniques (Fig. 3) as β-*p*-hydroxyphenyl propionic acid. The ultra-violet absorption spectrum of this compound gave a pattern identical with Fig. 1. This compound is known to occur in exhumed viscera[6,7] and has been suggested as a possible cause of interference with the ultra-violet spectrophotometric determination of barbiturates[8]. However, residues from fresh post-mortem samples also exhibiting a pseudo-barbiturate type spectra gave chromatograms without this particular spot. β-*p*-Hydroxyphenyl propionic acid is a degradation product of tyrosine[9]. Many other breakdown compounds of this amino-acid are known and could possibly be present in viscera. We have

Table 1. Ultra-violet Absorption Details of Compounds having 'Pseudo-Barbiturate' Type Spectra

Name	Structure	$E^{1\%}_{1\ cm}$				
		*p*H 10		*p*H 13		*p*H 2
		240 mμ	292 mμ	240 mμ	292 mμ	275 mμ
β-*p*-Hydroxyphenyl propionic acid		333	87	393	100	100
β-*m*-Hydroxyphenyl propionic acid		320	136	352	156	96
β-*o*-Hydroxyphenyl propionic acid		247	107	278	138	71
p-Hydroxyphenyl acetic acid		555	136	585	150	87
o-Hydroxyphenyl acetic acid		552	200	581	238	124
p-Hydroxyphenyl lactic acid		249	66	249	66	40
p-Hydroxyphenyl mandelic acid		200	48	200	48	33

measured the ultra-violet absorption spectra of some of these substances (Table 1) and have found that the particular pattern of Fig. 1 is common to many other phenolic compounds. We are therefore attempting to correlate these with the unidentified spots on the chromatograms.

Unusual ultra-violet absorption spectra have been previously described[10,11]. These also occurred in the acid ether extracts of post-mortem livers. Although the ultra-violet absorption spectrum of the interfering chromogen recovered by Curry[12] from fresh post-mortem liver specimens is identical with that of β-*p*-hydroxyphenyl propionic acid, the chromatographic behaviour is quite different. His unknown compound gave a spot, which had a blue fluorescence in ultra-violet light, near the solvent front, whereas β-*p*-hydroxyphenyl propionic acid gives an absorbing spot near the base line. It is interesting to note that one of our specimens of β-*p*-hydroxyphenyl propionic acid, originally synthesized in 1951 [6], now gives two spots when examined by paper chromatography and one of these is a fluorescent spot near the solvent front.

Another unusual spectrum was noted by Walls *et al.*[11] and afterwards identified as being due to a mixture of *p*-hydroxybenzaldehyde and *p*-hydroxy benzoic acid. *p*-Hydroxybenzaldehyde also gives a pseudo-barbiturate pattern plus an additional peak at 328 mμ. This may well explain one of the variations we have observed in our cases.

Ultra-violet spectrophotometric methods are widely used in clinical laboratories for the detection of barbiturate poisoning in patients, and many hospital biochemists are now undertaking the analysis of post-mortem specimens for barbiturates. In view of the possible occurrence of pseudo-barbiturates in post-mortem material we think it is important to emphasize that the detection of an absorption peak at 240 mμ (even when accompanied by a large optical density difference between *p*H 10 and *p*H 2) does not prove the presence of a barbiturate. This behaviour is characteristic of many other compounds. Further, the $E^{1\%}_{1cm}$ values of these pseudo-barbiturates is of the same order as genuine barbiturates. Thus, concentration of β-*p*-hydroxyphenyl propionic acid as low as 0·06 mg per cent will give an optical density reading of about 0·6 at



Fig. 2. X-ray diffraction pattern of β-*p*-hydroxyphenyl propionic acid

240 mμ. Measurement of the whole spectrum between 230 and 330 mμ is essential for the detection of these interfering compounds. Examination of the pseudo-barbiturate curve (Fig. 1) will show that the criteria mentioned by Broughton[13] are not fulfilled, therefore this spectrum could not be mistaken for a 5 : 5 di-substituted barbiturate spectrum. If only a limited portion of the spectrum is plotted, however, it may be mistaken for the curve of a 1 : 5 : 5 substituted barbiturate.

If both barbiturate and pseudo-barbiturate are present the only reliable method of assay is by eluting the barbiturate spot from a chromatogram and subsequent ultra-violet spectrophotometry.

We thank Dr. Ivor Smith and Dr. J. B. Jepson for providing us with samples of compounds involved in this work, and Mr. L. C. Nickolls for his encouragement and advice.

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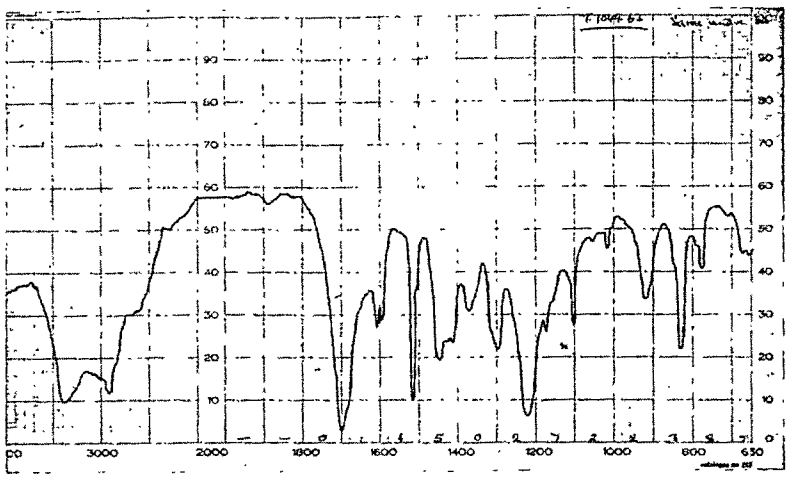


Fig. 3. Infra-red spectrum of β-*p*-hydroxyphenyl propionic acid

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CYTOCHEMICAL DEMONSTRATION OF UBIQUINONES IN ANIMAL TISSUES

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SUCCINATE dehydrogenase cannot be demonstrated histochemically in material embedded in paraffin wax[1]. Nor can it be demonstrated in freeze-dried blocks, or in air-dried cryostat sections, if these have been incubated for brief periods in low melting point waxes. While studying this enzyme inactivation we added a number of substances to the wax as anti-oxidants. We found that succinate dehydrogenase was apparently completely reactivated in cryostat sections incubated in wax to which a small quantity of hydroquinone had been added. Control tests showed that neither the wax nor the dehydrogenase was a contributory factor since fresh cryostat sections incubated in buffer containing only hydroquinone and our customary tetrazolium salt, 3-(4,5-dimethylthiazolyl-2)-5-diphenyl tetrazolium bromide (MTT), gave an equally strong reaction.

We considered and tested a number of different hypotheses before concluding that this reaction was due to ubiquinones and possibly closely related compounds. Our reasons for this conclusion form the first part of this paper and some results of the application of the method the second half.

Technical details of the reaction. The best results are given by the following technique. Fresh cryostat sections (8μ), dried in air at room temperature, are incubated in a medium composed of: 0·25 ml. MTT (1 mg/ml.) in distilled water; 0·05 ml. cobalt chloride (0·5 M); 0·40 ml. *tris* buffer (0·2 M, *p*H 7·4); 0·10 ml. hydroquinone (40 mg/ml., in *tris* buffer, *p*H 7·4); 0·20 ml. distilled water; 2 mg catalase.

Sections are incubated for 10–30 min at 37°. They are then fixed in 10 per cent formalin at room temperature for 15 min, and afterwards washed briefly in running water. After counterstaining the nuclei in 2 per cent chloroform-washed methyl green (2 min) the sections are rinsed in distilled water and mounted in glycerine jelly.

* British Council Scholar.

Hydroquinone. This solution must be absolutely fresh. The final concentration of 4 mg/ml. is that found to be best; but a concentration of 0·4 mg/ml. gives strong reactions in rat liver. Higher concentrations weaken the reaction.

*p*H. Below *p*H 5·2 the reaction is negative or very weak. Higher *p*H-levels than 7·4 were not tested because of their effect on reduction of tetrazoles by protein and carbohydrate tissue components.

Catalase. When the incubating medium (containing no catalase) is left in contact with glass or air, after a short period of time a black film of formazan appears. This spontaneous reaction is minimal at acid *p*H-levels, but so also is the histochemical reaction. The addition of catalase completely prevents the slow spontaneous reaction.

Other components. The concentrations of tetrazole, cobalt chloride and buffer are those used in present practice[2]. Cobalt (Co^{2+}) ions are chelated by the nascent formazan which is prevented thereby from crystallizing.

Alternative electron donors and acceptors:

Donors. Resorcinol, pyrocatechol, *p*-phenylene diamine, pyrogallol.

Acceptors. 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′biphenylene) ditetrazolium chloride (Nitro-BT). 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT). 2,2′,5,5′-tetra-*p*-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene) ditetrazolium chloride (Tetra Bitro-BT). These were used in the same concentrations as the hydroquinone and the M.T.T., respectively.

Resorcinol and pyrocatechol give scarcely any spontaneous reaction with MTT and the former gives little reaction with the other tetrazoles. Pyrocatechol-MTT mixtures gradually darken but give no reaction in tissue sections. With *p*-phenylene diamine MTT gives a histochemical reaction, resembling that obtained with hydro-

quinone; but the presence of brownish decomposition products makes interpretation difficult. Pyrogallol reduces MTT spontaneously.

Tests of alternative tetrazolium salts as final electron acceptors showed that only tetra-Nitro-BT gave any immediate reaction with hydroquinone. Nitro-BT and INT gave a feeble reaction after long incubation. With pyrogallol tetra-Nitro-BT was reduced spontaneously, but Nitro-BT and INT gave a histochemical reaction broadly comparable with that shown with hydroquinone-MTT. Only the latter gave results which could be used for detailed investigation at the cytological level.

Influence of different factors on the reaction.

Action of fixatives: (*a*) *15 per cent formaldehyde.* Sections fixed for 5–60 min at room temperature gave reactions as strong as those of unfixed sections. (*b*) *1 per cent osmium tetroxide.* Sections fixed for 15 min at 4° in Palade's[3] medium showed complete inhibition of the reaction. (*c*) *Absolute ethanol.* Treatment for 1 h at room temperature completely abolished the reaction. (*d*) *Absolute acetone.* Similar treatment as with ethanol only slightly reduced the reaction.

Other organic solvents were tried. Chloroform produced complete inhibition; ether and petroleum spirit (b.p. 40°–60°) had little or no effect.

Action of heat. (*a*) *Dry heat.* Treatment at 90° for 30 min produced no change. (*b*) *Moist heat.* Treatment with water or 15 per cent formaldehyde at 90° for 30 min did not prevent the development of the reaction.

Addition of coenzymes. Addition to the hydroquinone-MTT medium of NAD, NADP or cytochrome *c* (2 mg/ml.) had no effect on the reaction.

Enzyme inhibitors. The following substances were added to the incubation medium at *p*H 7: potassium cyanide (0·01 M), 'Amytal' (0·01 M), 'Dicoumarol' (0·01 M), 'Phenindione' (sat. soln.), hydroxylamine (0·03 M), NaN_3 (0·03 M). Potassium cyanide was used in the absence of cobalt, with which it forms an unstable electron-donating complex. The reaction was affected only by sodium azide.

Possible mechanisms for the reaction

We considered a number of different hypotheses to explain our observations. These included physico-chemical artefact (adsorption of preformed formazan), non-specific enzyme activity, specific enzyme activity (hydroquinone dehydrogenase), and non-enzymatic catalysis.

Physicochemical artefact. The spontaneous reaction between hydroquinone and MTT, which occurs in the presence of air, at the surface of the medium, presumably occurs in the following manner:

$$1/2\ O_2 + H_2O \rightarrow H_2O_2$$

$$\text{Hq (hydroquinone)} + H_2O_2 \rightarrow \text{Q (quinone)} + H_2O + (O) + (2H).$$

$$\text{MTT} + (2H) \rightarrow \text{MTF (formazan)}$$

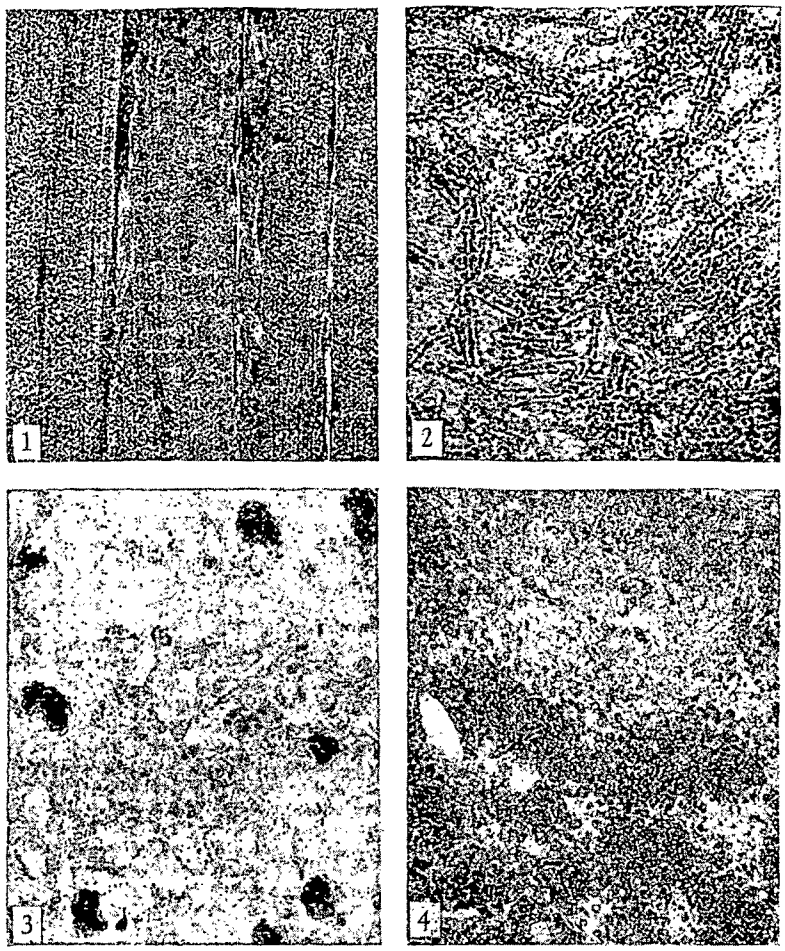
$$H_2O + (O) \rightarrow H_2O_2$$

In the presence of catalase hydrogen peroxide cannot exist and the foregoing reaction is therefore prevented. The cytochemical reaction, however, is not affected. On this evidence we rejected the first hypothesis altogether.

Enzyme activities. The cytological appearances of sections incubated in the hydroquinone-MTT medium appeared, at first sight, closely to resemble those obtained with techniques for succinate dehydrogenase and the two mitochondrial diaphorases. We were obliged, therefore, to investigate the possibility that enzyme activity might be responsible.

It is known that hydroquinone can be oxidized by tissue extracts such as the Keilin–Hartree heart muscle preparation[4,5]. Substantial results have been obtained, however, only after addition of cytochrome *c*. Among others Ogawa *et al.*[6] have shown that tetrazolium salts can accept electrons from different parts of the respiratory chain, including the region of cytochrome a^3, so that cytochrome oxidase could have been responsible, in theory, for the reaction. We considered, however, that its resistance to heat, formaldehyde and potassium cyanide, and the lack of effect from the addition of cytochrome *c*, must rule out the passage of electrons from hydroquinone to MTT by way of cytochrome oxidase.

It seemed possible that endogenous coenzymes such as NAD and NADP, reduced by hydroquinone, could transfer electrons by way of their respective diaphorases. This hypothesis was regarded as untenable, for various reasons. The oxidation-reduction potentials of the reactants are such that the reaction would proceed in the opposite direction to that required.



All photomicrographs are of 8μ cryostat sections, unfixed

Fig. 1. Rat; striated muscle. The distribution of ubiquinones is apparently sarcosomal (× *c.* 297)

Fig. 2. Rat; spermatozoon middle pieces. The product of the reaction is exclusively mitochondrial (× *c.* 776)

Fig. 3. Rat; intestinal lamina propria. Eosinophil leucocytes distinguished by a very intense reaction in their granules (× *c.* 776)

Fig. 4. Rat; lymph node. Above, large reticulum cells in a germinal centre show a strong non-mitochondrial reaction. Below, pyroninophilic plasmacytes show strong mitochondrial staining (× *c.* 136)

Hydroquinone E_0 = + 0·681 V (ref. 7)
NAD E_0 = − 0·113 V (ref. 8)

Furthermore, the concentrations of NAD and NADP in the tissues are very low and the addition of either coenzyme did not affect the reaction.

Hydroquinone itself might have been the substrate, transfering electrons by way of the diaphorases. We found, however, that no such transfer occurred when solutions of either bacterial diaphorase (Nutritional Biochemical Corporation) or a Straub diaphorase from heart muscle (courtesy of Dr. M. Dixon) were added to the medium. The further addition of $NADH_2$ produced immediate reduction of the tetrazole, in both cases, showing that the diaphorase remained active under the conditions of the experiment.

Since Agner[9] had shown that the 'M–Nadi reaction' was due to a peroxidase we investigated the possible role of the latter. Neither peroxidase nor catalase could produce the true reaction, but with catalase we noted inhibition of the spontaneous reaction which has been referred to already.

After dismissing the non-specific enzmye activities detailed here we postulated the intervention of 'hydroquinone' dehydrogenase. Enzymes of this type, the laccases, have been described in plants and lower organisms[10–12]. They have not been described in animal tissues, and would, in any event, have difficulty in surviving the strenuous inactivation procedures which we used. We were finally obliged, therefore, to consider the participation in the reaction of a non-enzymatic catalyst.

Catalysis by ubiquinones.

In our search for a possible catalyst or intermediate substance we tested once more the effect of alcohol in inactivating the reaction. Pieces of rat liver (about 1·5 g each) were freeze-dried in an apparatus recently described[10–12]. The dried tissue was powdered and extracted with 4 ml. ethanol for a few hours at room temperature. After centrifugation, some drops of the supernatant were placed on a cover-slip and the ethanol allowed to evaporate. The dry residue, immersed in a fresh hydroquinone-MTT solution, rapidly became black. Our 'inactivation' was thus shown to be extraction.

The observed properties of our 'catalyst' appeared to resemble those of the ubiquinones described almost simultaneously by Morton *et al.*[14,15] and by Crane *et al.*[16]. Table 1, shows the comparison.

Ubiquinone-6 (Q30) (L. Light and Co., Colnbrook, Bucks) was dissolved in ethanol. One drop (containing approximately 50 γ) was added to 2 ml. of the hydroquinone-MTT medium and incubated at 37°. Rapid reduction of the tetrazole occurred with deposition of the black formazan. No reaction occurred with menadione (vitamin K_3) or with various sterols, including dehydroisoandrosterone, ethenyl œstradiol, œstradiol, and 17*a*-ethenyl-D5-anterosten-3*b*l7*b*-diol.

We have concluded, on the foregoing evidence, that the cytochemical reaction described in this article is due to the presence of ubiquinones. We have not excluded, however, the possibility that closely related compounds, such as vitamins K_1 and K_2, may be capable of giving a positive reaction. Mitochondria from animal sources, however, contain very little vitamin K. Measurements of the sensitivity of the reaction have yet to be made. Its catalytic nature leads one to suppose that this may be very high indeed.

Table 1

Tissue component giving reaction	Ubiquinone (ref. 17)
Oxidation–reduction catalyst	Oxidation–reduction catalyst; coenzyme of flavoproteins
Oxidation–reduction potential intermediate between hydroquinone and MTT	E_0 = 0·542 E *p*H 7·3 = 0·089
Reduced form can reduce tetrazoles	Coenzyme Q10 acts as intermediate electron transfer agent in succinate tetrazolium reductase systems (refs. 18, 19). Reduced CoQ reduces INT (ref. 17, p. 379)
Detectable cytochemically in the majority of mammalian tissues	Found biochemically in most mammalian tissues—hence the name ubiquinone (ref. 14)
Cytochemical localization predominantly mitochondrial	Mitochondrial preparation rich in ubiquinones
Cytochemical reaction not affected by water or aqueous solutions	Insoluble in water
Cytochemical reaction stable to heat up to 90°	Stable at 90°
Extractable from sections by ethanol and certain fat solvents. Fraction extracted rich in lipids	Extractable from homogenates by ethanol/ether 3 : 1. Purification by various fat solvents. Ubiquinones soluble in lipids

It may be predicted that by selection of a suitable primary electron donor, and of a suitable tetrazolium salt as final acceptor, it will be possible to demonstrate the localization and level of a number of redox substances, making use of their activity as intermediates.

Mechanism of the reaction.

The system involved is formed by three redox couples

(*a*) Hydroquinone $\underset{+2e}{\overset{-2e}{\rightleftharpoons}}$ Quinone
E_0 = 0·68 V at 25° (ref. 7)
E_0 *p*H 7·3 = 0·273 V at 25° (ref. 20)

(*b*) Ubiquinol $\underset{+2e}{\overset{-2e}{\rightleftharpoons}}$ Ubiquinone
E_0 = 0·542 V (ref. 21)
E_0 *p*H 7·3 = + 0·089 V (ref. 17, p. 170)

(*c*) Formazan $\underset{+2e}{\overset{-2e}{\rightleftharpoons}}$ Tetrazolium
$E\frac{1}{2}$ *p*H 7·2 = − 0·11 V at 22° (ref. 2, p. 537)

(*a*) and (*b*) are reversible in aqueous media and in organic solvents. (*c*) is reversible in organic solvents, but will not proceed from left to right in aqueous media, where the formazan is insoluble.

The available data on redox potentials are incomplete and not strictly comparable, since they have been obtained under very different conditions. For system (*c*) only polarographic data have been published. Nevertheless, it is considered permissible to compare polarographic with potentiometric measurements[22].

If the two couples (*a*) and (*c*) are in a condition of reversibility, and if the concentrations of their oxidized and reduced forms are correct, electrons should be transferred from (*c*) to (*a*). In aqueous media (*c*) is not reversible, however. Furthermore, in the Hq/MTT solution there is an excess of the reduced form of (*a*) and of the oxidized form of (*c*). This should favour the passage of electrons from (*a*) to (*c*). That such a transfer does not occur spontaneously may be explained by the close agreement between the two redox potentials.

The role of couple (*b*) appears to be that of an intermediate or catalyst. It has been observed that ubiquinol (the reduced form of the catalyst) can reduce certain tetrazoles directly[18,19] and pyrogallol, the E_0 of which is 0·713 (ref. 22) can reduce ubiquinone[17]. It is therefore probable that hydroquinone, which has a similar redox potential, can function in the same way. As already mentioned, we observed a positive reaction when we substituted pyrogallol (and Nitro-BT or INT) for hydroquinone and MTT.

The cytochemical results are not dependent solely on the different redox potentials of the tetrazolium salts used but also on their solubility in lipids, their substantivity for proteins and, probably, their stereochemical characteristics. The very much better results obtained with MTT must be ascribed mainly to its lipid solubility, since it is in the lipid phase that the whole reaction occurs.

Localization of ubiquinones in animal tissues.

Our present survey has been confined to rat and human tissues. The results are presented here in abbreviated form, emphasizing only points of special interest. In general the reaction was stronger in rat tissues than in human material. As expected, ubiquinones appeared to

be present in the majority of tissues and the intensity of the reaction was comparable with the levels found by other workers using biochemical assays. The reaction was predominantly mitochondrial (Figs. 1 and 2), but in some cells, such as those of the exocrine pancreas and of the serous salivary glands, an extramitochondrial reaction was observed. In the proximal convoluted tubules there was also a non-mitochondrial reaction, located at the luminal border of the cells or in the brush border. This suggested that the product made visible was in process of excretion in the urine.

In both man and the rat, an outstanding localization of ubiquinones is in the granules of the eosinophil leucocytes (Fig. 3). Even with short incubation the reaction is intense. Since electron microscopic studies[23,24] have shown that eosinophil cell granules contain a central osmiophilic crystalloid body it is probable that the ubiquinones are located in this (lipid) region. Electron cytochemical studies should resolve the problem. Speculation as to the possible functions of the eosinophil with respect to ubiquinones must include: (1) a high rate of synthesis, accompanied by storage; (2) absorption and storage; (3) transport to areas with high requirements for the coenzyme; (4) utilization for anabolic or catabolic processes.

In the reticuloendothelial system of the rat the Kupfer cells contain a few discrete granules of reaction product, but in some large reticulum cells, in the germinal centres and sinusoids of the lymph nodes, there are accumulations of large granules which are manifestly not mitochondrial. These reticulum cells are not pyroninophilic, but an equally intense reaction for ubiquinones occurs in cords of cells surrounding the sinusoids (Fig. 4). These cells stain strongly with pyronin, and the majority can be classified as plasma cells or their immediate precursors. Mast cells are usually negative for ubiquinones but occasionally, in the lymph node capsule, some strongly reacting examples can be seen.

Strongly positive reactions are characteristic of epithelial cells, both glandular and otherwise, and in these the product is mainly or exclusively mitochondrial. Because of reports that ubiquinone levels in tumours were found to be lower than those of normal tissues[17] we investigated the matter. It seems that in many epithelial tumours the ubiquinone level per cell is as high as it is per cell in the parent tissue. In some metastases, on the other hand, the levels per cell are below those per cell of the parent tumour. Mesenchymal tumours have low ubiquinone levels (and incidentally low levels of most mitochondrial oxidative enzyme systems). The level of ubiquinone in tumour cells probably does not merely reflect the number of mitochondria in the cell though there is obviously a relationship to this.

We have the impression that ubiquinone levels are high in cells concerned primarily with protein synthesis, whether this protein is designed for self-perpetuation or for export.

Applications of the hydroquinone-MTT reaction.

In addition to its use as a qualitative or semiquantitative cytochemical reaction, the possibility of developing a fully quantitative cytochemical technique can be considered. This would depend on the establishment of the stoichiometry of the reaction as has already been done for $NADH_2$ oxidation with menadione as intermediate electron carrier[25].

In vitro estimation of ubiquinones is also a distinct possibility. The reaction can be carried out with an alcoholic solution of the quinone and an aqueous Hq-MTT solution.

We have been able to show, using low concentration spots on filter paper, that the reaction is fully sensitive enough to be used for chromatographic recognition of ubiquinones. Further developments along these lines are planned.

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5-HYDROXYTRYPTAMINE IN THE SPERMATOPHORIC SAC OF THE OCTOPUS

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THE male octopus, in common with other Dibranchiata belonging to the advanced molluscan class of Cephalopoda, possesses a single testis; this oval, whitish organ occupies together with the remainder of the reproductive tract (Fig. 1) a dorsal position in the visceral cavity and is attached to the cœlomic wall by a mesenteric fold of the peritoneum.

On leaving the testis the spermatozoa enter the long convoluted vas deferens through a slightly enlarged portion known as the ampulla, and from there pass into a thick-walled helically wound tubular gland, named by its discoverer de Cuvier[1], glandula vesicularis ('seminal vesicle'), and later examined by Marchand[2], Belonoschkin[3] and others. In the seminal vesicle the spermatozoa are enfolded by the glandular secretions into tube-shaped membranes and ultimately encased into the spermatophores. That task is performed by the seminal vesicle together with another closely located accessory gland, namely, the 'prostata' of de Cuvier, where the assembly of spermatozoa into the spermatophores is brought to completion. The seminal vesicle and prostate together constitute the so-called spermatophoric gland. Emerging from the prostate the spermatophores, each carrying numerous encased spermatozoa, pass along the vas efferens, sometimes also referred to as the distal vas deferens, into the capacious storage organ known after its discoverer in the eighteenth century, as Needham's sac, or simply the spermatophoric sac.

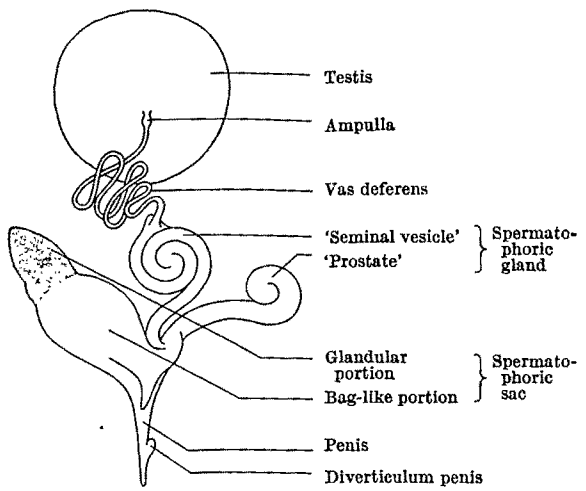


Fig. 1. Diagrammatic outline of the male reproductive tract (unravelled) of *Octopus vulgaris*, to illustrate the localization of 5-hydroxytryptamine (serotonin) in the glandular portion of the spermatophoric sac (Needham's sac)

On close inspection, Needham's sac of the octopus can be seen to consist of two distinct portions, one proximal to the efferent duct, shaped like a pouch or bag, and serving as a reservoir for the spermatophores, and another, more distally located, in appearance a secretory gland rather than a bag. The spermatophores lie in the bag-like portion in a match-box arrangement, all pointing outward with the same end. From the spermatophoric sac they are discharged into the penis, whence at the time of mating they are removed by one of the eight arms of the male octopus, the hectocotylized arm, to be transferred bodily into the mantle cavity of the female. The stimulus necessary for the discharge of the spermatophores is unknown; presumably it is furnished by the contraction of the spermatophoric sac itself, under the influence perhaps of a chemical stimulant. The lack of data in this respect prompted me to examine chemically the spermatophoric sac in *Octopus vulgaris*.

During a recent stay in the laboratory of Dr. Charles B. Metz, at Florida State University, I had the opportunity of examining the male reproductive tract in five specimens of *Octopus vulgaris*, caught in the Gulf of Mexico during February and March 1963, two of them small and three large, 0·5–2 kg in weight. A search was made in the various parts of the reproductive tract for substances known to be secreted by the male accessory glands in other animal species[4], including 5-hydroxytryptamine (serotonin, enteramine). I have shown earlier that this aromatic amine, which is capable of exerting a powerful stimulating effect on the uterus and other smooth-muscle organs, occurs in the secretion of at least one male accessory organ of aquatic animals, namely, the clasper-siphon sac of the spiny dogfish, *Squalus acanthias*[5]. An indole base, probably related to 5-hydroxytryptamine, which causes contractions of the oviducts of insects, has also been located in the male accessory secretions of certain insects such as *Rhodnius prolixus* and *Periplaneta americana*[6]. In mammals, on the other hand, no unequivocal evidence has as yet been obtained for the occurrence of 5-hydroxytryptamine in either whole semen or in the secretions of male accessory organs of reproduction[7].

The chemical analyses carried out on the two portions of Needham's sac separately have shown that the glandular portion of the spermatophoric sac, in distinction to the bag itself, contains a remarkably high concentration of 5-hydroxytryptamine, and that it also produces smaller quantities of at least one and possibly two other diazo-reactive and ultra-violet-absorbing substances. This demonstrates for the first time the presence of 5-hydroxytryptamine in the male genital tract of the octopus. The other organ of that animal in which the same indole base had previously been shown to occur is the posterior salivary gland[8].

Quantitative analysis of 5-hydroxytryptamine in the spermatophoric sac (glandular portion) of the five animals examined, based on the reaction given by that base with 1-nitroso-2-naphthol[9], gave values ranging from 12 mg/100 g tissue (fresh weight) in the smallest specimen to 169 mg/100 g in the largest one. The identity of the indole base was confirmed as follows. The spermatophoric sacs of the three large animals, totalling 9·6 g, were extracted by grinding with 70 ml. ethanol, the centrifuged ethanolic extract was evaporated to 5 ml., centrifuged, reprecipitated with equal volume of ethanol, centrifuged again, and the colourless water-clear solution (10 ml.) was analysed by three methods: spectrophotometrically, spectrophotofluorimetrically, and chromatographically. The ultraviolet spectrum measured in the Beckman recording spectrophotometer showed an absorption maximum at 273 mμ, and on addition of 0·04 N sodium hydroxide exhibited a small subsidiary absorption maximum at 325 mμ. Taking the optical density observed and the molecular extinction coefficient $\varepsilon = 5{\cdot}3 \times 10^3$ (at 275 mμ), the content of 5-hydroxytryptamine was calculated as 10·1 mg/10 ml. extract or 105 mg/100 g tissue. The fluorimetric analysis was carried out in the Aminco–Bowman spectrophotofluorimeter, in the presence of 3 N hydrochloric acid and 0·003 per cent ascorbic acid (activation maximum at 295 mμ; fluorescence maximum at 540 mμ). It gave a value of 9·2 mg 5-hydroxytryptamine in 10 ml. extract. Chromatograms were run at room temperature for 16 h on Whatman No. 1 paper, with butanol/acetic acid and *n*-propanol/acetic acid as solvents. On spraying with Ehrlich's reagent[10] and diazotized sulphanilic acid[11], a characteristic spot was revealed, with an R_F value identical with that of pure 5-hydroxytryptamine creatinine sulphate.

The demonstration of a large amount of 5-hydroxytryptamine in the Needham's organ of the octopus not only establishes the occurrence of that pharmacologically active amine as a normal constituent in the male genital tract of a mollusc, but also helps to bring out an interesting, hitherto unsuspected, chemical difference as between the glandular and the reservoir portion of the spermatophoric sac (Fig. 1). It also suggests that in the octopus, in any event, serotonin may play a part in the ejaculatory process. This might perhaps be envisaged as an induction of either the discharge of the spermatophores from the spermatophoric sac, or a stimulation of contractions within the male or female reproductive tract, assisting the passage of spermatozoa to the site of fertilization.

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A MOLECULAR THEORY OF MUSCLE CONTRACTION: CALCIUM-DEPENDENT CONTRACTIONS WITH HYDROGEN BOND FORMATION PLUS ATP-DEPENDENT EXTENSIONS OF PART OF THE MYOSIN-ACTIN CROSS-BRIDGES

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ENOUGH new information has become available recently to permit the development of a molecular theory of muscle contraction based on a reasonable sequence of known chemical reactions.

(I) Basis

This theory rests on the following observations and conclusions.

(1) Electron micrographs of fixed, stained, mammalian, skeletal muscles show that the actin and myosin are arranged as in Fig. 1. The thick filaments of myosin are in the central region of the sarcomeres, and have a six-fold screw axis of symmetry with a helical arrangement of the cross-bridges which contain the *H*-meromyosin ATPase. The *F*-actin filaments are attached to the *Z*-lines between each sarcomere, and can link with three thick filaments[1]. They are composed of an open double helix with 13 sub-units (*G*-actin) in each turn of each helix[2].

(2) The individual myosin molecules in the myosin filaments are oriented in opposite directions on either side of the centre of the filaments. The actin filaments are structurally polarized in opposite senses on either side of the *Z*-lines. This ensures that the individual filaments slide in one direction only during contraction[3].

(3) The interdigitating-filament mechanism represents essentially the anatomical description of contraction, that is, the thin filaments move along the channels between the thick filaments without overall changes in length. The ends of the muscle thus get nearer as in a collapsing telescope[1,4] (Fig. 2).

(4) When muscle is activated, calcium is apparently released from the vesicles of the sarcosplasmic reticulum into the cytoplasm, and is later removed by the action of the 'relaxing-factor' system[5].

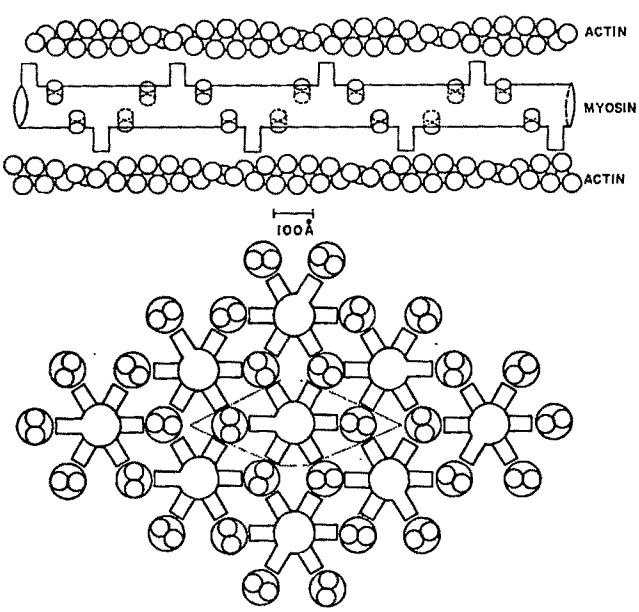


Fig. 1. Spatial arrangement of myosin and actin filaments in fixed striated muscle (rabbit psoas)

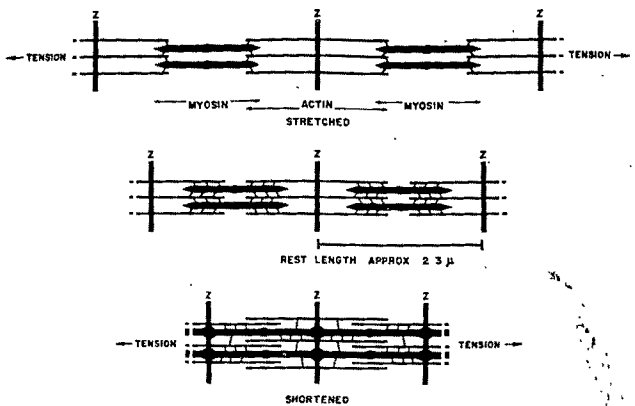


Fig. 2. Schematic diagram of contraction of skeletal muscle showing some completed cross-bridges at various lengths

(5) Tension development, shortening and work follow from the periodic making and breaking of cross-links between the myosin cross-bridges and the receptor sites on the actin[1,2,4].

(6) Adenosine triphosphate (ATP) is broken down both during smooth tetanic contractions and during single twitches. It is normally regenerated by ATP–creatine phosphoryl transferase and by myokinase during the rising phase of contraction. The net amount of 'high-energy' phosphate compound used is largely related to the work done by the muscles and, to a lesser extent, to the process associated with the active state[6].

(7) During the contraction cycle the actin of muscle remains in the form of double helical *F*-actin containing one bound ADP per *G*-actin sub-unit. This ADP is probably bound via calcium[2,7].

(8) The myosin filaments have one firmly bound ATP per H-meromyosin cross-bridge, and this ATP has a small but finite chance of dissociating[1,8]. It can be cleaved to ADP when it gets close to the *H*-meromyosin ATPase. The ADP formed can be rephosphorylated by, or exchanged with, free cytoplasmic ATP. The observed action of myokinase during a single contraction of frog muscle proves that this overall reaction must occur several times during the time the work is being done[6].

(II) Assumptions

The theory is based on the following assumptions.

(1) The binding site for ATP is at the end of a mobile polypeptide which is part of the *H*-meromyosin cross-bridge (Fig. 3). The bound ATP is electrostatically held by three positive charges which are probably three magnesium ions forming electrostatic links with negative groupings on the protein (cf. ref. 9).

(2) There is a fixed negative charge (perhaps phosphoryl-serine) inside the *H*-meromyosin cross-bridge and near the mobile polypeptide (Fig. 3) which repels the extra negative charge of the ionized ATP so that in the resting state the asymmetrically arranged polypeptide chain is on average extended (see section IV, 5).

(3) When the muscle is activated the depolarization of the sarcolemma and the sarcoplasmic reticulum liber-

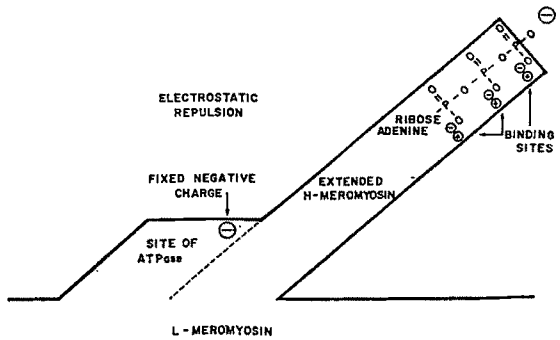


Fig. 3. Resting extended state. ATP bound to *H*-meromyosin

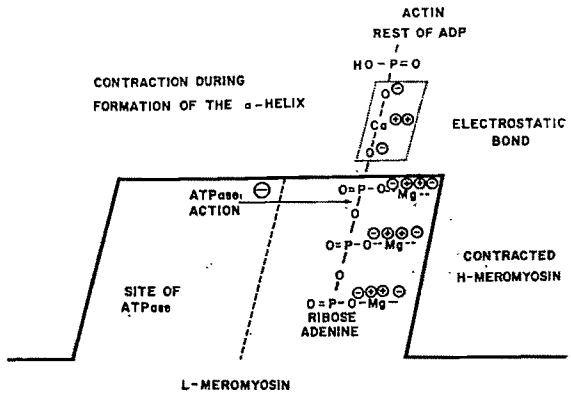


Fig. 5. Contracted *H*-meromyosin maintaining tension between itself and the bound ADP of actin

ates enough calcium to form chelate links between the bound ATP of the extended *H*-meromyosin polypeptide and the bound ADP of the *F*-actin (ref. 10) (Fig. 4). This step results in the formation of electrostatic bonds between the thick and thin filaments of the sarcomere, and by this fact causes the neutralization of the negative charge on the terminal phosphate and the abolition of the coulombic repulsion within the *H*-meromyosin. Thus the extended polypeptide in the cross-bridge contracts and forms an *a*-helix by twisting and forming hydrogen and hydrophobic bonds as in the elastic recovery of stretched *a*-keratin[11,12]. This process develops tension and, if the sum-total of the forces developed at any time by these ultramicro-contractions is sufficient to overcome the external load and the resistance of the series elastic elements, the actin filaments will be pulled along the myosin filaments into the A band and the muscle will undergo a quantal contraction (Fig. 4).

(4) The ATPase activity of the *H*-meromyosin cross-bridge is inside the cross-bridge and the active site does not have easy access to free cytoplasmic ATP. The contraction of the polypeptide chain brings the bound ATP into the region of activity of the *H*-meromyosin ATPase which then hydrolyses the ATP and breaks the link between myosin and actin[12] (Figs. 4–6). In the next step, the ADP bound to the cross-bridge is phosphorylated by cytoplasmic ATP in a process which probably requires magnesium. There could also be a slow exchange of the ADP with cytoplasmic ATP and this as well as 'product inhibition' could account for the inhibition of myosin ATPase by ADP (ref. 13). The change from ADP to ATP of necessity recreates the intense coulombic repulsion between the two negative charges so that part of the *H*-meromyosin is actively extended asymmetrically towards and along the nearby actin filament.

(5) The whole process repeats itself while calcium is available in the sarcoplasm (Fig. 4). The end of the 'active state' is taken to be the time when all the calcium has been pumped back into the sarcoplasmic reticulum by an active transport process which itself depends on ATP (relaxing factor)[5]. This requires that in the absence of stimulation the avidity for calcium is in the order: free solution < myosin ATP < inorganic phosphate in solution < ATP in solution ≈ link between actin ADP and inorganic phosphate < link between myosin ATP and actin ADP < sites for calcium in the sarcoplasmic reticulum. This sequence is reasonable on the basis of the apparent stability constants found by Smith and Alberty[14], and would result in low myosin ATPase activity at cross-bridges in regions where the myosin and actin filaments do not overlap, since the calcium would associate with the inorganic phosphate or ATP in solution rather than with the myosin ATP with its single free negative charge (see Section II, 1 and Fig. 5).

(III) Qualitative Conclusions

As has already been shown by A. F. Huxley[4], H. E. Huxley[1], Goodall[15], and Spencer and Worthington[15], many observations on muscles can be fitted to the inter-

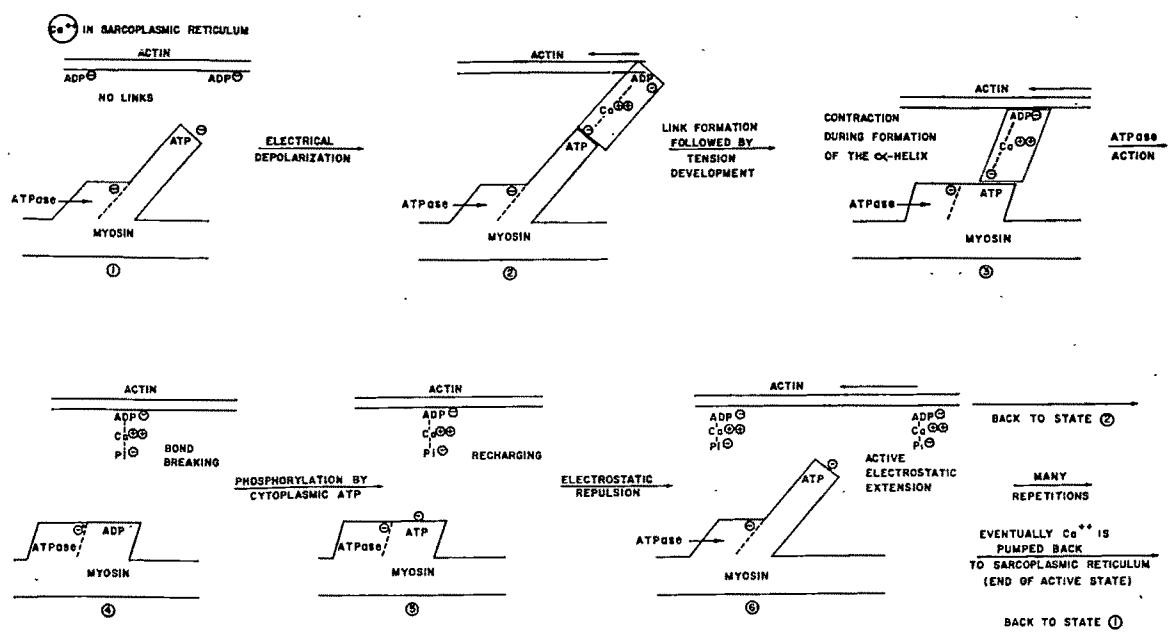


Fig. 4. Ca^{++}-dependent contraction during *a*-helix formation plus ATP-dependent extension of the cross-bridges

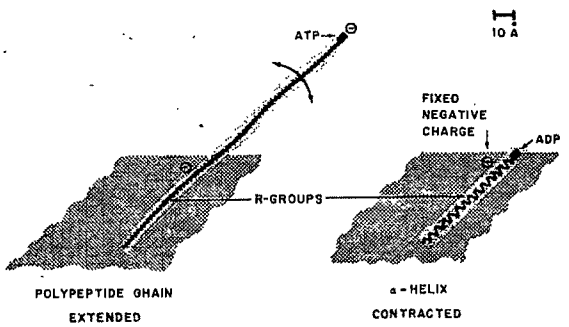


Fig. 6. Diagram of a flexible polypeptide chain of an *H*-meromyosin in the extended and contracted state

digitating filament theory of muscle contraction in which the chance of a local reaction between the myosin and actin filaments is inversely proportional to a function of the relative velocity of the filaments. These include: (1) the early increase in torsional rigidity; (2) the Hill equation relating force and velocity; (3) the loss and redevelopment of tension during 'quick release' of an activated muscle. The length-tension diagram has been explained by A. F. Huxley and H. E. Huxley as follows[1,4]. Tension is low at extreme lengths because the region of overlap of all the filaments is reduced and low at short-lengths both because the myosin filaments hit the *Z* lines and crumple, and because they are pushed apart laterally by the overlapping of the actin filaments which reduces the chance of link formation. Calculations of the rate of turnover of ATP necessary to drive such a model have been shown to be entirely reasonable on the basis of the enzyme activity in extracts of muscle[1].

The principle of the detailed theory presented here has some similarities to the possibilities put forward by Meyer[16], and Morales, Botts, Blum and Hill[16], and also to the explanation of the movement of the entropic-mechanochemical filaments of Kuhn[16]. However, only part of the cross-bridges are involved in the electrostatic extension and, in contrast to their views, the subsequent contraction causes a decrease of configurational entropy in the polypeptide chain. The energy for contraction comes from the formation of numerous hydrogen bonds which had been previously split by the action of ATP. These reactions could be part of the 'thermo-elastic' effect which is believed to account for the 'heat burst' during a 'quick release'[17], and the reversal of these reactions, as the peptides extended simultaneously, could be part of the absorption of heat during the subsequent spontaneous redevelopment of tension[17] (cf. Section IV, 6). The appearance of these reactions in activated muscles may well account for the apparent change in the thermo-elasticity of muscle from rubber-like when resting to steel-like when active[17].

The theory provides ready specific explanations for several other events occurring in muscle and leads to several predictions. These include:

(1) The amount of calcium that must be micro-injected into muscle to cause maximum contraction should be equal to at least the number of cross-bridges (see Section IV, 1).

(2) The length of the latent period should be the time needed for the calcium to get out from the sarcoplasmic reticulum to the sites where it links the actin and myosin and for the links to be formed (see Section IV, 2).

(3) The early increase then decrease in volume, and latency relaxation[18] are of the right order of magnitude to be manifestations of the expected changes in partial molal volume (compare ref. 19) associated with the differences in electrostriction between the states of the calcium tightly bound in the sarcoplasmic reticulum, in free solution in the sarcoplasm, and in the postulated bond between the nucleotides. Later volume changes can be associated with the splitting of ATP and phosphoryl-creatine, and with lactic acid formation[18].

(4) The increase of resistance to passive stretch[20] is associated with the formation of the calcium chelate links and occurs before contraction begins.

(5) Activation heat is a manifestation of the heat of dissociation of the calcium from its very firmly bound state in the sarcoplasmic reticulum. This follows depolarization and begins before tension development occurs (see Section IV, 3). It is little influenced by the length of the muscle. The heat uptake on forming the chelate links would be swamped by the output caused by the immediate contraction of the polypeptide (Section IV, 6). Work is needed later to pump back the calcium into the sarcoplasmic reticulum (see Section III, 9).

(6) The maintenance of tension would not *per se* require much ATP breakdown. This might explain the ability of some smooth muscles to remain in tension for very extended periods with only a small output of metabolic energy[21,22] (cf. Section III, 17).

(7) ATP and phosphorylcreatine breakdown are mainly associated with work[6,23]. ATP is broken down only if movement of the cross-bridges can occur. Since more links are formed and broken in a given time and over a given distance at low speeds of shortening with heavy loads than at high speeds with light loads both external work and power development depend on ATP splitting.

(8) In isometric contractions in fully pre-shortened and fully pre-extended muscles not much movement can occur and no significant ATP breakdown is detectable in rectus abdominis muscles with present techniques[24]. In any event, the work associated with any such movement would be low[25]. In maintained isometric tetani around rest length, micro inward movements and subsequent slippage of individual actin filaments could occur continuously since some filaments would have more and some less than the average muscle tension per square centimetre. Thus some ATP breakdown would be expected and does occur[24].

(9) The biochemical equivalent of the activation heat should be associated with the activity of the calcium pump which transports the calcium back into the sarcoplasmic reticulum[5]. The breakdown of 'high-energy' phosphate associated with this endothermic calcium binding (cf. Section IV, 3) would thus be delayed and seen during relaxation[6]. It is of the right order of magnitude to account for the expected amount of calcium involved (see Section IV, 1).

(10) In hypertonic solutions contraction fails or becomes very slow. It is known that the helical content of copoly-L-lysine-L-glutamic acid (1 : 1) is quite dependent on, and increases with, ionic strength. Such an attenuation of the electrostatic interactions of the charged groups would reduce the statistical length of the *H*-meromyosin polypeptide until it could not, or only occasionally, reach to the actin[26].

(11) 'Shortening heat'[17,27] is identified with the heat of formation of many hydrogen bonds during only the first contraction of each of the originally extended polypeptide chains (Figs. 4 and 6). This will continue during the creeping of the ends of the actin filaments along the myosin filaments as they form new links with each of the cross-bridges that they reach. This will be largely independent of length and load (but see Section IV, 6). Complete cyclic formation and breakage of the hydrogen bonds in the periodic contraction-extension steps will be thermally neutral, but during the decay of the active state all the chains will become re-extended with breakage of the hydrogen bonds. This will cause a heat absorption just equal to the earlier shortening heat because of the large increase in entropy. However, there is no net absorption of heat because of the larger heat output from the concomitant overall biochemical changes in the working muscle.

This reconciles Hill's observation of a shortening heat in moving, fully activated muscles with the absence of

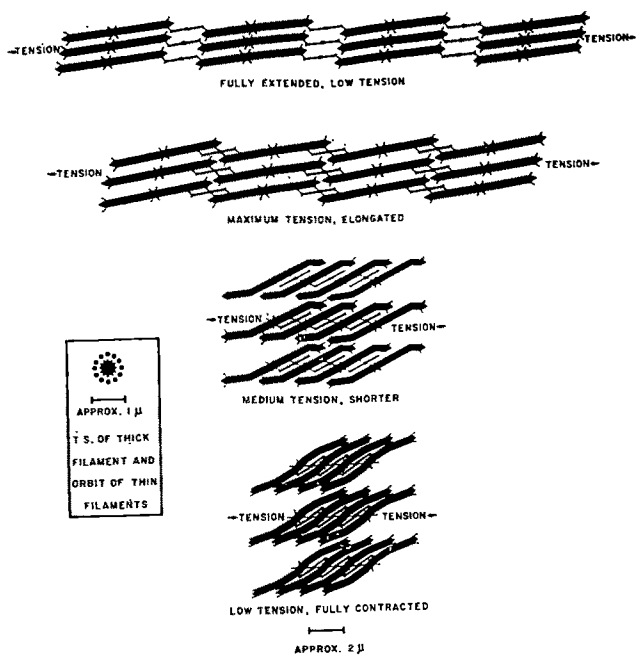


Fig. 7. Schematic diagram of contraction of smooth muscle (translucent adductor of oyster) showing some completed cross-bridges and developed tension at various lengths

such shortening heat in the complete cycle of contraction and relaxation in muscle twitches as found recently by Carlson, Hardy and Wilkie[23] (see Section IV, 6).

(12) Muscles in which most of the ATP has been broken down, and glycerinated muscle models in which the ATP but not the actin-bound ADP has been leached out, are in rigor and are stiff[28]. This is expected since the positive binding sites on the *H*-meromyosin would be strongly attracted to the actin ADP and the fixed negative charge of the *H*-meromyosin. The extended polypeptide would contract, actomyosin would be formed, and the whole system would become rigid. The addition of pyrophosphate, or ATP plus an ATPase inhibitor, plasticizes the muscle models in the presence of relaxing factors[28]. This latter would create state 1 of Fig. 4 with the actin and myosin repelling each other.

(13) Myosin ATPase requires magnesium and is strongly activated by calcium and by calcium plus actin, and there is an initial burst of ATPase activity[1,28,29]. This follows from Figs. 4 and 5.

(14) Relaxing factor is believed to operate by removing calcium from effective contact with the actin and myosin. Relaxation, or reduction of tension, also occurs on addition of excess ATP to muscle models. This would bind calcium and magnesium and tend to remove them from the myosin–actin link[5,26].

(15) The persistence of tension on stretching an activated muscle[1] is expected. The links would have less chance to be detached by the ATPase since the *H*-meromyosin polypeptide could not contract.

(16) The apparent 'disappearance' of energy during the forcible stretching of activated muscle[27] is identified as a reversal of the heat of formation of many hydrogen bonds during only the first extension of the largely contracted tension-bearing polypeptide chains (cf. Section III, 11), since for this reaction $-\Delta H$ is numerically greater than $-\Delta F$ (refs. 30–32) (see Section IV, 6). Stretching an activated muscle should, therefore, not lead to a resynthesis of ATP and such a muscle could readily absorb work with little energy output[24].

(17) This theory also makes possible a tentative unification of the basic mechanism of contractions occurring in skeletal and cardiac muscle with those in smooth muscle, vorticella, bacteriophage tails and perhaps flagellæ, cilia and sperm tails. There is good evidence for the operation of a sliding-filament mechanism in smooth muscles[21,33]. Fig. 7 shows a possible mechanism by which a particular smooth muscle could both be stretched by large amounts and contract by large amounts with formation of contraction bands (cf. ref. 34). This mechanism would also account for the helical arrangement of fibres in many smooth muscles. The existence of long thick filaments in many types of smooth muscle would account for the high tensions found because there will be large numbers of cross-links. For example, in the translucent adductor of the oyster, there are twelve thin filaments around each thick filament[21]. Hoffman-Berling[35] concluded that the contraction of vorticella stalks is due to the blocking of the excess negative charge on the contractile protein by calcium ions and that this is reversed in the relaxation phase by means of ATP. This is clearly very similar to the postulated contraction-relaxation cycle of part of the *H*-meromyosin presented here. Wahl and Kozloff[36] found that one nucleoside triphosphate and one calcium ion are associated with each sub-unit of the contractile part of the tail of T-even *E. coli* bacteriophages. The presence of the nucleotides seems to maintain the relaxed state, and contraction occurs only in the absence of ATP and is independent of ATP hydrolysis. This is similar to the rigor contraction of muscle. Several other types of contraction have been discussed by Weber[28].

(IV) Quantitative Conclusions

This theory can be used to make approximate quantitative estimations of: (1) the amount of calcium needed for maximum tension development; (2) the length of the latent period; (3) the heat of activation; (4) the force developed and the work done per ATP at the cross-bridges during muscle contraction; (5) the energetics of the individual extension–contraction steps; and (6) the heat changes during shortening and stretching of active muscle.

(1) According to H. E. Huxley[1] each half of each thick filament of rabbit psoas muscle has 18 sets of 6 helically arranged side pieces (Fig. 1). There are about $2{\cdot}5 \times 10^{14}$ thick filaments and $5{\cdot}4 \times 10^{16}$ cross-bridges per 1 ml. Since 1 g molecular weight contains 6×10^{23} molecules, the amount of calcium required to link with the ATP on each *H*-meromyosin is 0·09 μmoles/g. Heilbrunn and Wiercinski[37] obtained maximum contraction of small pieces of frog striated muscle fibres (adductor magnus of *Rana pipiens*) by intracellular injection of one-tenth volume of 1·25 mM calcium chloride, that is, 0·125 μmoles/g of muscle. Just discernible effects were obtained with 0·02 μmoles/g. Since some calcium is probably bound on other structures, this is a remarkably good agreement. Injection of higher concentrations caused local contraction and coagulation[37,38].

(2) This theory requires that, on activation, the calcium is removed from its binding sites and moves to the cross-bridges from the sarcoplasmic reticulum in twitch fibres or from the surface and invaginations of the sarcolemma in striated tonus fibres and in smooth muscle during the latent period, which is about 10 msec in frog sartorius at 0° C. Peachey (private communication) has shown that the diffusion distances involved in many varieties of fast muscle are of the order of 1 μ. Thus the diffusion time for calcium fits nicely in this period, even after allowing for a tortuous pathway around the filaments for several cycles of formation and breakage of bonds with cytoplasmic ATP and inorganic phosphate[39] and for the estimated time for the extended flexible ATP-bearing polypeptide chain to snake around into the exact position for link-formation. The intermediate chemical reactions would have much higher temperature coefficients than simple diffusion and this may account for the high temperature coefficient of the latent period[1]. The rediscovery of the sarcoplasmic reticulum has thus solved the apparent mystery of the problem of excitation–contraction coupling[40].

(3) The heat of activation is identified here as the heat associated with the removal of calcium from the sarcoplasmic reticulum. This amount of heat is not known at present, but an order of magnitude calculation may be made from the heat of dissociation of calcium phosphate which is +5 kcal/mole (ref. 14). Since some extra energy must be provided, probably from the influx of sodium ions on excitation, in order to free this tightly bound calcium, this heat is probably between 5 and 10 kcal/mole. If the total heat for this step were 10 kcal/mole the heat of activation would be 0·9 mcal/g muscle in agreement with the 1–1·5 mcal/g (ref. 41) found by thermal measurements.

(4) Recent results from these laboratories[6] show that under normal physiological conditions 0·64 μmole ATP/g muscle is used when 120 g-cm of external work is done in a single contraction of frog rectus abdominis muscle. Under these conditions the actual free energy of ATP hydrolysis to ADP is about 10 kcal/mole (ref. 42), but is less when the ratio of products to reactants increases[6]. Thus the normal efficiency of energy transduction is about 44 per cent (that is, this step is nearly thermally neutral[30]) and, of the 7×10^{-13} erg from each molecule of ATP, about 3×10^{-13} erg is used for work. H. E. Huxley[1] has found that the number of cross-bridges in each tension-bearing unit of half a sarcomere length in a 1 cm² cross-section of rabbit psoas muscle is about $6{\cdot}5 \times 10^{12}$. Since the maximum tension developed by skeletal muscle is approximately 2 kg/cm², and this would be when all the cross-bridges are linked to actin, the force per link is on average 3×10^{-7} dyne. Since the work is 3×10^{-13} erg the distance moved is about 100 Å per ATP. A similar conclusion has been reached by both H. E. Huxley[1] and A. F. Huxley[4], but their calculations were based on estimates from measurements of heat changes and work done during contraction, and are thus thermodynamically objectionable since entropy changes were neglected and, in any event, were unknown. However, if, on average, only half the cross-bridges could link to actin at maximum tension, the movement per ATP would be about 50 Å, that is, about the length of a *G*-actin sub-unit of a F-actin filament, and the force would be twice as great per link for the same work per ATP molecule. In any event, it seems possible that the actin may spin as it slides in, and thus maintain optimal steric conditions for cross-link formation.

(5) It is clear that when a muscle moves quickly there is less chance of any particular cross-link being formed in time since this depends on a precise hit of the actin site, the calcium and the bound ATP. If a muscle is stretched until the actin filaments just overlap the myosin filaments, and then stimulated and made to do maximal work during contraction until the ends of the actin filaments reach the innermost cross-bridge of the myosin filaments, this should require a movement of 18 × 400 Å (Fig. 1), that is, 7200 Å per half sarcomere. There would be an average of 36 contraction-relaxation cycles per cross-bridge for a movement equal to 63 per cent of the rest length of the muscle, which would cause a breakdown of $36 \times 0{\cdot}09 = 3{\cdot}2$ μmoles ATP/g. This can be tested experimentally. In the frog muscles[6] (rectus abdominis) which did 120 g-cm of work and contracted by 41 per cent for a breakdown of 0·64 μmole ATP/g, the average number of contraction-relaxation cycles was $0{\cdot}64/0{\cdot}09 = 7$ per cross-bridge. Thus, most of the cross-bridges did not form links continuously during all the total rapid movement of about 4700 Å per half sarcomere.

Assuming that for maximum velocity of shortening in unloaded vertebrate muscles only one link is needed on the average between each actin filament and a myosin filament then such a muscle, shortening from rest length by even 50 per cent would break down only about 0·1 μmoles ATP/g, but at a very rapid rate.

This accounts for why it has been found that the amount of ATP, measured as phosphorylcreatine, cleaved during single contractions of unloaded rectus abdominis muscle, is so low as to be not significantly different from zero with the present experimental errors and biological variability[6]. This could also explain similar results on the lack of increase of creatine in contraction of lightly loaded turtle muscles found by Mommaerts *et al.*[43].

It follows that there is no major 'shortening' ATP breakdown[6,23], independent of work, corresponding to Hill's 'shortening heat'[27].

This theory is based on the assumption that the work done by the repulsion of the (assumed) fixed negative charge on the *H*-meromyosin and the negative charge on the terminal phosphate of the bound ATP comes from the distortion of the ATP and exists as potential energy in the extended polypeptide chains. On neutralization of this negative charge of ATP by calcium ions, the structure contracts to an α-helix, develops tension, and delivers up as work the excess of the free energy of hydrogen-bond (and hydrophobic-bond) formation over that associated with the loss of configurational entropy of the structure. The potential energy gained by the structure can be calculated roughly from the data given by Schwarzenbach[44]. His results show that a dielectric constant of about 12 is reasonable for a region that would be full of organic material, since the repulsion acts through the extended polypeptide chain and its associated peripheral organic residues. Thus the system would carry the electric force like an insulated cable. It follows from his Fig. 1 that movements of the charges from a distance of 6 Å–110° Å would involve just 3×10^{-13} erg per pair of charges. Most of this energy change would occur in the first few ångströms and would be needed to start the splitting of all the hydrogen bonds to open the α-helix. Thus the amount of energy needed for the active extension of the cross-bridge polypeptide is in very satisfactory agreement with that available and is, of course, delivered to the system when the bound ADP is phosphorylated by cytoplasmic ATP (Fig. 4) near to the fixed negative charge on the *H*-meromyosin. Thus 40 per cent of the energy delivered would be used to extend the polypeptide chain. Clearly, the maximum amount of work which could be recovered from the system during the subsequent contraction (Fig. 4) is the free energy put into it during the active extension. However, the actual work would be less if the reformation of the α-helix delivered less energy during contraction. It is not possible to calculate or measure this precisely because the exact structural details are not yet known. However, if the situation is similar to that in Fig. 4, a reasonable conclusion can be reached.

Investigations of the helix-coil transformation of several synthetic polypeptides have shown the importance of hydrogen-bond formation, configurational entropy of extension, electrostatic interactions, hydrophobic-bond formation, and "end effects"[31]. Thus polyglutamic acid only exists as an α-helix at a low *p*H value. It becomes a random coil at high *p*H values because of repulsion between the ionized carboxyl groups which will tend to extend small lengths of the chain into the fully extended β configuration. α-Helices have a pitch of about 5·4 Å and contain 3·6 residues per turn, so if the terminal portion of the mobile *H*-meromyosin polypeptide contains 50 amino-acid residues it would have nearly 14 turns in the α-helix configuration and be 75 Å long (Fig. 6). In the randomly coiling form the electrostatic repulsion for such a small polypeptide would favour the existence of an extended form of the chain for part of the time. It would be a helix of 3·0 residues per turn with the amino-acid *R* groups extending out at 120° from each other. The backbone would be 3·6 Å per residue and, since its total length would then be 180 Å, it would have extended by 105 Å. The α-helical form would fit neatly into the observed height of the side pieces as seen in electron-micrographs of muscle.

The helix-coil transformation of polyglutamic acid of molecular weight 22,000 occurs in the *p*H range of 4·6–

5·2 at 20° C in 0·2 M sodium chloride and over a change in the degree of ionization of 0·36–0·64. This shows that the stabilization of the α-helix by the 3·6 hydrogen bonds per turn counterbalances the loss of configurational entropy on forming the rigid structure. This does not mean that for a 50-residue *H*-meromyosin α-helix to be unstable more than 18 ionized carboxyls need to be present on the periphery of the protein cylinder, small helices being inherently unstable due to 'end-effects' since four hydrogen bonds cannot be formed. Small helices probably have critical sizes of the order of 15–20 residues, neglecting residue interaction, but even so it is likely that several glutamic or aspartic acid residues would be needed to loosen this postulated structure to the point where it is almost unstable and then an extra negative charge would cause the unzipping of all the oriented hydrogen bonds with a consequent gain in configurational entropy[31]. Since the α-helix could be made more or less stable by appropriate changes in the *R*-groups, it follows that for this tension-bearing peptide chain, virtually all the work done by the repulsion of the fixed negative charge and that on the terminal phosphate of the ATP could be used effectively. On the neutralization of this negative charge on the ATP the system would spontaneously contract smoothly, assisted by Brownian vibrations, and gain energy for additional work by increments as each of the 46 hydrogen bonds was formed by the 50 residues. Most of the net free energy of the change to intramolecular hydrogen-bond formation (approx. 1,000 cal/mole)[32] would be balanced by charge interaction of the *R*-groups and by the loss of configurational entropy. The rest would do the work of muscle contraction, that is, 3×10^{-13} erg/cross-link, and would develop an average of 3×10^{-7} dyne during a 100 Å contraction of each mobile polypeptide. This is a simplified statement since there will be changes in the complex interactions between the amino-acid *R*-groups.

It is interesting that the work done by the ATP in extending the polypeptide, and the free energy released by the formation of hydrogen bonds on contraction are equal. However, the stretching force from the charged ATP varies inversely as the square of the distance whereas the contractile force will be the summation of many equal pulses as the peptide coils to form the α-helix. Thus the fully extended chain will be unstable and only a small percentage of the time will be spent in this configuration. In any event, as the extended chain snakes by thermal motion the chain will bend and allow some of the aqueous phase of the cytoplasm to get between the bound ATP and the fixed negative charge so the effective dielectric constant will increase, the repulsion drop and the chain start to contract to a helix. However, as soon as it had pulled itself back sufficiently it would have to straighten out again so the repulsion would increase again and return the chain to its more extended form (Fig. 6). Thus this polypeptide of the *H*-meromyosin with the bound ATP on its end would spend much of its time in a partly random coil configuration but, because of the charge repulsion and Brownian motion, would exist for part of the time as a fully extended chain. It would continuously sweep out the available volume and, in the presence of calcium, would be able to make a link with any *F*-actin ADP site within reach.

It is necessary to show that α-helices could bear the tension developed in muscle. Enomoto and Krimm[45] recorded values and calculated that the elastic modulus in several natural and uncharged synthetic polypeptides is of the order of 5×10^{10} dynes/cm² for α-helices and 2×10^{11} dynes/cm² for extended chains. This elastic modulus of 5×10^{10} dynes/cm² is based on 10^{14} helices/cm² with an overall helical diameter of about 10 Å. The peculiar structure of muscle with its effective $6{\cdot}5 \times 10^{12}$ sites/cm² means that there are about one-fifteenth as many helical cross-bridge polypeptides available as in normal fibrous proteins. Thus the maximum tension in muscle (approximately 2×10^6 dynes/cm²) would extend the cross-bridge α-helices by only about 0·1 per cent if they were similar to those studied[45] (cf. Section III, 17, for smooth muscle). This shows that the proposed tension-bearing structure would be able to bear the actual tension. It may even need to be made less stable by electrostatic repulsion of, for example, glutamic or aspartic acid residues (which occur in *H*-meromyosin[46]) so that the extra negative charge from the bound ATP could extend the structure and break the α-helix. Since the α-helix is well able to bear the tension, the extended chain, which has a higher elastic modulus, must also be able to do this. The strong electrostatic attractions operating over only a few Å which chelate the calcium to the *H*-meromyosin ATP and the *F*-actin ADP and which bind the ATP to the myosin are also clearly able to bear the tension of muscle[44,47].

(6) The frog muscles[6] (rectus abdominis) (Section IV, 5), which did a full smooth tetanic contraction, weighed 180 mg and contracted by 2·1 cm. The isometric tension, P_0, was 65 g-wt., so Hill's[17] constant a was 16 g-wt. and the experimental shortening heat[17] would be 34 g-cm, that is, 190 g-cm/g or 4·4 mcal/g (cf. Hill[41]). This contraction would cause all the cross-bridges to form α-helices during the shortening (Section IV, 5).

The initial formation of the calcium chelate link between the *H*-meromyosin and the actin (Fig. 4) probably causes a heat uptake of the order of 10,000 cal/mole (cf. Section IV, 3, and ref. 14). This, together with the 46 hydrogen bonds formed per bridge (Section IV, 5) and a heat output of 1,500 cal/mole of hydrogen bonds[31,32], gives a calculated heat of shortening of 5·3 mcal/g which would be reduced proportionately if the polypeptide were already somewhat contracted when the chelate link was formed. This is a very satisfactory agreement for such a rough calculation. The theory predicts that shortening heat should be less during very lightly loaded, and hence very rapid, contractions when many cross-bridges will not interact even once.

An activated muscle will have a similar but negative heat of extension from the reverse of these reactions (cf. Section III, 11), since the work (free energy) needed to stretch the partially contracted tension-bearing polypeptide cross-bridges (4,000 cal/mole bridges) is only about one-seventeenth the associated heat change (see Sections III, 16; IV, 5. 6, refs. 30–32). The stretch will eventually pull apart the calcium chelate bonds so the tension should, and does[1], rise above the maximum isometric tension. The extended chain with its bound calcium will start to contract freely since the negative charge on the bound ATP remains neutralized, but it could form another link with actin as it shortens and would again resist the external stretching force (Figs. 1–6). Most important, the cyclic breakdown of ATP (coupled to phosphorylcreatine) will not have occurred and this could account for the observation that Hill's constant a for the negative heat of lengthening[48] can be several times that for the heat of shortening.

It is thus expected that "mechanical work applied to a contracting muscle by stretching it may reduce the net energy liberated by the muscle itself, either to nothing, or to an amount much less than in a similar contraction without stretch"[48].

This theory proposes that the activation of muscle releases bound calcium from the sarcolemma and the sarcoplasmic reticulum. This calcium diffuses and forms chelate links between bound ATP at the end of an asymmetrically extended rapidly snaking polypeptide of the cross-bridges of the *H*-meromyosins of the myosin filaments and the bound ADP of the *F*-actin filaments. The calcium neutralizes the electric charge on the bound ATP of the polypeptide which spontaneously contracts to an α-helix by the energy of hydrogen-bond and hydrophobic-bond formation. This contraction drags the actin filament along the myosin filament. This brings the ATP into

the range of action of the *H*-meromyosin ATPase, which cleaves off the terminal phosphate and breaks the link. On rephosphorylation of the ADP the helix is pulled out to a largely extended chain by the repulsion of the negative charge on the ATP and a fixed charge on the *H*-meromyosin. This cycle is repeated during the active state, which ends when the calcium is pumped back into the sarcoplasmic reticulum and sarcolemma. It seems that, in principle, this type of mechanism, which includes both sliding and folding, can account for many qualitative and quantitative observations on contraction of skeletal, cardiac and smooth muscles and of several other biological systems. It leads to predictions which can be tested experimentally.

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A NEW STAPHYLOCOCCAL ANTIGEN

Its Preparation and Immunizing Activity against Experimental Infections

IN the course of investigations in these laboratories on protective antibody against the *Smith* strain of *Staphylococcus aureus* in human γ-globulin[1,2] and human serum[3], it appeared to one of us (M. W. F.) that *Staphylococci* of this type may be immunologically unique. We therefore investigated this and other staphylococcal varieties with respect to their ability to induce resistance to challenge infections. After testing and evaluating the mouse-protective immunogenicity of vaccines made from numerous isolates of *S. aureus*, we discovered that only certain infrequently occurring strains had this property. We further ascertained that such strains were generally of the *Smith* type and that by treating cultures of these strains in the manner to be described here, a distinctive highly potent immunizing polysaccharide could be produced. This polysaccharide, which we have designated 'SPA' (staphylococcal polysaccharide antigen), is described in the following communication with respect to its physical and chemical characteristics. This communication deals with the preparation of SPA from cells and presents some of its salient biological properties.

SPA is prepared from cells of an especially useful strain of *S. aureus*, designated by us as '05068'. Like the *Smith* strain, '05068' is highly virulent for mice, it produces diffuse colonies in serum-soft agar[4], and it is not lysed by standard phages. Trypticase soy broth (Baltimore Biological Laboratories) was used for relatively small-scale cultivation of this organism under static or shake flask conditions.

Two procedures were developed to produce SPA. One method consisted of mechanically blending a washed cell mass with 85 per cent phenol at 50°–55° C for 4–6 min.

The resultant phenol-insoluble precipitate was washed with 85 per cent phenol, suspended in water and dialysed to remove phenol. The water-insoluble portion, which contained most of the immunizing activity, was treated with 0·1 N acetic acid to *p*H 3·2 and autoclaved at 121° C for 15 min. This process resulted in a water-soluble preparation of high SPA content.

Another and simpler process consisted of directly treating the washed cell mass with 0·1 N acetic acid at 100° C for 5–20 min. This method also provided a water-soluble portion rich in SPA, with the cellular debris being essentially inactive. Procedures for the further purification of SPA as well as for its colorimetric assay will be reported in detail elsewhere.

The high degree of immunizing activity of SPA for mice is illustrated by the results in Table 1. In this typical test, groups of female *CF*-1 albino mice, 18–22 g, were injected subcutaneously with a single dose of various amounts of SPA. Seven days later these and untreated mice were acutely challenged with 10^4 LD_{50} of '05068' staphylococci given intraperitoneally in 5 per cent hog gastric mucin. The survival percentages, based on observations made 5 days later from initial groups of 20 mice, indicate a 50 per cent protective dose (PD_{50}) of about 0·004 μg/mouse.

'Immune paralysis', similar to that noted for pneumococcal polysaccharide[5], is also revealed by the results in Table 1. In this example, the 50 per cent 'paralysing dose' of SPA is estimated as 0·12 μg/mouse, or 30 times greater than the PD_{50}.

SPA will also immunize mice against challenge by other strains of *S. aureus*. Unequivocal resistance to challenge was found in experiments, like that previously described involving the following strains: *Smith, H and D, Castellani, 1-AV, S193N4, MCG-138, K6, K93, K93M, Adams* and *Welwood*. In addition to an apparent immunological homogeneity, these strains have the common feature of being unusually virulent for mice. A low degree of elevated resistance was provided by SPA to mice challenged with various isolates of phage type 80/81. However, the necessity for use of very heavy challenge doses of 80/81 staphylococci may have served to occlude or diminish immune effects. SPA did not alter the susceptibility of mice to challenge by any of the following bacteria: *Diplococcus pneumoniae, Streptococcus pyogenes, Strept. salivarius, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa*.

Challenge immunity was detectable in mice during 36–48 h after an injection of SPA and such resistance was still demonstrable 70 days later. SPA appeared to be of equivalent efficacy whether given subcutaneously, intradermally, intramuscularly or intravenously. However, multiple or divided doses of SPA proved to be less immunogenic for mice than a single dose.

The direct relationship between protective antibody and challenge resistance in mice is indicated by the results shown in Table 2. In this experiment groups of 40 mice were injected with various doses of SPA. Seven days later, half the mice were challenged with 10^3 LD_{50} of the *S193N4* strain, given intraperitoneally in mucin. The remaining 20 mice at each SPA dose-level were exsanguinated, their serum pooled, and a passive test run on each.

Further details of extensive biological studies on SPA will be reported at a later date. These other experiences have revealed that SPA will stimulate the appearance of protective serum antibody in mice, dogs, and human beings, but will not do so in guinea pigs, rabbits and monkeys. The ability of SPA to prevent or modify natural infections of animals and man is at present being investigated and the results of these studies should serve to resolve the disagreement[6,7] on the relevance of *Smith* strain immunology to clinical disease.

Table 1. Mouse Immunizing Activity of SPA

SPA dose (μg/mouse)	Per cent protected
None (untreated)	0
0·002	5
0·006	50
0·02	60
0·06	80
0·2	20
0·6	0

Table 2. Correlation of Active and Passive Immunity in Mice

SPA dose (μg/mouse)	Per cent protected vs. challenge	Serum titre*
None (untreated)	0	<2
0·001	20	20
0·01	75	40
0·1	100	180
1·0	100	350
10·0	65	65
100·0	5	<2

* Reciprocal of 50 per cent protective dilution of serum[3].

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2-Amino-2-deoxy-D-glucuronic Acid, the Main Constituent of SPA (Staphylococcal Polysaccharide Antigen)

In the preceding communication, Fisher *et al.* have reported on the preparation and biological evaluation of a pure polysaccharide antigen from the cells of a staphylococcal strain of unique immunogenicity. This product, designated SPA, was shown to possess active immunizing properties in mice, dogs and man. We wish to report some of the physical and chemical properties of SPA together with degradation studies leading to the elucidation of its main structural features. The polysaccharide consists of 2-acetamido-2-deoxy-D-glucuronic acid and 2-[(*N*-acetylalanyl)amino]-2-deoxy-D-glucuronic acid residues linked by 1 → 4 type linkage (probably β). Active antigenic preparations also contained about 7–8 per cent *O*-acetyl groups as determined by the quantitative hydroxamate test[1].

Acidic hydrolysis techniques were used by Fisher *et al.* to produce SPA from whole bacterial cells. This was most conveniently carried out by heating the cells with 0·1 N acetic acid at 100° for brief periods (5–20 min). We also have produced SPA by stirring the cells with 5 per cent trichloroacetic acid at room temperature for 20–24 h. The crude SPA was then purified by a combination of methods, including carbon chromatography and alcohol fractionation. Pure SPA was found to be an acidic polysaccharide free of nucleic acid, protein and lipid.

SPA could be detected and its electrophoretic mobility studied on both glass fibre[2] and paper strips. It appeared as a pink spot when sprayed with the *p*-anisidine–sulphuric acid in *n*-butanol reagent on glass fibre strips. Detection on paper strips was accomplished by utilizing the amide spray of Rydon and Smith[3]. At *p*H values greater than 4, SPA migrated as an anion. In the Tiselius apparatus, SPA migrated as a homogeneous entity with an observed mobility constant of $5{\cdot}87 \times 10^{-5}$ cm^2 sec^{-1} V^{-1} in *p*H 6·0 phosphate buffer. Homogeneity was also apparent from ultracentrifugal studies whereby a single symmetrical peak, $S^{w}_{20} = 1{\cdot}7$ Svedberg units, was obtained in 0·1 M phosphate buffer at *p*H 7·0 for material prepared by extraction with trichloroacetic acid.

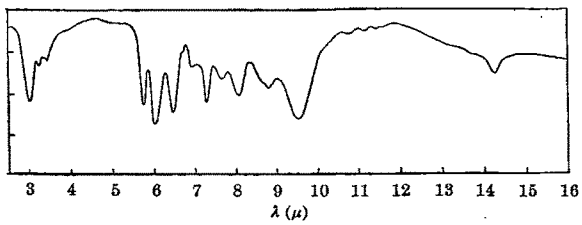


Fig. 1. Infra-red spectrum of SPA in compressed potassium bromide

Purified SPA is a white amorphous solid which in its salt form is freely soluble in water and is not readily precipitated from dilute salt solutions with alcohol. The polysaccharide shows no absorption maxima in the ultra-violet range. The infra-red spectrum of the free acid form samples of SPA prepared from various source materials is shown in Fig. 1. Elemental analysis of six different showed the following average values: C, 44·7; H, 6·30; N, 8·20; P, < 0·06, ash 0·38. Aqueous solutions were strongly lævorotatory ($[\alpha]_D^{23} - 91°$, *C* 1, in 0·1 M phosphate buffer at *p*H 7·0). The polysaccharide had an acidic dissociation constant (pK_a) of 3·3 in water. The failure to be adsorbed on sulphonic acid exchange resins demonstrated the absence of basic groups in SPA. Tests for uronic and sialic acids and with the Molisch or anthrone reagents were negative. The intact polysaccharide gave a negligible reducing value and failed to reduce periodate solutions.

Acid hydrolysis (4 N hydrochloric acid at 100°) revealed the presence of a neutral amino-acid, an amphoteric amino-sugar, and a considerable amount of humin. No other known sugar component was detected in hydrolysis studies under a variety of conditions. The amino-sugar moved by endosmosis during electrophoresis at *p*H 5 and gave positive tests with ninhydrin, Elson-Morgan[4] and silver nitrate[5] reagents. On paper chromatograms the amino-sugar had an identical mobility to that reported for synthetic 2-amino-2-deoxy-D-glucuronic acid[6]. Since efforts to crystallize this substance failed, the parent polysaccharide was converted to the methyl ester (0·07 N methanolic hydrogen chloride for 24 h or diazomethane in methanol–water). The ester was reduced with lithium borohydride in formamide–tetrahydrofuran solution until the 5·78-μ ester band had practically disappeared from the infra-red spectrum of the product. Acid hydrolysis of the reduced polysaccharide resulted in the formation of much less humin and a new amino-sugar corresponding to glucosamine by paper chromatography. Crystalline D-glucosamine hydrochloride was afterwards isolated from such hydrolysates and characterized by optical rotation and X-ray diffraction pattern data. The precursor of D-glucosamine and the only detectable carbohydrate present in SPA is therefore 2-amino-2-deoxy-D-glucuronic acid. The neutral amino-acid present in SPA was found to be L-alanine and was characterized by optical rotation, infra-red spectra and microanalysis.

Aminohexuronic acids have been previously isolated as decomposition products of bacterial cell walls. Heyns *et al.*[7] found 2-amino-2-deoxy-D-galacturonic acid to be the main constituent of the *Vi* antigen, while Perkins[8] reported the presence of 2-amino-2-deoxy-D-mannuronic acid in hydrolysates of cell walls of *Micrococcus lysodeikticus*. Perkins[8] also suggested the presence of 2-amino-2-deoxy-D-glucuronic acid in an antigen described by Morse[9] which is different from but possibly related to SPA.

Methylation and hydrolysis examinations of the parent and reduced polysaccharides confirmed the glycosidic linkage as 1 → 4. Thus the reduced polysaccharide was methylated (14·53 per cent OCH_3) by a modification of a method described by Stacey and Wooley[10] and on hydrolysis (2 N hydrochloric acid, 3 h) afforded a major methylated monosaccharide which was isolated by preparative paper chromatography. Selective *N*-acetylation then afforded crystalline 2-acetamido-2-deoxy-3,6-di-*O*-methyl-D-glucopyranose, identical with an authentic sample. The only other detectable methylated monosaccharide was formed in small amount and corresponded to 2-amino-2-deoxy-6-*O*-methyl-D-glucopyranose.

Alternatively the parent polysaccharide was methylated (9·0 per cent OCH_3) by a modification of the Kuhn procedure[11] and the product was then esterified and reduced with lithium borohydride. Acid hydrolysis followed by separation and N-acetylation of the single methylated monosaccharide product afforded crystalline 2-acetamido-2-deoxy-3-*O*-methyl-D-glucopyranose, identical in all respects to an authentic sample.

The identity of the repeating monosaccharide units in SPA was established indirectly by mild acid hydrolysis (1 N acetic acid at 108°, 16 h in a sealed system) of the reduced polysaccharide. Under these conditions, the totally dialysable products consisted predominantly of an *N*-acyl-D-glucosamine derivative. Small amounts of alanine, D-glucosamine and a sugar with identical electrophoretic and paper chromatographic mobility as 2-(alanylamino)-2-deoxy-D-glucose were formed. Purification and separation of the *N*-acyl-D-glucosamine component by chromatographic techniques afforded a colourless solid which, on acid hydrolysis, revealed alanine and glucosamine only. Since synthetic mixtures of 2-acetamido-2-deoxy-D-glucopyranose and 2-[(N-acetylalanyl) amino]-2-deoxy-D-glucopyranose could not be separated on paper chromatograms with a variety of solvent systems, optimum hydrolysis conditions for the liberation of alanine and glucosamine from above mixtures were established. Quantitative analysis of the *N*-acyl-D-glucosamine product showed an alanine–glucosamine ratio of 1 : 2. A solution of the *N*-acyl-D-glucosamine component in a water–methanol–acetone mixture deposited crystals of 2-acetamido-2-deoxy-D-glucopyranose. The mother liquors consisted predominantly of 2-[(*N*-acetylalanyl) amino]-2-deoxy-D-glucopyranose. The repeating units of SPA therefore appear to be equimolar amounts of 2-acetamido-2-deoxy-D-glucuronic acid and 2-[(N-acetylalanyl)amino]-2-deoxy-D-glucuronic acid.

Mild acid hydrolysis on the parent polysaccharide (SPA) as described here also produced a completely dialysable product. The main components were isolated by chromatographic techniques and consisted of a mixture of *N*-acyl-D-glucosaminuronic acid and its lactone as evidenced by electrophoresis studies at *p*H 5 and colorations with the Morgan–Elson[5] and hydroxylamine sprays[12]. Solutions of the lactone remained homogeneous on chromatography, while the *N*-acylated component equilibrated partially to the lactone.

Staphyloccal polysaccharide antigen, therefore, differs chemically from all other staphylococcal degradation products previously described in that it is composed of two main components, L-alanine and 2-amino-2-deoxy-D-glucuronic acid with *O*- and *N*-substitution by acetyl.

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INFLUENCE OF TEMPERATURE AND AGEING ON ETHYLENE PRODUCTION BY A SUB-CELLULAR FRACTION FROM TOMATOES

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THE production of ethylene by sub-cellular particles is affected by many conditions, including disintegration of the particles, and reaction mixture constituents[1–3]. In an attempt to resolve to some extent the evident complexity of factors governing the evolution of ethylene by sub-cellular fractions, studies were made of the effects of temperature and ageing on production of the gas. Preparation of the fraction separating at 35,000*g* from tomato homogenates, and the method of analysis for ethylene, have been described previously[4,5]. Confirmatory analyses were made periodically by direct sampling of the gas mixture in the reaction vessel without absorption in mercuric perchlorate, and by disappearance of the ethylene peak from the gas chromatogram when the sample was shaken with bromine water. Comparisons were made only among fractions from the same particulate preparation, since considerable variation in ethylene-producing activity may be expected with particles from different runs[4].

Meigh[2] reported that boiling or evacuating a mitochondrial suspension from tomatoes before sonication had little effect on hydrocarbon production whereas these treatments after sonication resulted in marked decreases. (He did not specify whether comparisons were made among particles from the same preparation.) In drawing conclusions about the ethylene-producing activities of boiled and unboiled particles, distinction must be made between pre-formed ethylene, liberated on disruption of the particles, and that actually formed after boiling. We found that when the particulate suspension was evacuated and then aerated to remove much of the ethylene initially present, boiled particles on sonication gave two-fifths the amount of ethylene produced by unboiled particles, identically evacuated, aerated and sonicated. Moreover, there was no further ethylene liberated by the boiled fraction on ageing for 20 h. The presence of active, unboiled particles was necessary for this evolution.

When the particles were sonicated before boiling, and the ethylene liberated during these processes was removed by aeration and evacuation, little or no ethylene was detectable. However, if collection of ethylene was made during boiling of the sonicated particles, large amounts of gas were obtained (Table 1, run 8). This high initial outburst might be suggestive of increased enzyme activity for the short time prior to denaturation of enzymes, or of thermal activation of strictly chemical or physical reactions. In all our experiments no ethylene was evolved by boiled particles after the first hour of collection, whereas unboiled particles continued to produce the gas. In our earlier work[1,4] also, we showed a continued production by unboiled particles during a 4–20-h reaction period.

Evacuation of the sonicated, unboiled particulate suspension and maintenance in a closed system[2] would be expected to result in lower ethylene production for a 2-h collection period. This might, for example, be due to removal of ethylene immediately evolved on disruption of the particles.

Further insight into the nature of the processes involved in ethylene production was sought from experiments in which the effects of intermediate temperatures on ethylene evolution were studied. Representative results are shown in Table 1. For these runs the sub-cellular particles were sonicated in the complete substrate and the reaction vessels were connected to the collection system before immersion in water baths of the appropriate temperature.

Table 1. Effects of Temperature on Ethylene Evolution by Sonicated Sub-cellular Particles from Tomatoes

		C_2H_4 (mμl.)							
	Run No.:	1		2		3		4	
Time (h)	Temp. (°C):	25	0	25	15	25	20	25	30
0–2		369	126	211	268	145	158	401	347
2–4		43	158	189	326	211	189	236	> 441
4–20		43	137	↓	↓	↓	↓	↓	↓
Total		455	421	400	594	356	347	637	> 788
		5		6		7		8	
		25	40	25	50	25	60	25	100
0–2		482	473	99	66	132	115	225	307
2–4		316	95	241	180	T	T	47	N
4–20		↓	↓	↓	↓	20	47	—	—
Total		798	568	340	246	152	162	272	307

Particles were suspended in the complete reaction mixture: 0·5 M sucrose, 0·125 M KH_2PO_4, 10^{-3} M $MgSO_4$, 10^{-3} M $MnSO_4$, 0·05 M L-malic acid, adjusted to *p*H 7·0 with NaOH; ATP (10^{-3} M) was added after sonication.
N = not detectable: T = trace.

For each run, half the reaction mixture was kept at 25° C, and the other half at a different temperature.

When ethylene evolution at 25° C was used as a basis for comparison, it was observed that the initial (0–2 h) gas production was less at 0° C and greater at 100° C, while at intermediate temperatures it did not differ greatly from that at 25°. Evolution for the subsequent period was greater at 0° and 15° than at 25° C, about the same at 20° and more at 30° C. At 40° and 50° it decreased, and at 100° there was no ethylene production during this period. Total evolution over the entire 20-h period relative to the 25° controls was highest at 15° and 30° (Table 1).

These results suggest that the initial production may not be entirely due to enzymatically controlled reactions, although, as mentioned previously, the high initial evolution at 100° could be the result of increased enzyme activity for a short interval before denaturation commences. Consideration of the ethylene yield for the 2–20-h period, as well as the total ethylene yield, reveals a common maximum in close agreement with the optimum temperature of 33° reported for ethylene production by tomatoes *in vivo*[6,7]. However, the second maximum at 15° C, and the variation in ethylene production between two halves of one particulate preparation on ageing[1], suggest the existence of some further controlling reactions, either enzymatic or non-enzymatic, in the sub-cellular system.

Experiments with intact rather than sonicated particles showed that, boiled or unboiled, they produced little ethylene. (Ethylene collections were made during the entire boiling period.) These results are in agreement with our previous experiments with unboiled particles, from which we showed that production of ethylene was related to disintegration of the structure of the particles[4]. When unboiled particles were aged overnight, then sonicated, a very large amount of ethylene was produced. This did not occur with boiled particles, once again illustrating the difference in behaviour between boiled and unboiled suspensions. The fact that boiled intact particles fail to produce ethylene or liberate significant amounts of the gas on sonication after ageing could be due to inactivation of the enzymes involved in the production of the volatile or to destruction of the components necessary for a non-enzymatic evolution of the gas.

Additional experiments were conducted to investigate the nature of the very large increase in ethylene production which occurred when particles were aged before soni-

Table 2. Effect of Substrate Components and Sonication on Ethylene Production by Aged Sub-cellular Particles from Tomatoes

C_2H_4, mμl. (mμl./mg N in brackets)

Run No.:	1				2	
	A	*B*	*C*	*D*	*A*	*B*
Time (h)	S		S		S	S
0–1	107·1 (15·1)	N	59·9 (5·5)	N	195 (12·0)	—
						S
1–2	34·6 (4·9)	37·8 (5·3)	94·5 (8·7)	31·5 (2·9)	N	36 (2·2)
						S
2–3					N	110 (6·7)
						S
3–17					T	353 (21·6)
17–18	T	T	34·6 (3·2)	31·5 (2·9)	N	T
S						
18–19	358·5 (50·5)	392·1 (55·1)	113·2 (10·5)	170 (15·4)	1,748 (107·1)	441 (27·1)
19–21	T	T	34·6 (3·2)	N	T	36 (2·2)
					S	
22–23					1,551 (95·2)	450 (27·6)
Total	500·2 (70·5)	429·9 (60·4)	336·8 (31·1)	233·0 (21·2)	3,494 (214·3)	1,426 (87·4)

N, not detectable; T, trace; S, sonicated.
In run 1, parts *A* and *B*, and run 2, particles were suspended in sucrose-phosphate: 0·5 M sucrose, 0·125 M KH_2PO_4, *p*H 7; in parts *C* and *D* of run 1, the complete reaction mixture given in Table 1 was used. All runs were at room temperature.

cation. The effect of reaction mixture components was measured by ageing the particles in a complete reaction mixture and comparing their ethylene-producing ability with that of particles aged in sucrose–phosphate buffer. Also, the effect of re-sonication after ageing, of previously sonicated preparations, was tested. For these experiments the sub-cellular particles obtained from 1 kg of tomatoes were halved, suspended in either sucrose–phosphate buffer or the complete reaction mixture, and each suspension was halved again for further treatment. Equal and homogeneous division of particles before suspension in any medium was, of course, impossible. Comparison of results expressed on a nitrogen basis may not be entirely valid since there is no evidence for a close relationship between total nitrogen content and the factors responsible for ethylene production. Despite these limitations of interpretation, it is evident from the results in Table 2 that a large increase in ethylene production occurred when particles were aged in sucrose–phosphate buffer, as well as when they were aged in the complete reaction mixture. Furthermore, initial sonication did not markedly affect the ethylene production on ageing and re-sonication of particles suspended in sucrose–phosphate (run 1, parts *A* and *B*). Larger total ethylene production by particles suspended in sucrose–phosphate was obtained with initial sonication.

When the particles were suspended in the complete reaction mixture, initial sonication resulted in reduced ethylene production after ageing and re-sonication (run 1, parts *C* and *D*). Considerable ethylene was evolved during ageing of the sonicated particles, and total production was either about the same, or greater than that of particles aged before any sonic treatment. (Re-sonication after short ageing periods did not result in production of a large amount of ethylene (Table 2, run 2). Similar results were obtained when the particles were suspended in the complete reaction mixture.)

Thus both initially intact and sonicated particles generate ethylene as they are aged. Reaction mixture components in addition to the sucrose–phosphate buffer are not necessary for the large increase in evolution of ethylene which occurs when aged particles are sonicated; experimental difficulties preclude conclusions as to whether the components actually assist in the production. Ethylene produced by the particles may be bound in some way, and released on further distintegration of the particles. Boiled particles are apparently incapable of this further production of ethylene.

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EFFECT OF DIURNAL FREEZE–THAWING ON SURVIVAL AND GROWTH OF SELECTED BACTERIA

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REPEATED freeze–thawing of micro-organisms is generally considered to be lethal[1,2] unless some protection is provided, such as immersion in glycerol. The experiments reported here demonstrate the ability of some organisms to survive and grow well under freeze–thaw cycles typical of day–night conditions at the equator of the planet Mars in summer. Martian conditions[3] were further simulated in terms of temperature extremes (24° C day to −75° C night) and time cycling. The temperature was cycled so that the organisms had only about 4·5 h during which the temperature was above 0° C with a maximum of 25° C. The remainder of the time was spent in a dry ice chamber at −75° C. In these experiments it was assumed that water is present on Mars, perhaps in some micro-environments, as suggested by Lederberg and Sagan[4], despite the fact that recent observations (unpublished) indicate water in the Martian atmosphere amounting to only about 1 per cent of the Earth's atmospheric water content, and except for the micro-environment possibility, no liquid water at the surface.

Preliminary experiments indicated no effect of simulated Martian pressures (0·1 atmospheres) on growth of micro-organisms so that pressure was not controlled in these experiments. The organisms used were *Aerobacter aerogenes* A.T.C.C. No. 129 and an organism tentatively identified as a pseudomonad collected from the rim of the crater of the volcano Stromboli. Both organisms are facultative anaerobes. *A. aerogenes* was grown on Difco heart infusion broth and the second organism on Difco brain–heart infusion broth. In a typical experiment, sterile broth was seeded with a small quantity of medium from an 18–22 h culture of the given organism grown anaerobically at 25° C. The seeded broth was thoroughly mixed and distributed in 1·5 ml. quantities to a number of 'Pyrex' glass tubes. The tubes were

sealed under nitrogen at one atmosphere by drawing out in a flame. A viability assay was made in duplicate on each of the two tubes immediately after sealing by preparing serial dilutions in broth and plating on solid agar. All experiments were run anaerobically at 25° C. Colony counts were made daily by plating on agar plates. The remaining tubes were placed in a dry ice chamber at −75° C. On the following day, and each day thereafter for the duration of the experiment, the tubes were removed from the dry ice chamber and thawed at 25° C in an incubator. Thawing required approximately 30 min, after which viability assays (in duplicate on each of two tubes) were made at intervals over the next 4·5 h and the remaining tubes were then returned to the dry ice chamber. Control experiments were run using the same technique but without any intervals of freezing.

Fig. 1 shows the results of a typical experiment using *A. aerogenes*. Each datum point on the graph represents an average of four plate counts of two individual experimental tubes. The growing time of the experimental tubes is shown to be approximately 4·5 h per day and the rate of killing due to night-time freezing can be determined. It can be seen that the experimental organisms grow very satisfactorily under these conditions with a fairly constant rate of killing due to freezing. These experiments were repeated several times under identical conditions, always with the same result.

Fig. 2 shows the results of a typical experiment using the organism tentatively identified as a pseudomonad. As can be seen, the results are essentially the same as the *A. aerogenes*. It is of interest to note in these experiments that there appears to be no significant lag phase during the commencement of growth at the beginning of each day-time period, except for the first day. It has also been observed that these two organisms are not unique in this response, but there appear to be others, including yeast, which grow under these conditions. These experiments indicate then the ability of certain of Earth organisms to not only survive but satisfactorily grow under simulated Martian conditions given a suitable medium, including water. The results show a generation time in the experimental bacteria which approaches that

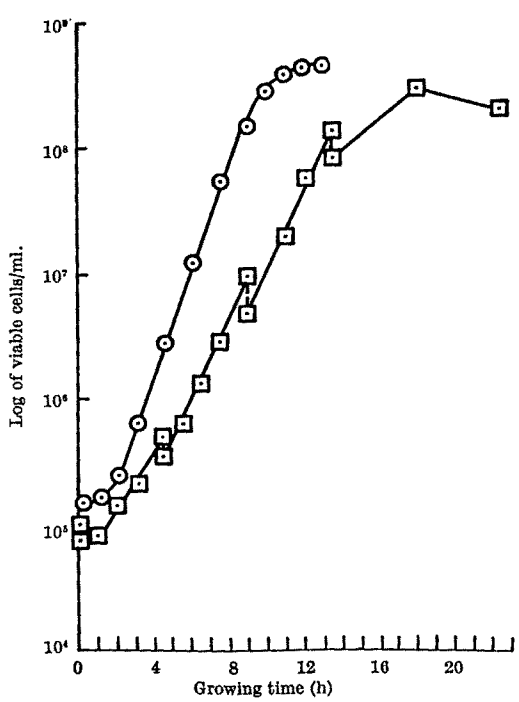


Fig. 1. *Aerobacter aerogenes*
Control, ○, uninterrupted growth; experimental, □, growth interrupted by freezing. Vertical discontinuity illustrates decline in numbers due to death as a result of freeze–thaw process

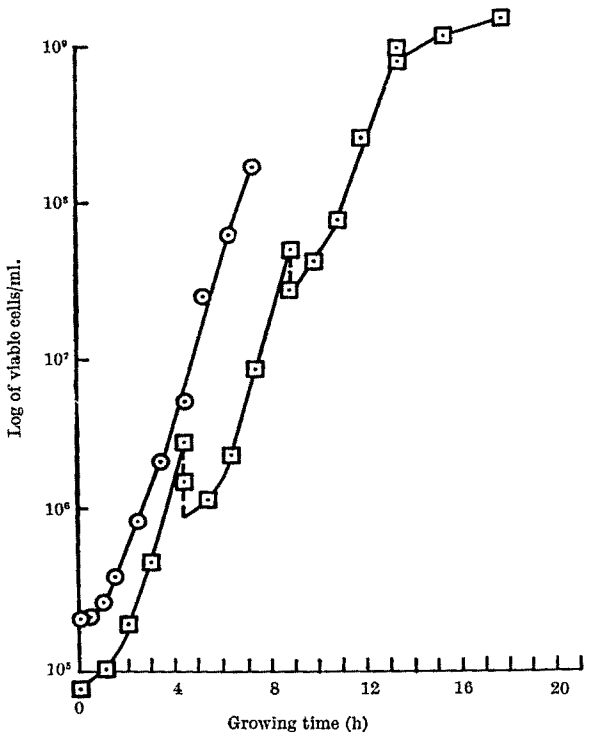


Fig. 2. *Pseudomonas* sp.
Control, ○, uninterrupted growth; experimental, □, growth interrupted by freezing. Vertical discontinuity illustrates decline in numbers due to death as a result of freeze–thaw process

of the controls during the growing period of 4·5 h, so that there is at least a 4,000-fold increase in viable experimental organisms during the 3½–4 days of the experiment, discounting the number of organisms killed by freezing and thawing. The completely lethal effect of repeated freeze–thawing of bacteria and other organisms to be found in the literature is not seen in these experiments and presumably because of the fact that sufficient time is provided for the organisms to reproduce. Preliminary experiments on other bacteria, including one which may be a variant in the *A. aerogenes* culture, show no killing due to freeze–thaw cycling under these conditions. On the other hand, some organisms (*Spirillum itersonii*, *Rhodopseudomonas gelatinosa*, *Pseudomonas ovalis*) tested are killed quickly. Experiments are now being carried out to determine the shortest period of growth time and the minimum nutritional requirements for such organisms to maintain themselves under these conditions.

Experiments are also being performed to determine the minimum water requirements for Earth organisms under simulated Martian conditions. If it can be shown that Earth organisms are able to survive and grow under the most rigorous simulation of Martian conditions possible in the laboratory, this should lend considerable emphasis to the need for space-vehicle sterilization as well as contribute information to our programme aimed at the detection of life on the planet Mars. At the same time, a great deal can be and is being learned concerning the adaptability of life as we know it on Earth to environmental extremes and perhaps, on the mechanisms of adaptation, selection and evolution of organisms on Earth or elsewhere in the universe. Obviously, anything or any conclusions which are made concerning the possible biology of Mars must be made and interpreted with great caution at this time.

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LETTERS TO THE EDITOR

ASTRONOMY

A Comparison of Terrestrial and Universal Expansion

Prof. L. Egyed has recently summarized[1] a number of hypotheses concerning the expansion of the Earth, and has suggested that the Earth's radius is expanding at a rate of 0·5–1·0 mm per year.

There appears to be a remarkably close agreement between the rate of increase of the Earth's radius and that of the universe according to Hubble's law. Using the at present accepted value for Hubble's constant, $H = 100$ km/s/megaparsec[2], which is $1{\cdot}65 \times 10^{-4}$ mm per year per mile, and substituting the value of the Earth's radius in the Hubble equation, $v = RH$, we obtain a radial expansion for the Earth of 0·66 mm per year.

While this agreement may be fortuitous it may suggest a fundamental concordance between expansion processes in the Earth's core and those responsible for the expansion of the universe.

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ASTROPHYSICS

Change in Rotation Period of Jupiter's Decameter Radio Sources

The hypothesis that Jupiter's decameter radio sources may have a constant period has been widely accepted since its initial independent suggestion by Gallet[1] and by Burke[2]. Estimates of this period have been made by many observers[1–8], and no significant departures from the adopted mean period (System III (1957.0)—9h 55m 29·37s) have been established. Upper limits of ± 2s to yearly fluctuations and of ± 1s to slow secular changes over an eleven-year period, 1950–61, were set by Douglas[7,8] and Douglas and Smith[9]. The apparent constancy of the rotation period, together with a constant difference in longitude between the three apparent activity regions, has supported the hypothesis that the radio sources are related to the non-gaseous body of the planet, perhaps through Jupiter's magnetic field.

Preliminary analysis of 22·2-Mc/s observations made at Bethany Observing Station of the Yale Observatory in 1962 and 1963 indicates that the rotation period of the radio sources has lengthened by approximately 0·8s. This may be demonstrated in two ways. Fig. 1 depicts the number of occurrences of Jupiter activity for each 5° interval of System III (1957.0) longitude of central meridian, for the years 1961, 1962 and 1963 (to July 7). A progressive drift in longitude of the major peak of approximately 10° per year is readily visible, corresponding to a 0·8s lengthening of the period. Fig. 2 is a Whittaker periodogram plot of the same material for a variety of assumed periods. The 0·8s lengthening is clear, and not explainable by statistical fluctuation. A change in the rotation period of the radio sources from approximately 9h 55m 29·4s to approximately 9h 55m 30·2s sometime in 1960–62 is consistent with the observations, a behaviour reminiscent of the abrupt changes in rotation period exhibited by the red spot. Detection of so small an

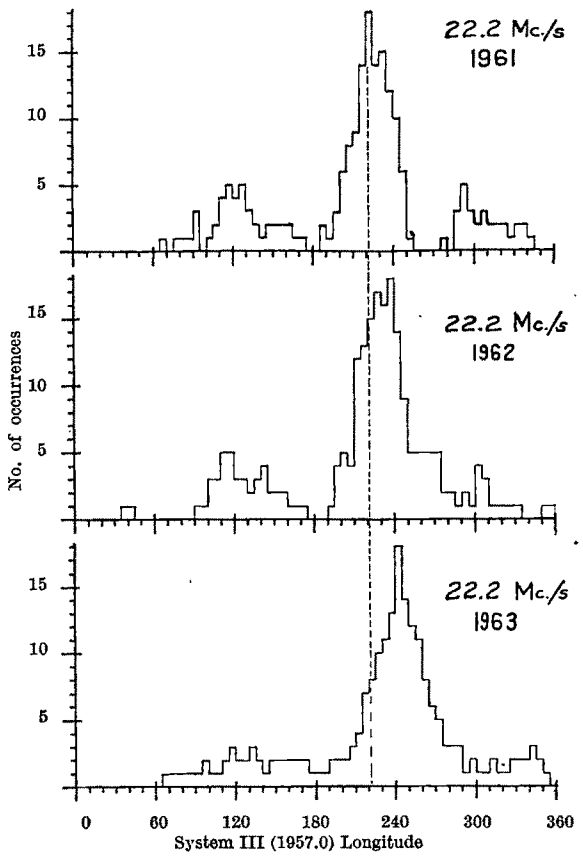


Fig. 1. 22·2-Mc/s occurrence frequency histograms, obtained at the Bethany Observing Station of the Yale Observatory, 1961–63

effect has been made possible by the profusion of Jupiter events recorded in the past three years, due to a substantial increase in Jovian activity, coupled with a new tracking antenna system. Previous period changes of this magnitude or smaller cannot be ruled out, as noted in the limits already quoted. It should be noted that a slow or fluctuating change in rotation period may also be consistent with present observations, as is a change in

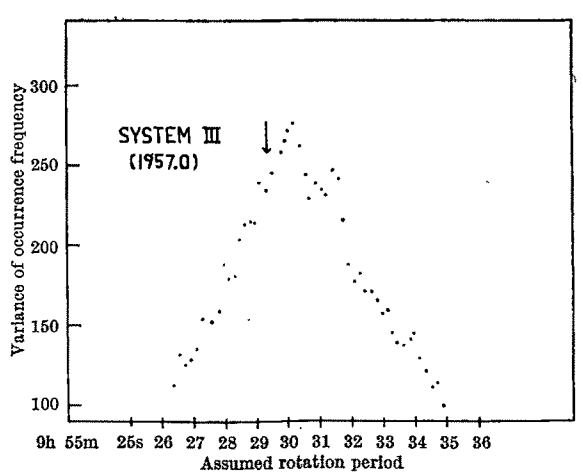


Fig. 2. Periodogram of 22·2-Mc/s Jupiter observations, 1961–63, showing deviation from System III (1957.0) period

apparent longitude of the activity regions; systematic long-term observations are important to clarify the matter.

Hide has hypothesized[10] that the red spot motion refers to that of a solid mantle under a deep atmosphere. In turn, the radio rotation periods may refer to a magnetic field rooted, as in the case of the Earth, to an inner, presumably liquid core. Exchange of angular momentum between core and mantle can then be made evident by studying relative period changes in the red spot and the radio sources[11,12]. Observations of the rocking plane of polarization of the decimetre non-thermal emission from Jupiter can establish the rotation period of the magnetic field. Such observations, as well as optical determination of the current period of the red spot, are now of very great interest, particularly in view of the decametre result reported here.

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PHYSICS

A Non-destructive Radioanalytical Method for the Determination of Gallium in Substituted Yttrium Iron Garnets

THE substitution of diamagnetic ions with a valence of three for iron in garnets modifies both the saturation magnetization and the magnetic anisotropy of these materials. So far, there has been no correlation between the extent of the changes in these parameters and the amount of substituted ion. For these reasons and because the amount of garnet material was extremely limited, usually of the order of micrograms, a rapid, non-destructive method for the determination of gallium in yttrium iron garnet was undertaken. The empirical formula of this substituted garnet is $(Fe_{5-x}Ga_x)Y_3O_{12}$, where x is typically in the range of 0·05–0·25.

Attempts to measure the gallium content by X-ray fluorescence methods failed because of the anomalous results obtained between single crystals and powders of the same composition. A non-destructive radioanalytical scheme based on neutron activation and γ-ray pulse-height analysis was developed in this laboratory.

Gallium exists as two stable isotopes, gallium-69 and gallium-71. Thermal neutron irradiation of the former produces the β-emitter (99+ per cent) gallium-70, but because γ-spectrometry was to be used for measurement of the induced activity, it was not of interest in this work. Under the same irradiation conditions, gallium-72 is also formed. Its principal γ-lines are found in good yield at 0·63 and 0·83 MeV; its half-life was found to be 14·2 h, in good agreement with reported values[1].

The spherically shaped, gallium-doped garnet samples weighing from 200 to 300 micrograms were placed in an irradiation container together with a suitable amount of pure gallium metal and a garnet (*YIG*) containing no gallium. Samples and standards were then irradiated in the pneumatic facility of the Massachusetts Institute of Technology Reactor for 1 min at a thermal flux of 8×10^{12} neutrons cm^{-2} sec^{-1}.

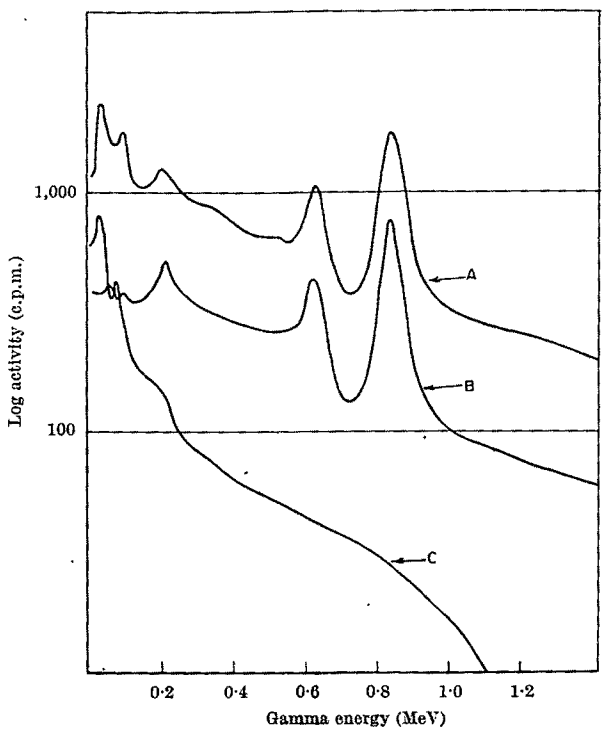


Fig. 1. Gamma spectra of three samples as indicated. Intensities are relative. Spectra taken 4 h after irradiation. *A*, Gallium yttrium iron garnet; *B*, gallium standard; *C*, yttrium iron garnet

The induced γ-activity was analysed for both energy and decay characteristics using a 512-channel analyser coupled to a 2-in. well-type sodium iodide (Tl) crystal detector.

Fig. 1 shows some typical results. The upper curve, *A*, is the γ-spectrum of the gallium-doped garnet. The middle curve, *B*, is that due to the pure gallium metal, and the lowest curve, *C*, is that of the pure gallium-free yttrium iron garnet. The principal peaks in the two upper curves are found to be at 0·63 and 0·83 MeV in agreement with the literature[2] γ-energy values for gallium-72.

Quantitative results are obtained by measurement of the areas under the samples and standard 0·83-MeV peaks after applying the usual background, decay, aliquoting and geometry corrections. An unusual correction, possible because of the versatility of the equipment used herein, was the subtraction of the activity under the aforementioned peak due to the pure yttrium iron garnet. This correction ranged from 2 to 4 per cent. Some results obtained are shown in Table 1.

Table 1. TYPICAL RESULTS OF THE RADIOACTIVATION ANALYSIS OF GALLIUM-SUBSTITUTED YTTRIUM IRON GARNETS

	C.p.m./μg in the 0·83γ peak*	Per cent gallium by weight
Sample *A*	61·5	12·2 ± 0·6
Sample *B*	50·4	10·0 ± 0·5
Sample *C*	47·9	9·5 ± 0·5
Standard	506	

* All corrections applied; time of determination was 4 h after irradiation.

Statistical, weighing, and other known errors amounted individually to less than 1 per cent, and the standard error of the method, given as the standard deviation, is calculated to be not more than 5 per cent.

A search of the literature[3] indicates that no single interfering element or combination of elements could give

a spectrogram with γ-energies and decay characteristics similar to that found.

Finally, it should be pointed out that because of the short irradiation time, residual activity, after a few days decay, is minimal and the garnets may be recovered for additional investigations.

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Utilization of Increased Sensitivity of X-ray Fluorescence Spectrometry due to Polarization of the Background Radiation

In X-ray fluorescence analysis, the limit of detection of an element is usually set by statistical fluctuations in the continuous background at the wave-length of the most 'sensitive' line of the element. Consequently, the sensitivity of detection of an element will be improved if the intensity of the continuum is decreased. A typical example is the use of pulse height analysis with a detector the output pulse height of which is proportional to the energy of the incident quantum. By this means, overlapping orders of background can be rejected, resulting in increased sensitivity.

A further improvement can often be made as follows:

The background continuum consists largely of photons which are scattered (both elastically and inelastically) by the sample. Since the angle of scattering accepted by the collimator of typical instruments is about 90°, the scattered radiation is highly plane-polarized[1]. On the other hand, the fluorescent radiation in the line, being emitted some time after the photoelectric absorption of the exciting radiation, is completely random in direction and is unpolarized. Therefore, a marked improvement in line to background ratio could be achieved if one had a polarization analyser.

Such a device is a latent component of most X-ray spectrometers, in the form of the analysing crystal used to disperse the spectrum. Unfortunately, the optics of commercial X-ray spectrometers are usually arranged so

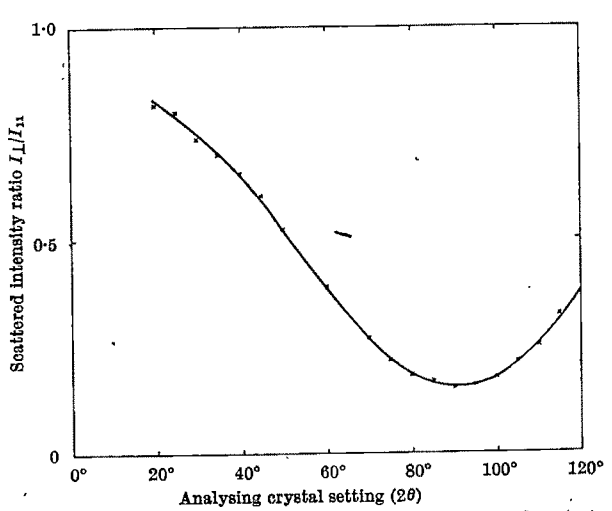


Fig. 1. Ratio of $I_\perp$ to $I_{||}$ of X-rays scattered by water, plotted against angle of tepaz analysing crystal

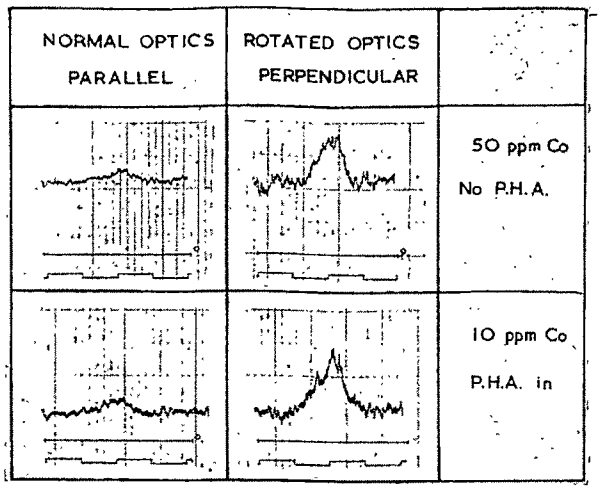


Fig. 2. Traces of cobalt K_α lines using conventional and perpendicular optics

that when the analysing crystal is at its optimum position for polarization ($2\theta = 90°$) the sample and analyser planes are parallel. It is not too difficult with the Philips *PW*1520 instrument to rotate the X-ray tube and sample holder about the axis of the collimator. When these are rotated by approximately 90°, the scattered radiation is considerably decreased for analyser settings in the vicinity of $2\theta = 90°$, as shown in Fig. 1.

The implications in analytical chemistry are illustrated in Fig. 2. The upper pair of traces show the significant improvement of detection of cobalt in aqueous solution (at $2\theta = 82{\cdot}5°$ for a topaz analysing crystal). For comparison purposes, the ratemeter was adjusted to give the same recorder reading for background in both cases.

The lower pair of traces indicates the extreme sensitivity of the method if both polarization of background and pulse height analysis are used.

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Influence of Electrode Coatings on Conduction Currents in Transformer Oil

Although the exact mechanism of conduction in dielectric liquids is still the subject of controversy, it has long been recognized that the surface state of the electrodes greatly influences the conduction process[1]. Recent experimental evidence[2,3] has shown that this conduction process is very sensitive to the surface state of the cathode, in particular to the presence of an insulating oxide layer. While it is known that oxide layers on the anode influence the electric strength of liquids[4], the influence of anode surface barriers or films on the conduction currents does not appear to have been studied.

This communication gives some preliminary results of conduction current measurements in transformer oil obtained using direct voltages, with both electrodes bare (*A*), both electrodes coated (*B*), cathode uncoated and anode coated (*C*), and cathode coated and anode uncoated (*D*). Further tests were made in which the coatings on both electrodes varied in thickness (*E*–*G*).

Both the coated and the uncoated electrodes were made of aluminium, polished to a 0·25-μ diamond paste finish. These had a uniform field profile and a nominal diameter of 2·5 cm. The coated electrodes were covered by a pore-free film of the thermoplastic, Penton; thicknesses of 0·5 mm and 0·75 mm were used. The transformer oil

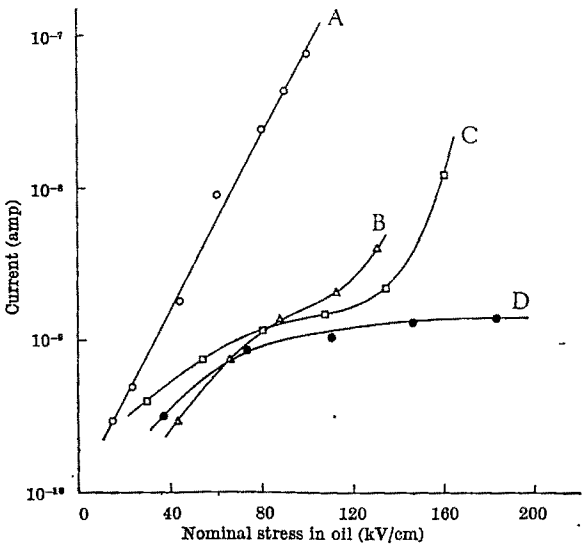


Fig. 1. Typical current–stress characteristics for coated and uncoated electrodes. Oil gap, 1·5 mm; film thickness, 0·5 mm. *A*, Both electrodes uncoated; *B*, both electrodes coated; *C*, cathode uncoated, anode coated; *D*, cathode coated; anode uncoated

was filtered several times through a sintered glass filter of 1-μ porosity. In each test the stress was maintained at a constant value until the gap became conditioned, giving, within the limits of experimental error, steady and reproducible values of current.

Typical experimental results are given in Figs. 1 and 2, the steady current values for each point being plotted to a base of nominal stress in the oil. Curve *A* in Fig. 1 shows that the current with uncoated electrodes increases in a log-linear manner with applied stress, indicating a process of emission. With a film on the cathode only (curve *D*), the emission is greatly restricted and tends to a constant value over the stress range considered. With a film on the anode, irrespective of whether or not there is a film on the cathode, the current, after following an ohmic characteristic at the lower stresses, increases rapidly above about 60 kV/cm.

A tentative mechanism of conduction in oil gaps, indicating the importance of the part played by the anode, is postulated as follows. On the application of voltage, space charges of both polarities are formed in transformer oil[5,6], the positive ions drifting towards the cathode and the negative ones towards the anode. In addition to the negative ions already present in the bulk liquid, electrons emitted at the cathode will form negative molecular ions by attachment. The stability of the negative space charge, and hence its intensity at the anode, will depend on the oxygen content of the liquid and the nature of the anode surface. Thus with air-saturated oil and an insulating film on the anode. a large anode space charge field will be set up giving rise to an ionization process in the vicinity of the anode. Evidence of intense ionization at uncoated anodes in liquids has been reported by Washburn[7] and by Swan and Lewis[4]. Such a process could feed back positive ions to the cathode, further enhancing the field there.

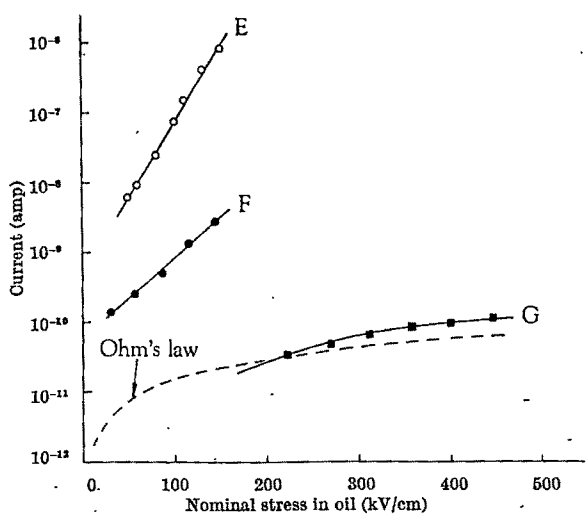


Fig. 2. Variation of current–stress characteristics with film thickness. Oil gap, 1 mm. *E*, Both electrodes uncoated; *F*, electrodes coated with 0·5 mm thick film; *G*, electrodes coated with 0·75 mm thick film

At the cathode two processes acting in opposition are possible: on one hand the presence of an insulating film will decrease emission, while on the other the establishment of a positive ionic space charge leads to a considerable enhancement of the field, resulting in an increased emission. The degree of field enhancement will depend on the number of positive ions available and on the blocking nature of the cathode surface. Hence the emission from the cathode and the formation of positive ions at the anode are interdependent and contribute to the conduction current.

Following the mechanism proposed here, the results given in Fig. 1 may be briefly interpreted as follows: Curve *A* is due mainly to emission from the cathode; the current increase at higher stresses in curve *B* indicates the onset of emission due to greatly enhanced cathode field and intense ionization at the anode; curve *C* is due to cathode emission and ionization at the anode; in curve *D* cathode emission is absent and the ionization at the anode is negligible. By increasing the thickness of the insulating film on both electrodes from 0·5 mm to 0·75 mm, emission from the cathode is almost completely suppressed (Fig. 2).

It has been shown that the presence of an insulating barrier on the electrode surfaces greatly modifies the current–stress relationships which, however, as already shown[3,8], are still characterized by an ionic and then an emission region. Details of this work and a full discussion of the mechanism outlined here will be published elsewhere.

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Time Characteristics of Light Pulses from Gallium Arsenide Lasers

Recent investigation of the light emitted by gallium arsenide lasers[1–3] when current pulses of widths in the tens of nanoseconds range are passed through the *p–n* junction have led to the observation in this laboratory that a certain charge must flow into the junction before laser action commences. For a given amplitude of the current pulse, there is a corresponding pulse width which will just induce laser action, the laser action commencing

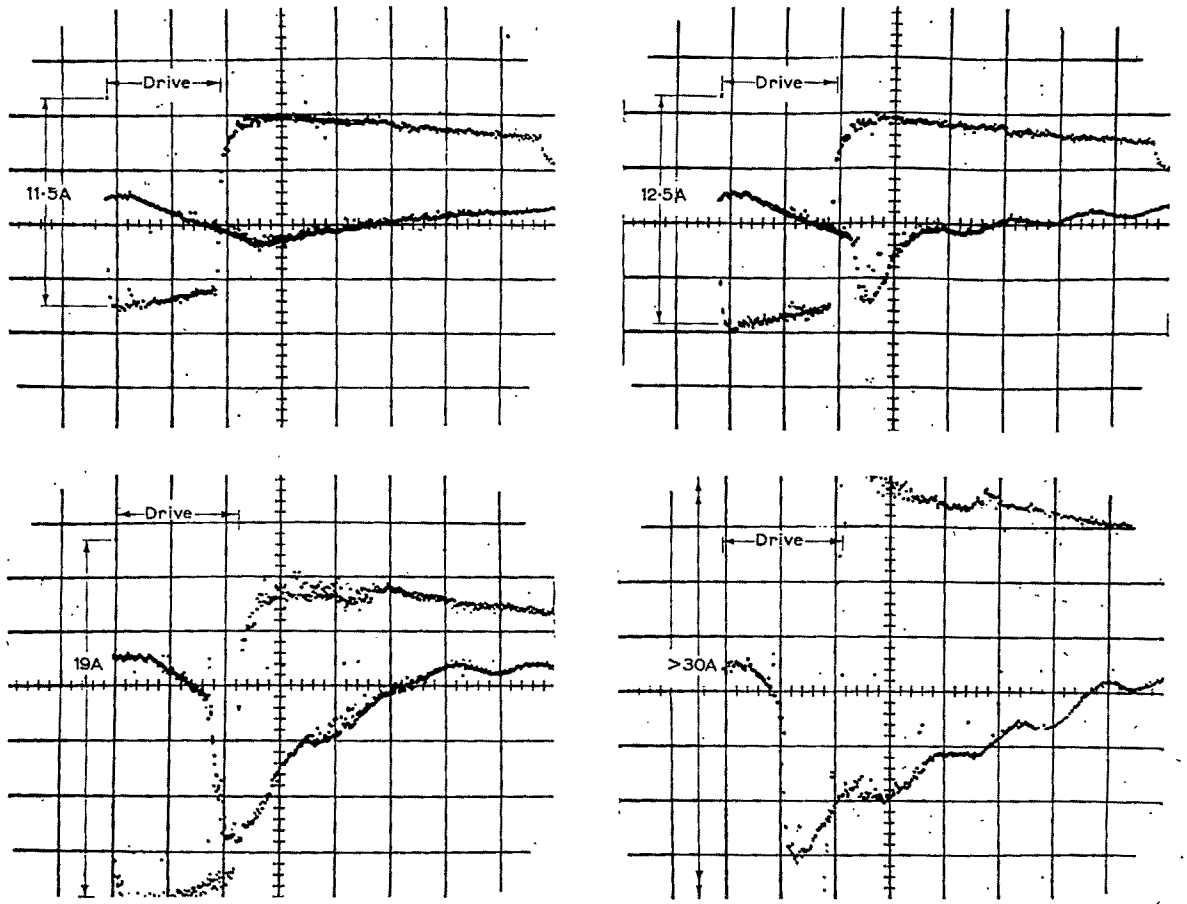


Fig. 1. Top left, no laser action; peak current, 11·5 amp; top right, laser threshold; peak current, 12·5 amp; bottom left, laser action in overdrive condition; peak current, 19 amp; bottom right, laser action in overdrive condition; peak current, >30 amp. In all photographs the upper trace represents the voltage pulse, and the lower trace the light pulse. Time-scale, 50 nsec/cm

on the falling edge of the pulse. The converse is also true. As pulse-width is decreased, the pulse-amplitude must be increased if laser action is to be achieved. Statements that the laser threshold current density under pulse conditions is x amp/cm^2 therefore become meaningless unless the pulse widths at which the measurements were made are also stated. Long time constants have also been observed in the light pulse emitted from the forward biased p–n junction.

The phenomenon is well illustrated by the photographs of oscilloscope traces shown in Fig. 1. The laser was made from n type gallium arsenide doped with selenium to an impurity concentration of 2×10^{18}/c.c., and zinc was diffused into the material to form a p region. The area of the junction was of order 10^{-3} cm^2. Polished faces were cleaved accurately perpendicular to the junction plane to form an optical cavity. The laser diode was operated at 77° K directly immersed in liquid nitrogen. Electrical pulses with rise and fall times of order 1 nanosecond were supplied by a wetted mercury relay-type pulse-generator to the primary of a pulse transformer in the secondary of which were connected in series the laser diode and a non-inductively wound 0·1 Ω resistor. The light pulses were observed using an R.C.A. 7102 photomultiplier with a limited number of dynodes in operation and a load resistor of 100 Ω in the output circuit. The electrical and light pulses were monitored by a Hewlett Packard model 185 A pulse-sampling oscilloscope fitted with a model 1878 dual trace vertical amplifier. Initial observation of the current and voltage pulses across the junction showed that these pulses were in phase and had fast edges.

In Fig. 1 (top left) the light is incoherent, and its pulse has a triangular shape with slow rise and fall times. The time delay between the initiation of the light and voltage pulses is of the order one might expect due to transit time in the photomultiplier (20 nanoseconds), but the maximum light emission occurs at least 10 nanoseconds after the electrical pulse has ceased, and light emission continues over a period several times the duration of the electrical pulse. This long duration of light emission is only characteristic of diode light at high current densities of order a thousand amp/cm^2. At current densities of order a hundred amp/cm^2 the electrical and light pulses have similar widths, and rise times of order 10 nanosecond have been observed on the light pulses.

Fig. 1 (top right) shows the light pulse when laser action commences. The coherent light appears as a peak on the maximum amplitude of the incoherent light, there being a distinct increase of amplitude and a change in the shape of the leading edge of the light pulse. That this was the threshold of laser action was corroborated by inspection with an image converter and a polarizer. The characteristic light spot emitting polarized light just appeared at the same current amplitude as that at which the peak appeared on the incoherent light pulse. Note that the laser action commences at threshold on the falling edge of the electrical pulse. As already observed earlier, this is true for any pulse width if the pulse amplitude is increased until laser threshold is reached.

Fig. 1 (bottom left) shows the light pulses which result when the drive is increased beyond that required to produce laser action. Note that the laser action occurs earlier in relation to the electrical pulse when the drive is increased, and the light peak moves inside the period of the electrical pulse. The decay time of the light pulse is always slow, and occupies a time in excess of the electrical pulse length.

This tail represents a considerable fraction of the total energy in the light pulse in the overdrive condition. It still remains to be established whether this tail is representing mainly stimulated emission or not.

The long time constants involved in the light pulse from the gallium arsenide p–n junction raise fundamental problems on the mechanism of the light emission. More detailed measurements of the phenomenon reported are being made, and the findings will be reported later.

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GEOLOGY

Rate of Adsorption of Water Vapour by Degassed Arizona Bentonite

It is perhaps well known that rate data for the rapid adsorption of gases are scarce[1]. What data have been published, moreover, are usually complicated by a varying adsorbent temperature. It would seem that this complication must always be present whenever the rate of adsorption is much greater than the possible dissipation rate of the heat of adsorption. This is the situation in virtually all determinations of very rapid adsorption rates. The only recourse, therefore, appears to be in finding a satisfactory method of analysing rate data for a fluctuating adsorbent temperature. (One must as a consequence in this case simply accept the fact that the temperature coefficient of the adsorption rates will always to some degree be uncertain.)

For some time we have been concerned with the temperature fluctuations accompanying the infiltration of porous media by water[2]. A quantitative description of the heat effects requires an estimate of the differential heat of adsorption (commonly called the isosteric heat of adsorption) of water vapour on the various media as well as the rate of adsorption. We were thus led into adsorption calorimetry and adsorption kinetics. One of our most recent undertakings was a determination of the rapid temperature fluctuation accompanying the adsorption of water vapour by degassed Arizona bentonite. From the result, we have obtained an estimate of the initial adsorption rate.

Arizona bentonite collected at the Cheto pit near Chambers, Arizona, was dried, powdered, and sifted untreated into a small, shallow, plastic dish supported by a thin brass plate in the centre of a cylindrical brass sample chamber. A lead sulphide infra-red radiation detector (Kodak 'Ektron' infra-red detector type Q-2. Time constant: 0·1–0·3 msec at 25° C) was mounted in the heavy brass lid of the sample chamber in such a way that it constantly monitored the temperature of the sample surface. The detector was wired through a sensitive resistance bridge network directly to the deflection plates of a dual trace, single sweep oscilloscope. This made it possible to measure and record photographically the very rapid temperature change of the porous clay surface as a function of time. Degassed, distilled water (about 1 g) contained in a sealed glass vial was placed directly beneath the sample-supporting platform. The clay was degassed under vacuum at 25° C for 24 h at a pressure of 10^{-5} mm mercury, isolated from the vacuum pump, and then exposed suddenly, while still under vacuum, to water vapour by breaking the glass vial. This was accomplished by a suitably arranged brass plunger. The brass sample-supporting plate shielded the clay from the violence of shattering the vial, and subsequent inspection showed that the sample support was also effective in preventing droplets of water from contacting the surface of the clay powder.

The resultant temperature rise was monitored in two ways: by means of the infra-red detector and resultant oscilloscope trace just described, and by means of a 0·001 in. diameter copper-constantan thermocouple embedded in the clay powder. The thermocouple was carefully positioned about 1 mm beneath the sample surface as the adsorption chamber was assembled. Voltages developed across the thermocouple leads were monitored by one trace of the oscilloscope and were afterwards interpreted as temperatures from a previously prepared calibration curve. After the maximum temperature rise had been ascertained by means of the slower responding thermocouple, the oscilloscope trace indicating the response of the lead sulphide detector at this time was interpreted in terms of the maximum thermocouple response. The data shown in Fig. 1 were obtained in this manner.

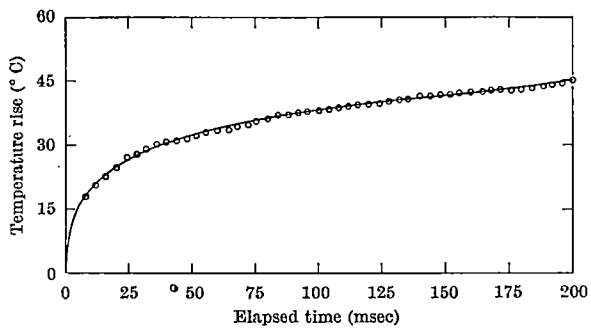


Fig. 1. Rise in temperature during the adsorption of water vapour by Arizona bentonite. Data points are from an oscilloscope trace. The curve drawn through the data was calculated from equation (1)

As can be seen, the rate of temperature rise during the first few msecs was very rapid, amounting to, let us say, about 2,350° C per second. A maximum temperature rise of 50·1° C above ambient (25° C) was observed within about 0·5 sec, after which the temperature declined very slowly. The empirical relation:

$$\Delta T = 50{\cdot}1\ [1 - \exp(-0{\cdot}15t^{0{\cdot}5})] \qquad (1)$$

where ΔT is the temperature rise above ambient in ° C and t is the time (msec), was found to fit the data and is superimposed for comparison in Fig. 1.

An estimate of the rate of adsorption and the sticking coefficient of water vapour on Arizona bentonite may be made by formulating a simple heat balance equation. Assuming that the heat loss from the adsorbent is negligible during the initial stage of adsorption (the loss of heat from the sample at 70° C in the presence of water vapour alone at a pressure of 23 mm mercury was experimentally determined to be about 1·4 cal/sec; this is entirely negligible during the initial rapid adsorption period).

The rate of adsorption is:

$$\frac{dm}{dt} = \frac{c_p}{q_{diff}} \frac{dT}{dt} \qquad (2)$$

in which dm/dt is the rate of adsorption (g water/g clay/sec); c_p is the specific heat capacity (cal/g clay); q_{diff} is the differential heat of adsorption (cal/g water); and dT/dt is the observed rate of temperature rise (° C per sec). Taking $c_p = 0{\cdot}22$, $q_{diff} = 940$ (a value we determined in another experiment during this investigation, the details of which will be published elsewhere), and $dT/dt = 2{,}350$, an initial rate of adsorption of 0·55 g water/g clay/sec is obtained. This corresponds to about $4{\cdot}3 \times 10^{16}$ molecules

per cm² of clay surface area/sec, assuming an external area of 42·4 m²/g clay[3]. The sticking coefficient may be estimated in the usual way[4] as the ratio of the number of molecules sticking to those striking the adsorbent. The latter is approximately $1{\cdot}1 \times 10^{22}$ molecules/cm²/sec, the kinetic theory estimate for water vapour at 25° C, indicating an average sticking coefficient of $3{\cdot}9 \times 10^{-6}$ for the first few msecs. Evidently, roughly all but about one water molecule in every 250,000 striking the clay surface are reflected.

The combination of equations (1) and (2), after differentiating the former with respect to time, leads to:

$$\frac{dm}{dt} = a(t) \exp(-0{\cdot}15t^{0{\cdot}5}) \qquad (3)$$

where $a(t) = 3{\cdot}76\ c_p/q_{diff}\ t^{0{\cdot}5}$ and (after the first msec) is a slowly varying function of time. Here $\frac{dm}{dt}$ has the units g water/g clay/msec.

Equation (3), in some respects, bears a formal resemblance to the well-known Elovich–Taylor–Thon equation for the rate of chemisorption[5]. If the correspondence is more than coincidental, it suggests that the initial stage of adsorption of water vapour by bentonite (probably the hydration of the exchangeable cations[6]) is a chemisorption process. The high initial heat of adsorption (about 17 kcal/mole water) and the low sticking coefficient lend support to this hypothesis.

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Occurrence of Karroo System Sediments in Ethiopia

The continental sediments which comprise the Karroo System of southern Africa are formed by thick, dominantly clastic rocks deposited throughout the Palæozoic–Mesozoic transition in a period of general epeirogenic uplift and accompanying cratonic subsidence. Sediments belonging to the Karroo System have previously been recognized as occurring as far north as coastal Kenya, where they are termed the Duruma Sandstone, an essentially continental series of shales and sandstones unconformably overlain by the Mesozoic transgressive marine sediments.

Sediments related to certain members of the Duruma Sandstone and with identical relationship to the Mesozoic marine formations have recently been discovered by Mr. D. H. Jepsen while mapping the geology of the Abbai (Blue Nile) basin for the joint Ethiopia–U.S. Water Resources Board. This information has generously been fully placed in the hands of the author for interpretation. A very brief summary is given here.

Various Italian surveys during the period 1936–40 have shown that previous ascriptions of the Adigrat Sandstone formation to the Karroo System were incorrect. The Adigrat Sandstone is a diachronous facies marking the initial sedimentation phase of the Mesozoic marine transgression from the south-east; although its basal members are not infrequently cross-bedded their conformity with the higher marine sands, mudstones, evaporites and limestones indicates an environment quite distinct from that of the Karroo sandstones.

The sediments discovered by Jepson form two irregularly elongated patches each approximately 5 km × 20 km exposed meridionally across the bottom of the Abbai canyon in the vicinity of the confluences with the Guder and Finchoa rivers. These sediments are overlain unconformably by the planar base of the Adigrat Sandstone and occur in pre-Adigrat valleys aligned north–south in Basement gneiss. These valleys were eroded to a depth of 300–400 m below the peneplained surface of the Basement rocks prior to filling by the pre-Adigrat phase of sedimentation. In the exposures at present known, the upper 150 m of the pre-Adigrat sediments have been removed by the denuding action of the Abbai river.

The remnant 200 m of sediments are composed of mostly thin-bedded bluish-grey calcareous mudstones overlain by massive dark grey sandstone beds. Down-warping of the sediments in the centres of the ancient valleys is considered due to gravitational compaction. The unconformably overlying Adigrat Sandstone, which can be continuously traced from Shoa (where it thickens as it plunges beneath the Rift Valley) to the Sudan border region, is a quite distinct facies; massive, fine to coarse grained, red or buff sandstones with some interbedded variegated shales and thin lenticular beds of conglomerate or pebbly cross-bedded sandstone.

The discovery of proved pre-Adigrat sediments lying on the Ethiopian Basement, a matter previously much disputed, has the following implications: (1) Towards the end of the Palæozoic, an era of peneplanation in the Horn of Africa, there was an uplift of the order of 400 m in central Ethiopia; (2) Consequent on this uplift was the erosion of broad river valleys which were formed meridionally, parallel to the tectonic grain of the foliated Basement rocks; (3) Gradual and accelerating subsidence of the central Ethiopian region enabled a filling of the eroded valleys with sediments derived from an unknown uplifted source, probably to the west; (4) These same epeirogenic tilting movements were perhaps responsible also for the ensuing initiation of the Mesozoic marine transgression north-westwards across Ethiopia, and which reached Shoa by the late Liassic or early Bajocian. The increased supply of sediments was now deposited in a marine environment to form the Adigrat Sandstone.

The occurrence of these pre-Adigrat sediments, probably of Permo-Triassic age considering their relations to the Palæozoic peneplanation and to the Adigrat Sandstone itself, is considered to suggest that Karroo-type sedimentation extended north into Ethiopia, though of restricted occurrence. It is now eminently desirable that these sediments be examined in more detail, particularly their petrography, and that search for fossils be made. It is also necessary that the Waju Sandstone of Chercher, similarly debated as of pre-Adigrat Sandstone age, be re-examined so that a regional knowledge of the existence of Karroo-type sediments over the whole of Ethiopia be obtained.

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CRYSTALLOGRAPHY

Structure of α-methyl D-galactoside 6-bromhydrin

As part of a crystallographic investigation of D-galactose and its derivatives, a determination of the structure of α-methyl D-galactoside 6-bromhydrin, $C_7H_{13}O_5Br$, has been carried out. The compound was prepared by Valentin[1] and the unit cell dimensions and symmetry were measured by Cox, Goodwin and Wagstaff[2].

Our measurements were made at 140° K, the crystal being cooled by a stream of dry, cold nitrogen gas provided by apparatus similar to that previously described[3]. The

compound crystallizes as orthohombic tablets, space-group $P\ 2_1\ 2_1\ 2$ with four molecules in the unit cell. We have interchanged the a and c axes given by Cox *et al.* to agree with the convention used in the *International Tables* (1, 104; 1952). The cell dimensions are:

a	At 140° K	11·14 Å	cf. Cox *et al.*	11·23 Å
b		7·815 Å		7·81 Å
c		10·61 Å		10·58 Å

Three-dimensional data was collected using the Weissenberg equi-inclination technique and 1,203 independent hkl reflexions were measured. Patterson projections down the three axes showed that the bromine atom had co-ordinates which were very close to $y=\frac{1}{4}$ and $z=0$ (or $\frac{1}{2}$). Harker sections were then calculated, using the three-dimensional data, and the bromine atom was found to be at $x=\frac{5}{60}$, $y=\frac{14}{60}$ and $z=\frac{1}{120}$. The three-dimensional Fourier synthesis, computed using the phase-angles decided by the position of the bromine atom, had a certain amount of spurious symmetry resulting from the near-special position of the bromine atom. The five most definite peaks were chosen and, together with the bromine atom, were given two cycles of least squares refinement. A second three-dimensional Fourier synthesis was carried out; in this the pyranose ring could be identified, together with a mirror image, and all the remaining atoms with the exception of the carbon atom of the methyl group. Seven cycles of least-squares refinement were then carried out, with isotropic temperature factors, for all the atoms but with the methyl group carbon atom not included. A difference Fourier synthesis then gave the position of the methyl group carbon atom. This atom was introduced into the calculations, together with anisotropic temperature factors for all the atoms and four more cycles of refinement were carried out, the final cycle giving the residual, $R=0{\cdot}108$. The electron density distribution for one molecule is shown in Fig. 1; the contour lines for the carbon and oxygen atoms are drawn at $1e/Å^3$ intervals starting at $1e/Å^3$ and for the bromine atom at $5e/Å^3$ intervals. A diagram of the molecule is shown in Fig. 2. The calculated bond lengths are listed in Table 1.

Table 1. BOND-LENGTHS (Å)

Br–C6	1·974	O1–C1	1·430
C1–C2	1·509	O1–C2	1·448
C2–C3	1·551	O3–C3	1·414
C3–C4	1·502	O4–C4	1·425
C4–C5	1·524	O5–C5	1·461
C5–C6	1·492	O5–C1	1·421

The pyranose ring has the expected chair form with the configuration 1a 2e 3e 4a, exactly as found for β-arabinose by Furberg and Hordvik[4]. In the crystal the bromine

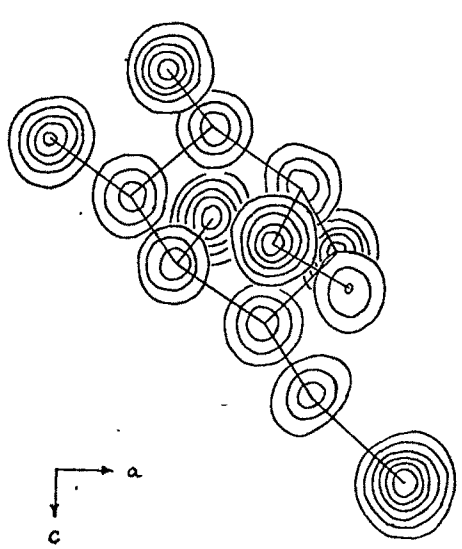


Fig. 1. Electron density distribution

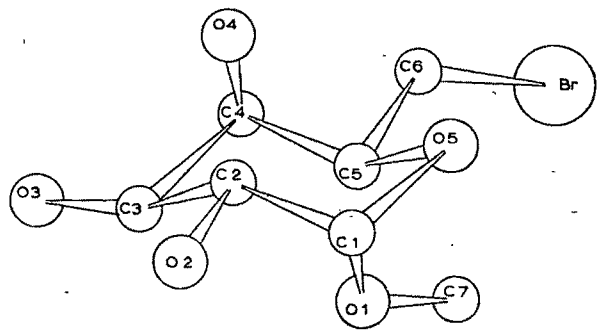


Fig. 2. Stereochemical arrangement

atoms are arranged in sheets parallel to (001), along which cleavage occurs, with the galactose molecules connected to each other by hydrogen bonds. The average carbon–carbon bond length is 1·516 Å and the average carbon–oxygen bond length is 1·434 Å. In previous measurements of sugars having O1 as a hydroxyl group, by McDonald and Beevers[5], McGeachin and Beevers[6], and Furberg and Hordvik[4], the bond between C1 and O1 was found to be significantly shorter than a normal carbon–oxygen single bond; in this work, where the hydrogen atom is replaced by a methyl group, the carbon–oxygen bond distance is quite normal.

The calculations were carried out on the University of Leeds Ferranti *Pegasus* computer and we gratefully acknowledge the use of programmes written by D. W. J. Cruickshank (S.F.L.S.) and by D. W. J. Cruickshank, Diana E. Pilling, A. Bujosa, E. M. Lovell and Mary R. Truter (Fourier synthesis)[7]. We also thank Dr. E. G. Cox for his initiation of this work.

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Chain Structure of the *Cyclo*pentadienils of Monovalent Indium and Thallium

THE *cyclo*pentadienils of monovalent indium and thallium have been prepared by E. O. Fischer *et al.*[1,2]. The In-C_5H_5 is very sensitive to oxidation and must be handled in complete absence of air; the Tl-C_5H_5 is more stable and its decomposition follows slowly. The stoichiometric convexion 1/1 between metal and C_5H_5 has been interpreted by E. O. Fischer[2] as a 'half sandwich' structure with transfer of π electrons from the aromatic ring to the metal. On the other hand, F. A. Cotton and L. Reynolds[3] believe the complex to be ionic, on the basis of molecular orbital treatment.

Tyler, Cox and Sheridan[4] have examined the microwave spectra of the gaseous Tl-C_5H_5. The thallium-ring distance is 2·41 Å.

We have examined the single crystals, obtained by sublimation in the vacuum, closed under nitrogen in Lindeman glass of both complexes, and determined the structure by use of two-dimensional Fourier methods on the diffractions of X-rays. The complexes are monoclinic and isotypical; systematic absences were found for hkl with $h+k\neq 2n$; $h0l$ with h and $l\neq 2n$; $0k0$ with $k\neq 2n$.

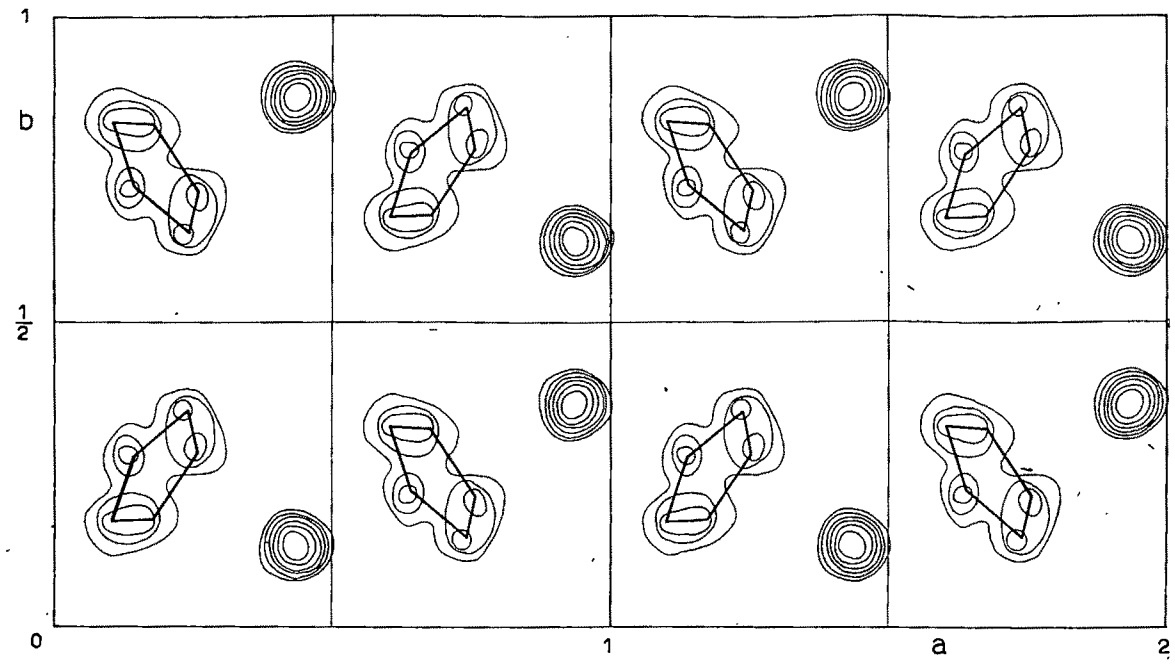


Fig. 1. Electron density projection (001) for indium-C_5H_5. Contours at 2*e*. Å^{-3}. Upper levels for indium omitted

The space group is therefore the non-centrosymmetric monoclinic C_s^4-Cc with four molecules in the unit cell. The lattice constants are listed as follows:

In C_5H_5: $a = 9{\cdot}08 \pm 0{\cdot}02$ Å; $b = 10{\cdot}01 \pm 0{\cdot}02$ Å; $c = 5{\cdot}86 \pm 0{\cdot}01$ Å
$\beta = 102° 30' \pm 30'$ $d = 2{\cdot}30$ g cm^{-3}
Tl C_5H_5: $a = 9{\cdot}13 \pm 0{\cdot}02$ Å; $b = 9{\cdot}96 \pm 0{\cdot}02$ Å; $c = 5{\cdot}94 \pm 0{\cdot}01$ Å
$\beta = 100° 20' \pm 30'$; $d = 3{\cdot}37$ g cm^{-3}

The picnometric density for the thallium-C_5H_5 is $d = 3{\cdot}33$ g cm^{-3}. The picnometric density of the indium-C_5H_5 was not determined owing to the difficulty of handling this compound.

The *h*0*l*, 0*kl* and *hk*0 reflexions for the indium-C_5H_5 and thallium-C_5H_5 were collected with Weissenberg and precession cameras and the intensities read with a photometer fitted with a potentiometric recorder.

Patterson projections were calculated on (001), (010) and (100). Electron density projections were obtained

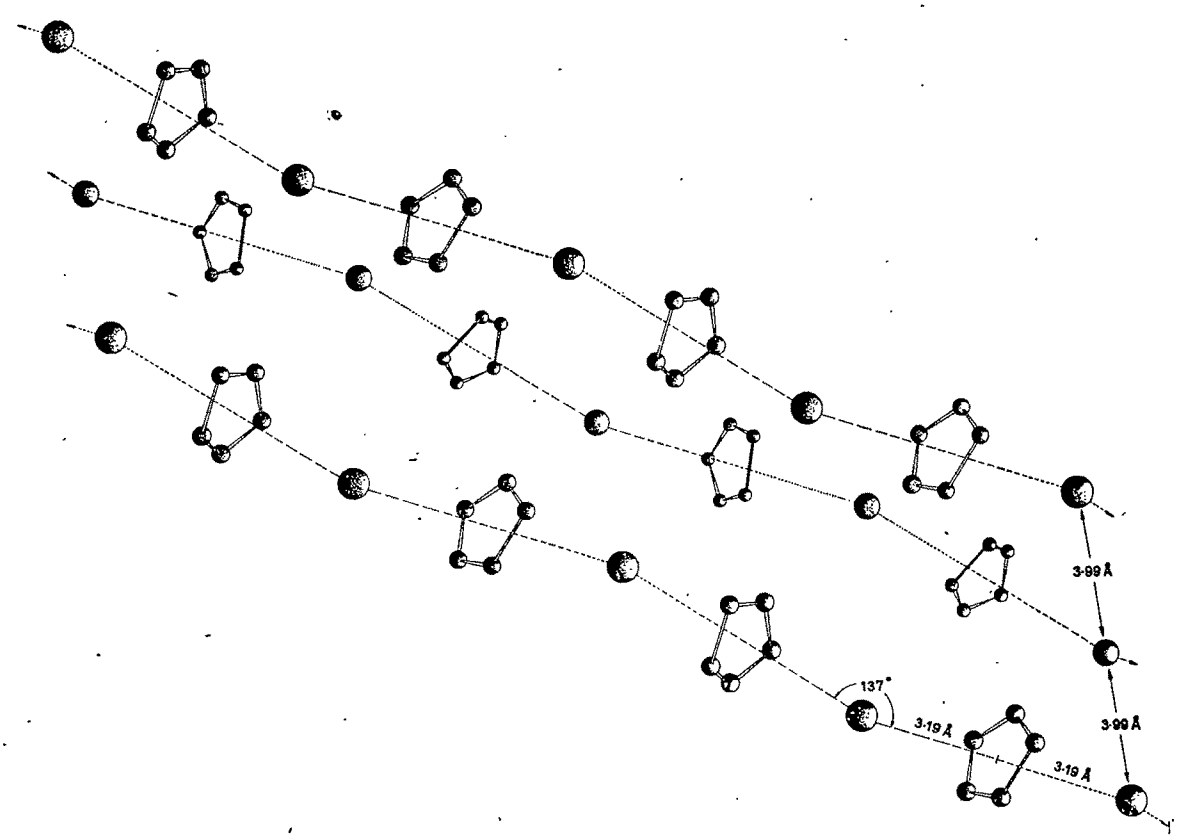


Fig. 2. Packing of the indium-C_5H_5 in the crystal

using at first the heavy atom method. Afterwards the contributions of the light atoms were introduced in the calculated structure factors. Fig. 1 shows the electron density projection on (001).

Both structures are similar, therefore we have chosen the indium-C_5H_5 for successive refinement because the lighter metal-atom would give the possibility of reaching smaller standard errors in the position of the aromatic ring. Using the Cochran method of difference syntheses at the present stage we have obtained the following reliability factors:

$hk0$ reflexions: $R = 0{\cdot}08$, $0kl$ reflexions: $R = 0{\cdot}07$

We have calculated that the actual co-ordinates are affected with the following standard errors:

In: $\sigma_r = \pm 0{\cdot}01$ Å and C: $\sigma_r = \pm 0{\cdot}07$ Å

The packing of the molecules in the crystal can be better explained with the aid of Fig. 2, where three chains of molecules are partially represented.

The structure is characterized by a chain disposition of the metal atoms and of the rings enclosed between them, at the same distance from both metals. The angular disposition of the system ring-metal-ring is characterized by an angle of $137° \pm 30$ min at the metal. The metal-ring distance is $3{\cdot}19 \pm 0{\cdot}10$ Å. The packing of the chains allows a minimal distance of 3·99 Å between metal-atoms belonging to adjacent chains. As can be seen, there is a zig-zag of equidistant metal-atoms, the angle M–M–M being about 100°.

The difference between the metal–ring distances in the gas phase[4] and in the solid-state is very large. This difference determines a great change of structure: in the gas phase there is the monomeric 'half sandwich' structure formed by one metal-atom and one aromatic ring; in the solid phase there is a polymeric structure formed by zig-zag chains. The chains are held together by a zig-zag of metal-atoms: the metal-metal distance is such as to allow the possibility of interaction between them.

It should be noted that every metal-atom is surrounded by two rings with the geometrical characteristics of the angular 'sandwich' structure.

The polymeric character explains the insolubility of the complex noted at first by E. O. Fischer.

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CHEMISTRY

Anhydrous Ruthenium Chlorides

The contrasting magnetic behaviour (Table 1 and Fig. 1) of the α- and β-forms of $RuCl_3$ is of interest in relation to the different types of stereochemical arrangements[1] shown by the transition metal halides: it has also allowed the presence of α-$RuCl_3$, produced in the usual method of preparation of β-$RuCl_3$, to be detected. A material, hitherto considered[2,3] as yet another form of $RuCl_3$, is now shown to be $Ru_2^{IV}OCl_6$ (Table 1), a member of a class of oxide chlorides of binuclear ruthenium in which the average oxidation state of ruthenium changes from +4 through +3·5 to +3.

α-RuCl₃. This is produced[3,4] by a slow reaction between the metal and chlorine in siliceous vessels at temperatures above 600° C. However, contact of the metal with the silica causes the α-$RuCl_3$ to be contaminated with the soluble and relatively volatile oxide chloride, Ru_2OCl_6 (since this is diamagnetic its presence lowers the susceptibility observed and accounts for the values previously reported[5] being 16–19 per cent lower than ours). To avoid this contamination, α-$RuCl_3$ has now been prepared by heating β-$RuCl_3$ at or above the transition temperature for the β → α transformation. We have found, using the susceptibility as a means of analysis, that this transformation occurs slowly ($t_{\frac{1}{2}}$, about 1 h) at about 450° C, a temperature appreciably lower than the > 500° previously noticed[4]. The molar susceptibility, χ_M, of α-$RuCl_3$ between 77° and 300° K is shown in Fig. 1; at very low temperatures it decreases:

Temperature (° K)	77·3	20·4	4·2
$\chi_M \times 10^6$, E.M.U.	9,500	15,400	9,950 (all ± 200)

suggesting that α-$RuCl_3$ becomes antiferromagnetic. Neutron diffraction measurements (by G. E. Bacon) at 4·2° K confirm this behaviour and indicate a small magnetic moment (about 0·2 *B.M.*) on each ruthenium atom. The Néel temperature, T_N, about 30° K, is of the same order as those, 17° and 15° K respectively, reported[6] for $CrCl_3$ and $FeCl_3$.

Table 1

Compound	Appearance	Solubility	Magnetic behaviour	Structure
α-$RuCl_3$	Black, lustrous	Insoluble	With $A = 1{,}000$ cm^{-1}, μ_{eff} is 2·14 B.M. at 300° K, and θ is 32–40°. Antiferromagnetic, T_N, about 30° K	Hexagonal, layered (ref. 4); $a = 5{\cdot}99$, $c = 17{\cdot}22$ Å. Ru atoms have octahedral bonding with d_ε^5 configuration
β-$RuCl_3$ (metastable)	Dark-brown fluffy	Soluble in EtOH	χ_M low and decreases as temperature falls (Fig. 1)	Hexagonal, layered; $a = 6{\cdot}12$, $c = 5{\cdot}66$ Å. Ru atoms probably have tetrahedral bonding with $d_\gamma^4 d_\varepsilon^1$ configuration.
Ru_2OCl_6	Dark-brown hygroscopic	Soluble in H_2O and EtOH	Diamagnetic	Probably linear Ru—O—Ru, cf. $[Ru_2OCl_{10}]^{4-}$

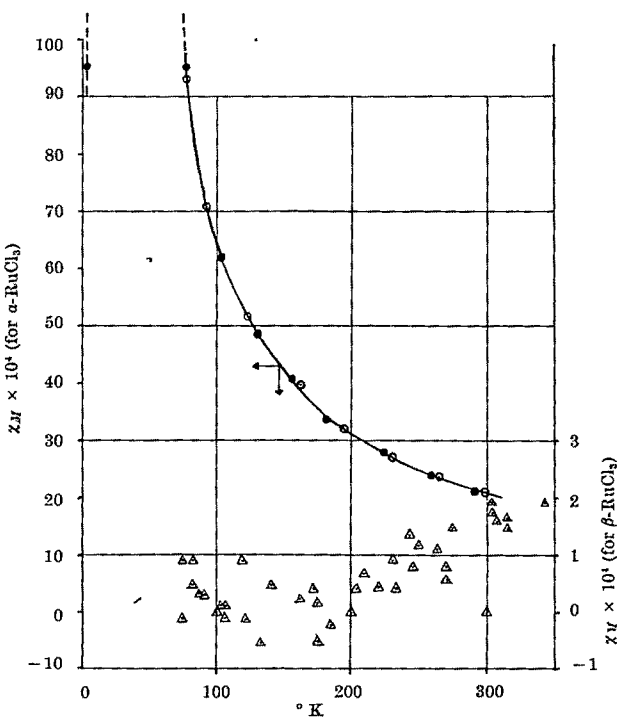


Fig. 1. Observed molar susceptibilities: ⊙, ●, Results for two separate preparations of α-$RuCl_3$; △, results from five separate sets of measurements between 77° and 345° K on the same preparation of β-$RuCl_3$, heated for 4 h at temperatures between 400° and 440° C between each successive set of measurements

β-*RuCl*$_3$. This is made[7] by heating ruthenium metal in a stream of carbon monoxide and chlorine. The susceptibilities of preparations made at 360°–390° C indicate the presence of 10–15 per cent of α-$RuCl_3$; this was not detected in the X-ray patterns until the materials had been annealed. β-$RuCl_3$ (found: Ru, 48·3; Cl, 50·5 per cent), almost free from α-$RuCl_3$ and certainly free from residual metal, has therefore been made at a temperature (340° C) lower than formerly used but sufficiently high for the carbonyl intermediate, probably $Ru(CO)_2Cl_2$, to be absent. The small increase in the observed susceptibility below 100° K (Fig. 1) is attributed to a trace (< 2 per cent) of α-$RuCl_3$ in the sample.

Oxide Chlorides. Ru_2OCl_6 is one of several relatively volatile oxide chlorides which can be formed from the water-soluble mixture of aquochloro complexes[8] of polynuclear Ru(IV) known as "commercial ruthenium trichloride". This, when dried at 110°, consists largely of $(Ru_2O)^{6+}$ complexes, and after further heating at 300° in a stream of chlorine, leaves a dark-brown hygroscopic residue, mainly Ru_2OCl_6. Such a residue had a chlorine/ruthenium ratio of 3·04; the average oxidation state of ruthenium was +3·96; and the absorption spectrum of a solution in 1 N acid, had λ_{max} 390 mμ, $\varepsilon_{Ru} = 5{,}600$, characteristic[8] of Ru_2O^{6+} complexes. It appeared amorphous to X-rays and was diamagnetic. When, however, the heating at 300° is carried out in a vacuum instead of a stream of chlorine, other oxide chlorides, Ru_2OCl_5 and Ru_2OCl_4, which have ruthenium respectively in the +3·5 (ref. 9) and +3 oxidation states, are formed. A typical product had the ratio chlorine/ruthenium = 2·60, and an average oxidation state of + 3·74: these, together with the spectrum of its solution, indicate a mixture of the following mole fractions: 0·62 Ru(IV) as $(Ru_2O)^{6+}$, 0·06 Ru(IV) as RuO_2, 0·22 Ru 3·5 as $(Ru_2O)^{5+}$, and 0·10 Ru(III) as $(Ru_2O)^{4+}$. The material was paramagnetic, χ_g being $0{\cdot}74 \times 10^{-6}$ e.m.u. at 299° K and rising to 1·57 at 90° K. The brown hygroscopic substance observed[2,3] to accompany α-$RuCl_3$ when prepared from the metal and chlorine at 600°–700° C was identified as Ru_2OCl_6 by the absorption spectrum of its aqueous solution and by the oxidation state of ruthenium.

Interpretation of Susceptibilities. The layered structure and cell dimensions[4] (Table 1) of α-$RuCl_3$ imply that each ruthenium atom occupies a position in which it is bonded octahedrally to six chlorine atoms. We assume: (1) that a strong ligand field exists for Ru^{3+} in the compound giving it a d_ε^5 configuration; (2) that there is a fairly strong spin-orbit interaction[10,11], such that the coupling constant, A (in ° K), is $> T$. It then follows[10] that $\mu^2_{eff} = 3 + 8T/A$ and that $\chi'_M = \dfrac{3 + 8T/A}{8(T - \theta)}$, where θ is a measure of the net magnetic interaction between the ruthenium ions. Although A for Ru^{3+} has been assessed[1] at 2,250° K, results[12] for Ru(III) complexes (for which θ should be nearly zero) give values in the range 1,000–2,000° K. Substituting $A = 1{,}500°$ K (≡ 1,000 cm^{-1}), and adjusting the values of χ_M to χ'_M by adding 100×10^{-6} e.m.u. for the diamagnetism of the ion cores, it is found that θ is 40° at 300° K and decreases to about 32° at 80° K (cf. θ = 31° for $CrCl_3$) (ref. 13). μ_{eff} is 2·14 Bohr magnetons at 300° K. The positive value of θ implies that the antiferromagnetic coupling between the ruthenium atoms in adjacent layers is weak compared with the ferromagnetic coupling between the ruthenium atoms within a layer.

The low values of χ_M shown by β-$RuCl_3$ are explained by each ruthenium atom occupying a position in a layered structure where it is bonded tetrahedrally to four chlorine atoms: this configuration may well be favoured by a 4-co-ordinated complex, $Ru(CO)_2Cl_2$, being an intermediate. On Kotani's[10] theory, Ru^{3+}, when tetrahedrally bonded and spin-paired, takes a $d_\gamma^1 d_\varepsilon^1$ configuration and, provided again that $A > T$, its magnetic moment is low, μ_{eff} being given by $\sqrt{4\ T/A}$ and χ'_M by $[2A(1-\theta/T)]^{-1}$: using again the value of 1,500° K for A, χ'_M would be $\sim 330 \times 10^{-6}$ e.m.u. at 300° K. In fact, χ'_M (that is, $\chi_M + 100 \times 10^{-6}$ e.m.u.) for β-$RuCl_3$ is found to be about 280×10^{-6} e.m.u. at 300° K. The fall in χ_M (Fig. 1) and hence in χ'_M as the temperature is lowered implies that θ in this case is negative, but a reasonable value for θ does not account for the observed fall.

It appears that β-$RuCl_3$ is metastable, the β→α transformation being exothermic[4] and irreversible, and its density[4] being lower than that of α-$RuCl_3$.

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Electron Spin Resonance in Polymer Carbons: Effect of Halogens and Interhalogen Compounds

The effect of adsorbed gases on the electron spin resonance signals of amorphous carbons has been reported by a number of workers[1–3]. In general, it has been found that whereas gases such as H_2, Ar, N_2 and Cl_2 (ref. 4) have no effect on the resonance signals, paramagnetic gases such as O_2 and NO have been found to have a large reversible effect at room temperature. This behaviour has been ascribed to the magnetic properties of the paramagnetic gas molecules.

Recent work in these laboratories has shown that the electron spin resonance signals of certain polymer chars are considerably modified by the adsorption of halogens and interhalogen compounds on to the carbon surface. Thus the adsorption of iodine from solution or from the vapour phase at room temperature by carbons prepared from polyvinylidene chloride (in the heat treatment range 300–1,000° C) caused a marked reduction in spin concentration at almost constant line width. The original signal was not restored by outgassing the carbon specimens at room temperature, but could be partially recovered by reheating the specimens in vacuum to 100° C.

The effect of Cl_2 and Br_2 was similar to that of I_2, leading to a partial or complete reduction in spin concentration depending on the carbon examined. In the reaction with chlorine at room temperature hydrochloric acid was detected in the gas phase using infra-red methods.

It should be noted that these effects are not observed with all amorphous carbons, a fact which may be explained on the basis of some of our earlier work[5], in which it was

shown that the reaction of paramagnetic oxygen molecules, with the unpaired electrons of the carbon, may be used as an indicator of the porous nature of the carbon structure. We found that carbons prepared from polyvinyl cyanide have negligible surface area, and as they are virtually inaccessible to gas molecules, show no characteristic electron spin resonance effects with paramagnetic gases. We attribute the failure of earlier workers to observe any effect with halogens to the inaccessibility of the carbons examined. This idea is supported by the observation that a polydivinyl benzene carbon (heat treatment temperature 900° C), initially showing no halogen effect, was found to have the signal partially removed after development of pore structure by activation with carbon dioxide at 880° C.

On the basis of our preliminary results we tentatively suggest that halogens adsorbed by a suitable carbon will interact with the unpaired electrons giving rise to some type of charge transfer complex. This has already been suggested as[6] possibly occurring in the adsorption of molecular oxygen by amorphous carbons. The formation of such charge transfers or molecular complexes between aromatic molecules and halogens is well established[7,8] and in many cases strong electron spin resonance spectra have been observed[9,10]. It is thought that the apparent disappearance of unpaired electrons in amorphous carbons occurs for the same reason as their production from initially neutral organic molecules, that is, an intermolecular charge transfer between the carbon and the halogen occurs, resulting in the removal of the unpaired electron from the carbon, and the formation of a complex which no longer exhibits electron spin resonance.

This could possibly arise as a result of a broadening of the resonance line beyond detection when the unpaired electron is localized on the iodine molecule. It has been observed that the charge transfer complexes between oxygen and aromatic molecules[11] show electron spin resonance spectra characteristic of the Ar^+ ion, but non-corresponding to the O_2^- ion. Similarly, Bennett *et al.*[12] have shown that the O_2^- ion in superoxide has an extremely broad resonance line. However, it should be noted that irradiation of potassium chloride crystals doped with potassium iodide produced a narrow electron spin resonance line which has been attributed to the I_2^- ion[13] though we feel the two systems are so different as not to allow any reasonable comparison.

The stability of charge transfer complexes of halogens and interhalogen compounds with organic molecules depends on the relative electron acceptor strengths of halogens, and decreases in the order $ICl > IBr > I_2 > Br_2 > Cl_2$ (ref. 14). We have been able to show that ICl and IBr produce analogous effects to I_2, though no estimate of the relative stability of the complexes has been made.

In the case of hydrogen containing chars, the stability of the complexes will also depend on the ease with which the halogen can react further with the hydrogen sites. Iodine, owing to its high electron acceptor strength and low affinity for hydrogen, might be expected to form a stable charge transfer complex. Chlorine, on the other hand, having a much greater affinity for hydrogen and low electron acceptor strength, will be more likely to react via the charge transfer complex with adjacent hydrogen sites producing hydrogen chloride, chemisorbed chlorine, and a regeneration of the signal in the absence of excess chlorine.

For certain carbons there is experimental evidence for believing this to occur, the chlorine being unable to react with the spin sites to affect the resonance either because the concentration of molecular chlorine is too low or because of pore restriction by chemisorbed chlorine.

If one assumes that the unpaired electrons in carbon represent sites of abnormally high reactivity, then the removal of these sites by the formation of a charge transfer complex may be the reason for the reported inhibition of graphite oxidation by chlorine[15].

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Action of Mercuric Chloride on Strained Keratin

Further evidence that the strained disulphide bonds of stretched animal fibres are more reactive than unstrained bonds has been obtained in experiments with mercuric chloride. Purified human hair fibres of measured cross-sectioned area were immersed in a solution of mercuric chloride (0·1 M) in hydrochloric acid (0·1 N) for 24 h at 22·2° C and then stretched different amounts in the same medium. After being released, the fibres were washed for 7 days in running water before their load/extension curves were determined up to the breaking point in distilled water at 22·2° C. Values for the work required for 55 per cent extension were derived from the load/extension curves and give the lower curve in Fig. 1.

For reference purposes, other fibres were stretched twice in distilled water, and it is because such fibres normally break at 55 per cent extension, whereas fibres stretched in the solution of mercuric chloride have higher extensions at break, that the work was calculated for this extension in all cases. Comparison of the two curves of Fig. 1 shows that the fibres are weakened far more by extension in the mercuric chloride solution than in water, and since fibres which had been immersed in the mercuric chloride solution for 24 h at 22·2° C, but without extension, were found after prolonged washing to give load/extension curves which were almost identical with those of untreated fibres in water, it seems probable that

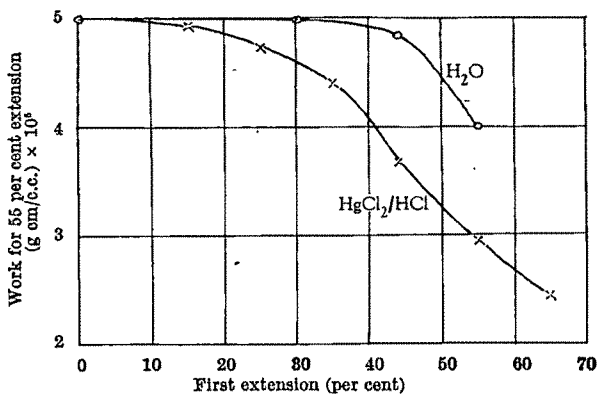


Fig. 1

the weakening is brought about by the fission of strained disulphide bonds according to the reaction:

$$R - S - S - R + HgCl_2 \rightarrow RSCl + RSHgCl \text{ (ref. 1)}$$

This view is supported by the higher extension at break of fibres stretched in the mercuric chloride solution, as well as by the general similarity between these results and those obtained with fibres which were stretched in presence of adsorbed iodine[2]. In both cases the breakdown occurs mainly in those regions of the fibre which give the higher modulus of the third part of the load/extension curve.

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BIOCHEMISTRY

Examination of Optically Excited Amino-acids by Electron Spin Resonance at Very Low Temperature

We have recently[1] investigated the irradiation by ultra-violet light of amino-acids at low temperature. Preliminary results of an investigation by electron spin resonance were given. We mentioned the difficulties of a correct interpretation of the signals obtained on microcrystalline powders of phenylalanine and tryptophan. Here are the first results of an investigation concerning solutions of aromatic amino-acids (tryptophan, phenylalanine, tyrosine).

At low temperatures transparent glasses are obtained with certain solvents. The purified and degassed samples are directly irradiated in the cavity of a Varian 4502 02 spectrometer at the liquid nitrogen temperature. The source of light is an Osram *HBO* 200 lamp without filter. There is a great difficulty in obtaining sufficiently concentrated solutions as the solubility of these amino-acids in ether–pentane–alcohol (EPA) is very low. But it can be raised by addition of alcohol and thus it is possible to obtain an electron spin resonance signal with tryptophan for $g \simeq 2$ after a few minutes of irradiation.

But the following results show that the signal is not specific of the solute because there is certainly a photosensibilization of the alcoholic part of the solution. Alcohols alone give better solubility (10^{-2} M with methanol, ethanol). Great care must be taken in avoiding crystallization. Two kinds of electron spin resonance signals are then obtained.

Electron spin resonance signals of the triplet state of phenylalanine, tyrosine and tryptophan. We know[2] that it is possible to induce a resonance transition corresponding to $\Delta m = 2$ in several organic molecules; Smaller succeeded

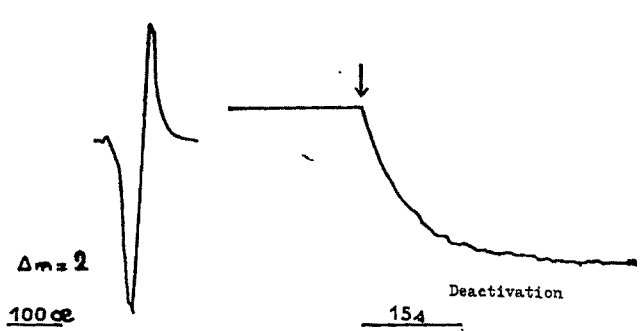


Fig. 1. Triplet state of tryptophan in methanol at low temperature

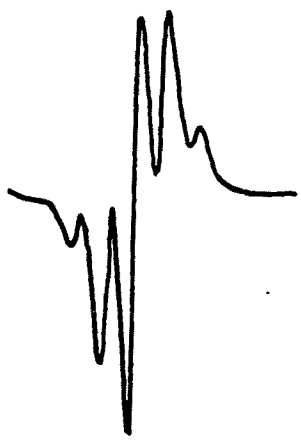


Fig. 2. Electron spin resonance signal of photosensibilized methanol

in investigating the triplet state of indole[3] and benzene[4] by electron spin resonance.

With these three amino-acids in alcoholic solution, at low temperature, we obtained signals which are specific of the state $S = 1$ (Fig. 1). Their form, position and deactivation kinetics are characteristic of the molecule considered. More information will be available with the complete calculation of the spin Hamiltonian.

Electron spin resonance signals of free radicals produced with solvents. For the normal transition $\Delta m = 1$, intense signals are obtained, and if we investigate their structure we can make the following conclusion: for sufficient concentrations of amino-acids we observe the photosensibilization of the alcohols by appearance of free radicals (Fig. 2).

The nature of these radicals, their appearance kinetics, their development with temperature are quite complex[5]. In the first seconds of irradiation we have a rather weak signal, similar to that of the methoxy CH_3O radical. Then it grows notably in intensity and complexity, giving a signal which must be that of the CH_2OH and CHO radicals. Smaller observed similar reactions with indole solutions in methanol.

In conclusion, it is difficult to make out the existence of radicalar metastable configurations in amino-acids by electron spin resonance. With new experimental conditions we might be able to obtain a characteristic electron spin resonance signal of the solute and to give its right interpretation.

Recent results of Gill and Weissbluth[6] on the thermoluminescence of polycrystalline samples of amino-acids induced these authors to admit the existence of such metastable configurations. But the results obtained in the same time by electron spin resonance are much more delicate to analyse. This conclusion and our observation of a triplet state pose the problem of the relationship between the triplet and the metastable states of the molecules of aromatic amino-acids: we are now investigating this matter.

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Inhibitory Action of Adenine, Adenine Nucleosides and Nucleotides on the Biosynthesis of Porphyrins by *Rhodopseudomonas spheroides*

Our earlier investigations have shown that adenosine-5-monophosphoric acid (AMP) exerts a strong inhibitory action on excess porphyrin formation in rats with experimental porphyria[1,2] or in lead-poisoned rabbits[3] as well as *in vitro* during incubation of reticulocytes or liver homogenates of rabbits with glycine[4]. Rimington has also observed this effect of AMP in the porphyria induced in rats by hexachlorobenzene[5]. To obtain further results concerning this inhibition, we have investigated the effect of adenine, adenine nucleosides and nucleotides on the biosynthesis of porphyrins by *Rhodopseudomonas spheroïdes*. As it is known, this micro-organism synthesizes, in semi-anaerobic conditions and in the light, porphyrins (and bacteriochlorophyll) through the same biochemical mechanism as animal cells.

Rhodopseudomonas spheroides, obtained from Dr. Lascelles (Oxford), were grown semi-anaerobically in the light at room-temperature for 48 h in medium *S* of Lascelles[6]. Then, the organisms were gathered by centrifugation at 0° and washed with cold 0·05 M phosphate buffer $pH = 7{\cdot}4$. The washed cells were suspended at a final concentration of 0·2–0·8 mg/ml. either in mixture *I* of Lascelles (containing α-oxyglutarate and glycine), or in mixture *Ia* of Lascelles (containing succinate and glycine), or in mixture *II* of Lascelles (containing δ-aminolævulinic acid as substrate). The adenine nucleosides and nucleotides were added in various amounts. These different mediums were then exposed to the light in semi-anaerobic conditions for 48 h. After this time, the dry weight of micro-organisms was determined by the method of Cohen-Bazire *et al.*[7]; porphyrins and bacteriochlorophyll were extracted and measured respectively following the methods of Lascelles[6] and Cohen-Bazire *et al.*[7].

Adenine, adenosine, inosine, AMP, ADP and ATP, at a concentration of 3 mM, exerted an inhibitory action in mixture *I* on porphyrin synthesis. Inhibition was weak for adenine, of about 50–70 per cent with adenosine, inosine, AMP and ADP, very strong with ATP (Table 1). The growth of the micro-organism and the formation of bacteriochlorophyll were not significantly modified by these compounds, except adenosine which inhibited constantly the synthesis of bacteriochlorophyll. Thus the decrease in porphyrin concentration after incubation in the presence of the investigated compounds cannot be explained by an increased synthesis of bacteriochlorophyll.

Table 1. Effect of Adenine, Adenosine, Inosine, AMP, ADP and ATP on Growth and Synthesis of Porphyrin and Bacteriochlorophyll in Mixture *I* of Lascelles (Incubation, 48 h)

Medium	Dry weight of micro-organism (mg/ml.)	Porphyrin (μmM/ml.)	Bacteriochlorophyll (μmM/ml.)
Mixture *I* of Lascelles	1·2	36·9	1·69
Same mixture + adenine, 3 mM/l.	1·1	29·5	1·25
Same mixture + adenosine, 3 mM/l.	0·9	14·6	0·84
Same mixture + inosine, 3 mM/l.	1·2	19·3	1·65
Same mixture + AMP, 3 mM/l.	1·2	7·9	1·35
Same mixture + ADP, 3 mM/l.	1·0	10·9	1·40
Same mixture + ATP, 3 mM/l.	1·1	0·5	1·35

The inhibitory effect has been also observed in mixture *Ia* (Table 2). Here again, ATP exerts the strongest inhibition.

The relation between the concentration of purine derivatives and the inhibition of porphyrin synthesis was examined with ATP. The inhibition increases rather regularly with the increasing amounts of ATP. The most efficient concentration seems to be 3 mM/l. In either of the concentrations investigated the growth of micro-organism and the synthesis of bacteriochlorophyll were significantly modified (Table 3).

Table 2. Growth and Synthesis of Porphyrin and Bacteriochlorophyll in Mixture *Ia* of Lascelles. Effect of AMP, ADP and ATP (Incubation, 48 h)

Medium	Dry weight of organism (mg/ml.)	Porphyrin (μmM/ml.)	Bacteriochlorophyll (μmM/ml.)
Mixture *Ia* of Lascelles	2·66	21·7	3·22
Same mixture + AMP, 3 mM/l.	2·66	13·2	3·55
Same mixture + ADP, 3 mM/l.	2·76	3·9	3·97
Same mixture + ATP, 3 mM/l.	2·83	1·8	4·18

Table 3. Effect of ATP in Increasing Amounts on Growth and Synthesis of Porphyrin and Bacteriochlorophyll (Incubation, 48 h)

Medium	Dry weight of micro-organism (mg/ml.)	Porphyrin (μmM/ml.)	Bacteriochlorophyll (μmM/ml.)
Mixture *I* of Lascelles	1·2	39·3	1·75
Same mixture + ATP, 250 μM/l.	1·2	33·2	1·56
Same mixture + ATP, 500 μM/l.	1·3	22·1	1·61
Same mixture + ATP, 750 μM/l.	1·3	19·8	1·88
Same mixture + ATP, 1,000 μM/l.	1·3	17·0	2·21
Same mixture + ATP, 3,000 μM/l.	1·3	2·4	2·40

The inhibitory effect of the adenine derivatives seems to be located, as suggested by our earlier observations[4], to the first step of porphyrin formation, namely, to the synthesis of δ-aminolævulinic acid. In fact, no inhibition has been noted when these compounds are added to suspensions of *Rhodopseudomonas spheroides* in mixture *II*, containing δ-aminolævulinic acid as substrate (Table 4).

Table 4. Effect of AMP, ADP and ATP on Growth and Synthesis of PBG, Porphyrin and Bacteriochlorophyll in Mixture *II* containing 100 μg ALA per ml. (Incubation, 24 h)

Medium	Dry weight of organism (mg/ml.)	ALA unchanged (μmM/ml.)	PBG (μmM/ml.)	Porphyrin (μmM/ml.)	Bacteriochlorophyll (μmm/ml.)
Mixture *II* of Lascelles	0·5	603	3·1	0·49	0·61
Same mixture + AMP, 3 mM/l.	0·5	603	3·1	0·47	0·60
Same mixture + ADP, 3 mM/l.	0·5	617	3·5	0·50	0·59
Same mixture + ATP, 3 mM/l.	0·5	656	3·3	0·61	0·61

The inhibition of porphyrin synthesis by adenine, adenine nucleosides and nucleotides may represent a feed-back regulation. Δ-aminolævulinic acid is not only a precursor of porphyrins, but it participates also, by its fifth carbon atom, in the biosynthesis of purines, as was shown by Shemin *et al.*[8]. Thus it can be assumed that, according to the definition of the feed-back inhibition, the purine derivatives examined act as end-products on the first step of their formation, with the resulting decrease of biosynthesis of δ-aminolævulinic acid and porphyrins. In this respect, it was interesting to note that pyrimidine derivatives—as thymine, uracil or orotic acid—did not exert any inhibition on porphyrin biosynthesis by *Rhodopseudomonas spheroides* (Table 5).

Table 5. Effect of Tymine, Uracil and Orotic Acid on Growth and Synthesis of Porphyrin and Bacteriochlorophyll in Mixture *I* (Incubation, 48 h)

Medium	Dry weight of micro-organism (mg/ml.)	Porphyrin (μmM/ml.)	Bacteriochlorophyll (μmM/ml.)
Mixture *I* of Lascelles	1·5	24·9	2·44
Same mixture + thymine, 3 mM/l.	1·6	27·7	3·02
Same mixture + uracil, 3 mM/l.	1·6	25·2	2·34
Same mixture + orotic ac., 3 mM/l.	1·5	25·8	2·68

The observations recorded here confirm and complete our earlier findings showing the inhibitory effect of

adenine nucleosides and nucleotides on excess porphyrin formation. This inhibitory effect may constitute one of the physiological mechanisms regulating porphyrin biosynthesis.

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Specificity of Pepsin and its Dependence on a Possible 'Hydrophobic Binding Site'

The specificity of pepsin has been extensively investigated, and the information obtained from work using synthetic di- or tri-peptides indicates that pepsin preferentially hydrolyses peptides which contain a tyrosine or phenylalanine residue[1,2]. However, Sanger, using the *A* and *B* chains of insulin, found that peptide linkages which did not involve tyrosine or phenylalanine were hydrolysed by pepsin[3,4]. Bovey and Yanari[5] recently reviewed the action of pepsin on synthetic peptides and proposed several general rules. It was pointed out, however, that these rules "do not necessarily apply to the peptic digestion of proteins and large polypeptides, which is often considerably more extensive than these rules would lead one to expect". In order to understand the action of pepsin on protein substrates, we have made a systematic examination of the sequence of amino-acid residues adjacent to the site of peptic hydrolysis in several proteins of known amino-acid sequences. In this communication, a concept is proposed which explains the specificity of pepsin for protein substrates in terms of a hypothetical 'hydrophobic binding site'.

Information on the site of hydrolysis of pepsin on insulin was obtained from the work of Sanger *et al.*[3,4], on α-corticotropin from Li[6] on ribonuclease from Hirs *et al.*[7], and on the α-chain of human hæmoglobin from Konigsberg and Hill[8]. A tabulation of the sequence of the first five amino-acids on either side of the site of cleavage by pepsin was made for these four proteins. An example of a part of the tabulation is shown in the following:

← To N-terminal					To C-terminal →				
CO5	CO4	CO3	CO2	CO1	NH1	NH2	NH3	NH4	NH5
Asp	Lys	Thr	Asp	Val	Lys	Ala	Ala	Tyr	
	Lys	Ala	Ala	Tyr	Gly	Lys	Val	Gly	Ala
Ala	His	Ala	Gly	Glu	Tyr	Gly	Ala	Glu	Ala

↖ Peptide linkages hydrolysed

If another site of hydrolysis was found before the fifth residue, the sequence was discontinued. The results of the hydrolysis of sixty-five bonds were tabulated, and the percentage of the total number of residues of each amino-acid found in a given position is shown in Table 1.

On the basis of the percentile distribution, the amino-acids were classified in three groups: (1) The first group (highly susceptible amino-acids) consists of, in the order of preference by pepsin, phenylalanine, leucine and tyrosine, all of which appear in relatively high frequencies in the CO1 and NH1 positions. Since these three amino-acids bind to all amino-acid residues in a random fashion, the high susceptibility of peptide linkages containing these three amino-acids must be due exclusively to their presence. (2) The second group (susceptible amino-acids), which includes glutamic acid (+glutamine), alanine, aspartic acid (+asparagine), methionine, valine, threonine, lysine, serine, and histidine, consists of those amino-acids which are susceptible to peptic hydrolysis but which are distributed rather evenly throughout the ten sequential positions. (One exception was observed for histidine at the CO4 position. However, its significance is uncertain.) (3) The third group includes glycine, isoleucine, proline, arginine and cysteic acid. None of them appears in the CO1 position and only three appear in the NH1 position (two with very low frequencies). This group is thus designated as non-susceptible amino-acids. An examination of nineteen peptide bonds involving phenylalanine, leucine, or tyrosine (members of the highly susceptible group) not affected by peptic hydrolysis revealed that in thirteen of these bonds the other amino-acid is one of the three amino-acids belonging to the third group: proline, arginine and cysteic acid. These three amino-acids should therefore be considered as 'anti-susceptible amino-acids'.

It seems clear from the foregoing information that the probability of a peptide bond being hydrolysed by pepsin depends mainly on the nature of the residues on the carbonyl side (CO1 position) of the peptide linkage, and, to a somewhat less degree, on the amino side of the linkage (NH1 position).

Since the side-chains of phenylalanine and leucine, two of the most highly susceptible amino-acids, consist of

Table 1. Frequency of Appearance of Amino-Acids at Ten Sequential Positions around the Hydrolysed Linkage*

Sequential positions	CO5	CO4	CO3	CO2	CO1	NH1	NH2	NH3	NH4	NH5
First group (highly susceptible):										
Phenylalanine	0	0	0	8 (0)	81	47	0	38 (0)	24 (12)	16 (0)
Leucine	0	0	5	14 (14)	59	33	25 (25)	35 (9)	14 (14)	29 (10)
Tyrosine	16 (0)	13 (0)	8 (0)	15 (8)	24	24	6 (6)	7 (7)	11 (11)	15 (15)
Second group (susceptible):										
Glutamic acid (+ Glu—NH_2)	10	15	18	34	28	28	31	21	13	18
Alanine	14	33	27	22	21	21	29	15	5	20
Aspartic acid (+Asp—NH_2)	9	7	24	23	16	22	18	0	6	24
Methionine	40	0	0	0	14	0	0	17	0	0
Valine	37	0	17	33	10	20	23	20	13	9
Threonine	28	0	33	6	10	15	17	6	0	13
Lysine	31	24	48	9	7	8	9	9	14	0
Serine	18	0	24	23	6	13	14	22	6	33
Histidine	16	77	0	29	6	12	14	21	34	15
Third group (non- and anti-susceptible):										
Glycine	17	64	38	15	0	6	6	0	22	15
Isoleucine	0	0	65	30	0	0	0	0	0	0
Proline	53	54	32	8	0	19	21	15	0	33
Arginine	0	0	12	11	0	0	0	0	0	24
Cysteic acid	37	15	17	0	0	7	7	48	0	17

* The data represent the percentage of an amino-acid residue appearing at a given position. The calculations were made according to the following formula: for example, leucine at position CO2 is calculated:

$$\%\ \text{of leucine at CO2} = \frac{\text{No. of leu found at CO2} \times \dfrac{\text{Total No. of tabulated peptide bonds hydrolysed}}{\text{Total No. of residues found at CO2}}}{\text{Total No. of leucine found in four proteins}} \times 100$$

The data in parentheses are the percentages of the residues after discounting those which are directly involved in the hydrolysis of other bonds.

only hydrocarbon groups, the affinity of pepsin toward these groups must be assumed to be apolar in nature. It is thus hypothesized that there exists a site on the pepsin molecule which binds the hydrocarbon side-chains of the amino-acid residues in the substrate through a hydrophobic bond as an essential step of enzymatic action. (For a recent review in the hydrophobic bond see ref. 9.) The following observations and discussion support the foregoing hypothesis.

A grouping of the amino-acids according to the nature of their side-chains is presented in Table 2. There exists an apparent correlation between the number of carbon atoms in the side-chains and the frequency of appearance at the CO1 position. The tendency for the less water-soluble residue to be more abundant in the susceptible positions supports the possibility of hydrophobic binding site between the enzyme and substrate. The standard free energy of formation of hydrophobic bonds between some isolated side-chains has been calculated by Némethy and Scheraga[9]. A correlation between free energy of formation of hydrophobic bonds and the frequencies of appearance of the amino-acids in group *A* (Table 2) is obtained only when it is assumed that the hydrophobic binding site of pepsin is phenylalanine. The striking low frequency of the amino-acids in group *B* seems to be due to steric effects, since the arrangement of the carbon skeleton in the side-chain of isoleucine and valine is basically different from those belonging to group *A*.

Table 2. CORRELATION OF THE NO. OF CARBON ATOM IN THE SIDE-CHAIN OF AMINO-ACIDS AND THE FREQUENCY OF THEIR APPEARANCE AT CO1 POSITION

Side-chains	Amino-acid	No. of carbon atoms in the side-chain	Standard free energy of formation† (kcal/mole)	Solubility in water* in mole fraction (No. × 10^4)	% of residues appeared at CO1
A Hydrocarbon, with all or part of benzyl group	Phe	7	−1·4	32	81
	Leu	4	−0·5	32	59
	Ala	1	−0·2	327	21
	Gly	0	—	566	0
B Hydrocarbon, not part of benzyl group	Ileu	7		56	0
	Val	3		135	10
C Contain oxygen	Tyr	7		0·7	24
	Glu (+Glu—NH_2)	3		54‡	28
	Asp (+Asp—NH_2)	2		34‡	16
	Thr	2		31	10
	Ser	1		86	6
D Contain nitrogen	His	4			6
	Lys	4			7
	Arg	4			0
E Others	Met	3		41	14
	Crys—SO_3H	2			0
	Pro	3		2,028	0

* Calculated from the data in Cohn and Edsall (ref. 10).
† Taken from Némethy and Scheraga (ref. 9), assuming that the side-chain of phenylalanine is the 'binding site' of pepsin.
‡ The solubility data for glutamine and asparagine are used, since the carboxylic group does not ionize below *p*H 2. It is thought that glutamine and asparagine would be more representative.

The presence of acidic or basic groups in the side-chains, known to hinder the formation of hydrophobic bonds, decreases the 'frequencies' of amino-acids in groups *C*, *D*, and *E*. The effect of the hydroxyl group is apparent when tyrosine is compared with phenylalanine, and serine to alanine. The lower susceptibility of the nitrogen-containing residues in group *D* could be attributed to the positive charge of nitrogen-containing groups at *p*H 2, the *p*H of peptic hydrolysis, whereas the carboxylic and hydroxyl groups do not ionize at that *p*H. The resistance of a cysteic acid residue seems also to be due to its highly acidic sulphonic group. In the case of proline, however, the low affinity could be explained by the difference in the bond angle in the back bone of the peptide linkages.

Although the proposed 'hydrophobic binding site' appears to determine the point of hydrolysis in protein substrates, it is unlikely that this 'site' participates in the mechanism of hydrolysis. The binding of the side-chain of the substrate to the hydrophobic site of the enzyme may be visualized as the first step in the enzyme action, which is followed by hydrolysis of the peptide bond through a yet unknown mechanism. The formation of new amino- and carboxylic-groups, however, could reduce the effectiveness of the hydrophobic binding of the side-chain to the 'site' and thus release the product from the enzyme.

It should be pointed out that in the present analysis the rate of hydrolysis of different peptide linkages is not considered. However, during the incubation of proteins with pepsin, if time is not allowed for completion of the hydrolysis, then the bonds hydrolysed would be those preferred by the enzyme. This appears to be the case in the hydrolysis of ribonuclease[7] by pepsin, since the resulting peptides are quite large. Insulin[3,4] did not present a problem, since in the work of Sanger[3,4] care was taken to distinguish the faster hydrolysed linkages from the slower ones. In α-cortico-tropin[6] and α-chain of human hæmoglobin[8], some of the more preferable peptide linkages still could be determined. If one finds in the hydrolysate a long peptide and several smaller peptides which apparently result from the further degradation of the long peptide by the enzyme, the peptide linkages cleaved at the terminals of this long peptide must be preferred by the enzyme over those at sites cleaved to give smaller peptides. Attempts were made to tabulate and analyse the result from only the more preferable peptide linkages. The conclusions drawn, however, are not significantly different from the analysis which utilizes all peptides cleaved.

It is possible that a 'hydrophobic binding site' may be present in other proteolytic enzymes. Chymotrypsin, for example, also prefers to attack a peptide linkage which contains an aromatic amino-acid. However, like pepsin, the hydrolytic action is not restricted to the bonds which contain an aromatic residue. Carboxypeptidase *A* and some cathepsins, which have affinity toward aromatic amino-acids, may also contain such sites.

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Preparation of an Insulin *A* Chain Derivative with an Intact Intra-chain Disulphide Bond

THE insulin molecule contains three disulphide bonds according to Sanger's structure formula[1]. Two of these connect the peptide chains; the third forms an intra-chain linkage between residues 6 and 11 on the *A* chain. Various authors have shown that there are differences in the reactivities of the three disulphide bonds[2]. However, in the presence of concentrated solutions of urea or guanidine hydrochloride complete cleavage is observed as well by oxidative sulphitiolytic as by reductive procedures with thioglycolate or 2-mercaptoethanol[3]. The recombination of completely reduced insulin chains by re-oxidation with air gave only low yields in the experiments of several groups of investigators[4–6], in accordance with theoretical statistical calculations made by Kauzmann[7].

Table 1. Physical and Chemical Data of the Isolated Insulin Chain Derivatives

Procedure	Reaction product	Cystine content (%)	Optical rotation	Elementary analysis	Electrophoretic mobility (cm^2/V sec)
—	crystalline zinc insulin, 25 I.U./mg (beef)	*calc.* 11·15 *pol.* 11·0 *col.* 11·2	$-62° \pm 1°$ *c.* 0·55 0·05 N NaOH	*calc.* 15·84 % N *f* 15·62 % N *f* 3·35 % S *f* 3·29 % S	3·1 cathode
Partial sulphitolysis without urea under N_2	reaction mixture	—	—	—	—
Passage through 'Dowex 50—*X*2'	*A* chain 6,11-di-sulphide-7,20-*bis*-thiosulphate	*calc.* 7·81 *pol.* 7·63 *col.* 7·95	$-50° \pm 1°$ *c.* 0·28 0·05 N NaOH	*calc.* 14·85 % N *f* 14·78 % N *calc.* 8·15 % S *f* 7·85 % S	0·29 anode
Elution of 'Dowex 50—*X*2' with 1 N ammonia	*B* chain 7,9-*bis*-thiosulphate	*calc.* 0 *pol.* 0 *col.* 0·22	$-79·5° \pm 1°$ *c.* 0·35 10% HCOOH	—	1·95 cathode
Complete sulphitolysis of *A* chain with intact intra-chain SS bond with urea in the presence of Cu^{++}	*A* chain 6,7,11,20-tetrathiosulphate	*calc.* 0 *pol.* 0 *col.* 0	$-66° \pm 1°$ *c.* 0·28 0·05 N NaOH	*calc.* 14·51 % N *f* 14·42 % N *calc.* 10·88 % S *f* 10·31 % S	1·32 anode
Oxidative sulphitolysis of insulin in presence of Cu^{++}/air and 8 M urea followed by separation	*A* chain 6,7,11,20-tetrathiosulphate	*calc.* 0 *pol.* 0 *col.* 0	$-65° \pm 1°$ *c.* 0·10 0·05 N NaOH	*calc.* 14·51 % N *f* 14·38 % N *calc.* 10·88 % S *f* 10·90 % S	1·26 anode

Abbreviations: *calc.*, calculated; *f*, found; *pol.*, polarographic determination of cystine; *col.*, determination of cystine by the method of Folin–Shinohara.

Recently, Cecil *et al.*[8] demonstrated by polarographic studies of the sulphitolytic disulphide cleavage, such as:

$$R.SS.R + SO_3^{--} \rightleftharpoons RS^- + R.SSO_3^-$$

a definite preferential cleavage of the two inter-chain disulphide bonds with sodium sulphite in aqueous solution at *p*H 7. They gave analytical evidence that the remaining intact SS bond is the 6–11 intra-chain ring of the *A* chain. We have been able to isolate in a preparative scale the *A* chain with the intact disulphide bond and to characterize the compound by cystine analysis using two independent methods, by elementary analysis, and by optical rotation (Table 1).

For the preferential selective cleavage of the two inter-chain disulphide bonds and the isolation of the *A* chain with the intact intra-chain SS ring crystalline zinc insulin (1 g) was treated at *p*H 7·2 and 35° C with a 250-fold excess of sodium sulphite. The reaction mixture was stirred mechanically for about 5 h while a stream of nitrogen was passed over the surface of the solution. After exhaustive dialysis in 'Visking' tubes 18/32 against 25 charges of distilled water at 5° C the *A* and *B* chain derivatives were precipitated with dilute hydrochloric acid at *p*H 4·8. The centrifuged solid mixture was redissolved in *p*H 1·9 buffer (50 ml.) containing 5 per cent acetic acid and 5 per cent formic acid and then passed through a 'Dowex 50—*X*2 column' (200–400 mesh, 2·2 cm × 14 cm), which had been equilibrated with the same buffer. The chromatogram was developed at room temperature with a flow rate of 25 ml./h and a volume of 5 ml. per fraction. The eluates were analysed by determination of the ultra-violet absorption at 278 mμ. Two peaks were obtained. The contents of the peak tubes (4–15 and 26–34) were lyophilized and the yields of *A* and *B* chain derivatives were 82 and 90 per cent respectively. The cystine content was determined by means of the polarographic Brdička reaction[9], and by the colorimetric method of Folin–Shinohara. The data presented in Table 1 demonstrate that the isolated *A* chain derivative is *A* chain 6,11-disulphide-7,20-*bis*-thiosulphate.

When this material was subjected to further treatment with sodium sulphite in the presence of 8 M urea the resulting reaction product was identical in electrophoretic behaviour with *A* chain tetrathiosulphate which we isolated in earlier investigations. We suggest that the *A* chain derivative with the intact intra-chain SS bond may be used with some more success in recombination studies. Hitherto, we obtained 4–7 per cent of original insulin activity on recombination of completely reduced insulin chains prepared by the oxidative sulphitolytic method.

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Intestinal Degradation of Gamma-globulin in the Dog

Increased catabolism of plasma proteins by leakage into the gastrointestinal tract and subsequent digestion by the intestinal enzymes may play an important part in normal plasma protein degradation[1,2]. All classes of plasma proteins seem to leak into the gastrointestinal lumen, but only albumin has been examined quantitatively[3–5]. In the work summarized here a quantitative estimation of the transfer of plasma γ-globulin to intestinal lumen has been made and suggestive evidence is presented that the amount of γ-globulin catabolized in the intestine of the dog is determined mainly by the capillary permeability of γ-globulin.

γ-Globulin was isolated from the serum of normal dogs by column chromatography on diethylaminoethyl cellulose[6] and labelled with iodine-131 (ref. 7). The turnover and exchange rates of γ-globulin were calculated by the method of Matthews[8] after intravenous injection of labelled γ-globulin in seven dogs. In the same dogs, four to seven weeks later, following a new intravenous injection of iodine-131 labelled γ-globulin, the transfer of plasma γ-globulin to intestinal lumen was determined by measuring the transfer of protein-bound radioactivity to isolated segments of the small intestine. Three segments (30–50 cm each, corresponding to 8–18 per cent of

Table 1. DATA OF γ-GLOBULIN TURNOVER AND INTESTINAL DEGRADATION OF γ-GLOBULIN IN THE DOG. Cl_{int}/Cl_{met} INDICATES THE FRACTION OF TOTAL TURNOVER CATABOLIZED IN THE SMALL INTESTINE

	Sex	Weight (kg)	Fractional turnover rate (%)*	Exchange rate (%)*	Metabolic clearance, Cl_{met}/min (μl./min)	Intestinal clearance, $Cl_{int/min}$ (μl./min)			$\frac{Cl_{int}}{Cl_{met}} \times 100$ (%)
						Proximal† segment	Middle† segment	Distal† segment	
1	F	13·9	23	35	111	29	32	9	21
2	M	12·5	17	26	67	17	15	16	24
3	M	10·7	21	58	71	27	44	40	52
4	M	9·2	24	46	70	33	19	29	39
5	F	6·4	18	46	38	19	6	—	33
6	M	6·3	23	37	50	6	18	8	21
7	F	4·3	29	56	52	38	34	43	74
Mean			22	43	66	24	24	24	38

* Per cent of plasma pool of γ-globulin. † Cl_{int}/min calculated as if the segment occupied the total length of the small intestine.

the total length of the small intestine) of the small intestine were isolated between tape ligatures, one orally, one anally and one about the middle. The segments were filled with 22·5 ml. saline containing soybean trypsin inhibitor (0·5 mg/ml.) through a thin 'Nelaton' catheter introduced through an enterostomy orally to the segment. The solution was aspirated after 10 min; protein-bound radioactivity was measured and by means of plasma radioactivity the intestinal clearance of γ-globulin (Cl_{int}/min) was calculated. The turnover rate of γ-globulin may be expressed as metabolic clearance per day[9] and indicates the volume of plasma containing the amount of γ-globulin catabolized per day (Cl_{met}/day). The fraction of total turnover of γ-globulin catabolized in the intestine may now be expressed by:

$$\frac{Cl\ /min}{Cl_{met}/min}$$

assuming that the intestinal clearance of the segment is representative for the whole of the small intestine.

Results of these experiments are presented in Table 1. Fractional turnover rate varied from 17 to 29 per cent (mean 22 per cent) of the plasma pool a day and the exchange rate to extravascular compartments from 26 to 58 per cent (mean 43 per cent) of the plasma pool a day. The metabolic clearance was from 38 to 111 μl. (mean 66 μl.) per min. The intestinal clearance varied from 6 to 43 μl./min (mean 24 μl./min) calculated for the whole of the small intestine, and no difference was found between the proximal, middle and distal sections; the intestinal clearance constituted 21–74 per cent (mean 38 per cent) of the metabolic clearance. In other words, these experiments indicate that about 40 per cent of the total amount of γ-globulin catabolized is broken down in the lumen of the intestinal tract. A significant correlation was found between exchange rate and the fraction of total γ-globulin turnover catabolized in the intestine ($r = 0{\cdot}84$; $0{\cdot}01 < P < 0{\cdot}02$) (Fig. 1). Since these two parameters are determined independently in two different experiments, this suggests that the intestinal exudation of γ-globulin found was not caused by the experimental procedure, because it is highly improbable that an artefact should be correlated to a physiological parameter. The exchange rate of γ-globulin, as determined by the method of Matthews[8], reflects the capillary permeability of γ-globulin. The correlation between exchange rate and intestinal degradation of γ-globulin, therefore, suggests that capillary permeability of γ-globulin is an important limiting factor in the passage of γ-globulin from plasma to intestinal lumen. This might indicate that the intestinal mucosa and epithelium are of minor importance as a barrier to intestinal exudation of γ-globulin and implies that altered capillary permeability and not changes in the intestinal epithelium should be in the cause of protein-losing gastroenteropathy, a disease of hitherto unknown ætiology.

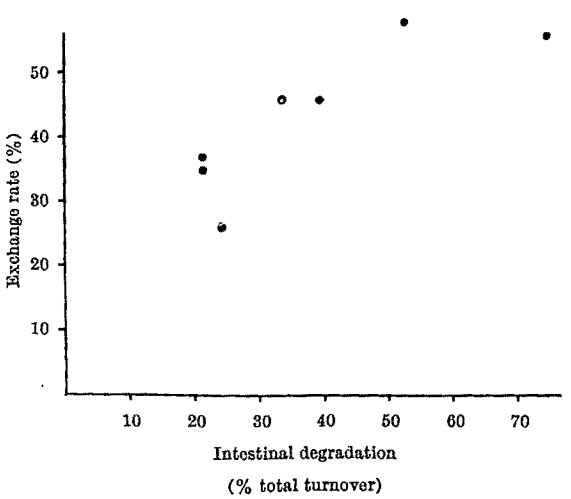


Fig. 1. Intestinal degradation of γ-globulin in per cent of total turnover (Cl_{int}/Cl_{met}) in relation to exchange-rate of γ-globulin between intravascular and extravascular compartments. $r = 0{\cdot}84$; $0{\cdot}01 < P < 0{\cdot}02$

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A Low-temperature Emission Band from Dilute Solutions of Pure Chlorophyll a

PREVIOUSLY published fluorescence spectra of chlorophyll *a* in dilute ethyl ether solution show a major band with maximum at 668 mμ and a minor band with maximum at 723 mμ, at room temperature[1,2]; at 77° K in ether–isopentane–ethanol (EPA), chlorophyll also exhibits two emission bands, with maxima at about 684 and 735 mμ[3].

Dilute acetone solutions of crystalline chlorophyll *a*, prepared by us according to either the method of Jacobs, Vatter and Holt[4] or Anderson and Calvin[5], also yield emission spectra containing two major bands—with maxima at 668 and 725 mμ at 77° K (Fig. 1, curve I). However, on further purification, an additional band with

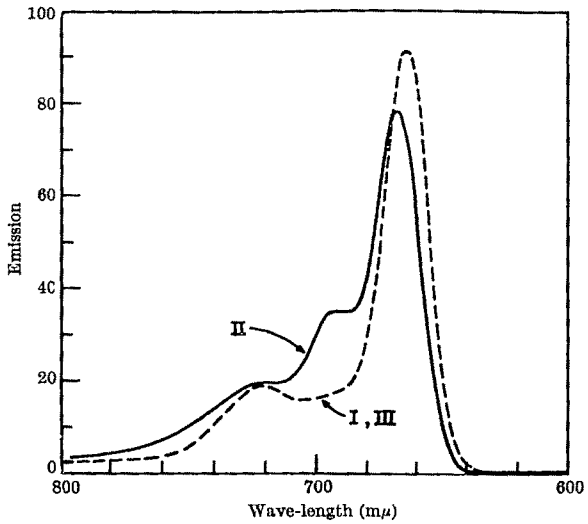


Fig. 1. Emission spectrum of a 10^{-5} M solution of chlorophyll *a* in acetone at 77° K; exciting wave-length 436 mμ. Curve I, emission spectrum of chlorophyll *a* before purification; curve II, emission spectrum of purified chlorophyll *a*; curve III, quencher added to sample shown in curve II (see text). The spectra have not been corrected for the spectral response of our Dumont 6911 photomultiplier tube. However, its response varies by only 10 per cent in the wave-length region shown

maximum at 698 mμ is observed in acetone at 77° K; in this solvent the two previously mentioned bands exhibit maxima at 671 and 725 mμ (curve II). No other emission band could be detected although measurements were made out to 1,100 mμ[6]. At room temperature the new band is not observable. The three-peaked emission spectrum characteristic of the purified chlorophyll *a* was found at 77° K, in dilute solutions of chlorophyll in acetone, ethanol, benzene, pyridine and ethyl ether. (The solvents were reagent grade, but were not further purified or dried.)

The purification technique which we used consisted of re-adsorbing the previously chromatographed chlorophyll on diatomaceous earth, washing it exhaustively with petroleum ether, and recrystallizing it in the fashion of Jacobs *et al.*[4]. Since the concentrated washings from 100 mg of chlorophyll *a*—which exhibit a pale yellow colour—yield an almost imperceptible residue on drying, the substance in question must be present in very low concentration. No appreciable change in the absorption spectrum or extinction coefficient of chlorophyll is observed after purification.

Adding the petroleum ether washings to the purified chlorophyll completely quenches the emission at 698 mμ (curve III); there appears to be a slight change in intensity of the emission maximum at 671 mμ (curves II and III). Nitrobenzene and *p*-phenylenediamine also quench the new band. The orange and yellow materials ordinarily extracted from plants do not quench the 698 mμ emission; included among those we tested were commercial preparations of β-carotene, α-carotene, and vitamin K, as well as xanthophylls and carotenes, which we extracted from spinach.

Since the naturally occurring quencher (which we obtained from our petroleum ether washings) is not readily removed from chlorophyll *a*, even after repeated chromatography, it is likely that it exists as a complex with chlorophyll. This contention is also supported by the fact that even in very minute amounts it will quench the 698-mμ emission. We are in the process of identifying this quencher, and have begun measurement of the lifetime of emission from the new band (698 mμ).

The possibility of some fluorescent material being introduced by the diatomaceous earth was eliminated by washing the earth with petroleum ether, extracting with ethyl ether and then examining the concentrated ethyl ether fraction for emission at 698 mμ at 77° K; no emission was observed.

Since the emission band at 698 mμ is readily quenched, the excited state giving rise to it may be effective in sensitizing photoreactions. Perhaps this emission band originates from a metastable state of chlorophyll such as postulated by Franck[7] in his theory of photosynthesis.

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Resolution of a Racemic Pteridine during Paper Chromatography

When ethyl pyruvate and 4,5-diaminopyrimidine were refluxed in water, between *p*H 1 and 3, a solid of molecular weight about 320 was formed which was seen as two spots[1] after dissolution in 0·1 N sodium hydroxide and chromatography on a sheet of Whatman No. 1 paper in water at *p*H 5·5 or 8·5. The spots (Fig. 1*A*) had R_F values of 0·55 and 0·65 respectively and appeared identical when viewed in ultra-violet light. When eluted, and rechromatographed, each spot generated only one spot, which corresponded in R_F to the spot from which it was eluted (Fig. 1*B* and *C*).

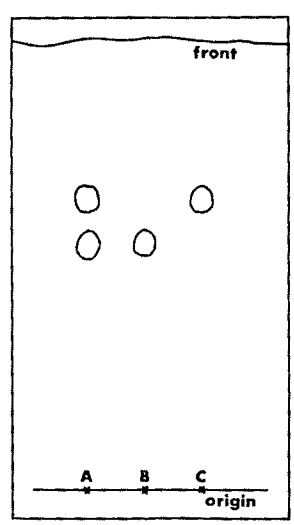
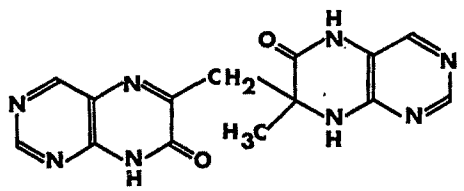


Fig. 1

When a larger quantity (50 mg) was chromatographed on a column of cellulose (diameter 4·7, and length 120 cm), two fractions were obtained which, after recrystallization from boiling water, were found to have the same four pK_a values and ultra-violet spectra as the material prior to chromatography. But whereas the original material was optically inactive, crystals from the faster-running fraction had the specific rotation $[\alpha]_{546}^{20} = +180° \pm 5°$, and those from the other fraction had $-180° \pm 5°$ (both measured at *p*H 9·1).

It was later found that the original racemate could also be made by refluxing 6-hydroxy-7-methylpteridine with 7-hydroxy-6-methylpteridine in water at *p*H 2·5. Further investigations, to be reported in detail elsewhere, showed that it was 6-(7,8-dihydro-6-hydroxy-7-methyl-

pteridin-7-yl)methyl-7-hydroxypteridine (I), formed by an acid-catalysed addition reaction of the two simpler pteridines. The molecule contains one asymmetric carbon atom.



(I)

Cellulose is an optically active substance and several authors[2] have reported that various DL-amino-acids (for example, tryptophan and histidine) are resolved in non-optically active solvents during chromatography on a sheet of paper. Resolution was demonstrated by showing that one of the two spots formed was identical with that given by the L-acid. But so far as we know, pairs of enantiomorphs have not previously been eluted from a paper sheet or a cellulose column, nor has the degree of resolution been demonstrated by measurements of optical rotation.

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PHYSIOLOGY

Influence of 2,4-Dinitrophenol on Anion Transport by an Intestinal *in vitro* Preparation

RECENT observations carried out on isolated and *in vitro* perfused preparations of the small intestine of the rat have confirmed that sodium is actively transported through the intestinal wall from the mucosal to the serosal side[1-5]. For such a transport glucose must be present in the mucosal solution; some metabolic inhibitors, 2,4-dinitrophenol (DNP) or monoiodoacetate, when added to the perfusion liquid, inhibit this transport[2,6]. The chloride ions cross the intestinal wall together with cations and for the transport of these ions, too, an active mechanism has been assumed[3,7].

The behaviour of the anion transport when DNP is added to the preparation seems also to support the view of an active chloride transport. Preliminary results[8] were obtained by perfusing the intestine on both sides with an apparatus similar to that used by Smyth and Taylor[6]. The results reported here were obtained on jejunum everted sacs of albino male rats (Wistar strain) according to the technique of Wilson and Wiseman[9]. The intestinal preparations were incubated for 1·5 h at a temperature of 28° C. Krebs–Henseleit solution[10] aerated by a mixture of 95 per cent oxygen and 5 per cent carbon dioxide was used as incubating fluid. The sodium concentration in the serosal fluid at the end of the experiment was determined by means of an 'Optica *CF4*' flame spectrophotometer ($\lambda = 589$ mμ). The calculation of the net sodium transport across the intestinal epithelium was made on the assumption that the concentration of sodium in the serosal liquid and in 20 per cent of the fresh tissue are the same. Lactic acid was determined both in the mucosal and serosal fluids according to the method of Barker and Summerson[11]. In order to calculate the amount of lactic acid present in the serosal side, an equal distribution in the serosal fluid and in 80 per cent of the fresh tissue was assumed. The total carbon dioxide in the mucosal fluid at the beginning (25 mM/l.) and at the end of the experiment was determined according to Van Slyke and Neill[12]. In order to calculate the amount of bicarbonate transported into the mucosal epithelium, the amount of carbon dioxide set free by acidification due to the presence of lactic acid in the mucosal fluid was subtracted from the total amount of carbon dioxide which disappeared in the mucosal fluid. The volume of the transported solution across the intestinal wall was determined by weighing the intestinal sac before and at the end of the incubation period.

The results (Table 1) clearly show that in the control experiments a fraction of the total amount of transported sodium ions was accompanied by lactate and bicarbonate ions, the remainder being presumably accompanied by chloride ions. The ratio sodium transported/bicarbonate entered into the mucosal epithelium is 1·8, in agreement with the results given by Gilman and Koelle[1,2]. In the experiments carried out after DNP poisoning the sodium net transport was markedly reduced and the sodium ions seemed to cross the wall accompanied only by lactate. In this case, the ratio sodium transported/bicarbonate entered into the mucosa or the ratio sodium transfer/lactic acid present in the mucosal fluid is close to 1.

As the lactic acid concentrations in the mucosal as well as in the serosal side do not vary in the DNP experiments, we may suppose that even in the mucosal epithelium the lactate concentration does not vary after DNP poisoning. If this assumption is correct, a chemical interference of DNP must be admitted in order to explain the complete suppression of the transport of chloride after DNP poisoning; therefore, an independent active mechanism seems to be implicated in the chloride transport through the intestinal mucosa.

The gradient, which occurs between the serosal and mucosal lactic acid concentrations, does not change in the

Table 1. Everted small intestine of albino male rats (Wistar strain) weighing about 250 g. Jejunum portions starting 20 cm from pylorus and weighing 0·5–1 g were chosen. In each everted sac 0·3 ml. of the Krebs solution without glucose was initially introduced and the sac was then incubated for 1·5 h in the same Krebs solution containing 13·9 mM/l. of glucose. Initial quantity of incubating solution, 50 ml. Temperature of incubation, 28 ° C. Oxygenation with a mixture of oxygen, 95 per cent, and carbon dioxide, 5 per cent. The number of experiments (n) for each group and the mean values ± *S.E.* referred to 1 g fresh weight and to 1 h are reported

Incubating mucosal fluid	Serosal lactic ac.		Mucosal lactic ac.		Transported HCO_3^- (μM g^{-1} h^{-1})	Transported Na (μequiv. g^{-1} h^{-1})	Ratio Transported Na: Transported HCO_3^-
	Total (μM g^{-1} h^{-1})	Concentration (μM/ml.)	Total (μM g^{-1} h^{-1})	Concentration (μM/ml.)			
Krebs + 13·9 mM/l. of glucose. Na concentration 143·5 m. equiv./l. $n = 8$	76·6 ± 5·7	32·8 ± 1·0	68·4 ± 6·3	1·77 ± 0·2	104·7 ± 16·9	188·0 ± 24·3	1·82 ± 0·1
Krebs + 13·9 mM/l. of glucose. Na concentration, 143·5 m. equiv./l. DNP, 0·1 mM/l. $n = 7$	64·2 ± 10·8	36·0 ± 2·4	73·8 ± 7·5	1·55 ± 0·11	71·7 ± 15·9	58·4 ± 7·3	1·01 ± 0·17

DNP experiments, hence is in agreement with the observations made by Pfleger, Jacobi and Rummel[13].

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Comparison of Fœtal Pulmonary Fluid with Fœtal Plasma and Amniotic Fluid

The fluid in fœtal lungs has been considered to be aspirated amniotic fluid[1], an ultra-filtrate of plasma or an exudate from the lungs or bronchial system[2]. Scattered and casual reports have appeared in the literature to the effect that, after delivery of a fœtus by Caesarean section, fluid continues to be formed in the lungs on such a scale as to suggest that the lungs may be an important source of amniotic fluid[3]. However, except for one instance[4], no observations on the nature of the fœtal pulmonary fluid have been reported. It has been possible to collect tracheobronchial fluid during the course of experiments on mature fœtal lambs for comparison with plasma and amniotic fluid in this laboratory. The experiments were performed for a different purpose and the observations reported here are consequently limited in scope.

Mature lamb fœtuses were delivered by Caesarean section from ewes anæsthetized with chloralose (30 mg/kg initially). A saline-filled rubber bag was placed over the head of the fœtus and the trachea was surgically exposed. After the trachea was carefully opened, a polyethylene catheter was passed into the trachea to the carina or slightly beyond and the fluid contained therein collected in a dry syringe. The trachea was then cannulated and the chest was opened on the left side. At no time during the procedure did the animal gasp or make respiratory movements. During the following surgical procedure, the left main bronchus was ligated and a small polyethylene catheter was placed in the left bronchial system through a small puncture in the left main bronchus below the ligature. Fluid from the lung was allowed to drain freely into a graduated cylinder, the end of the catheter being placed at the level of the lung or 2–5 cm below the lung. The collection period ranged from 15 min to 1 h. The initial sample of tracheo-bronchial fluid and the subsequent bronchial collection were analysed for osmolarity by the method of freezing point depression, as were samples of amniotic fluid obtained before delivery by needle puncture of the amniotic sac. When it was possible, samples of blood were obtained from the fœtus at the end of the experiment and plasma osmolarity was also determined. The results are presented in Table 1.

It is evident that the osmolarity of the fluid contained in the fœtal lungs at delivery is intermediate between that of amniotic fluid and that of plasma and is certainly not unadulterated amniotic fluid. Following delivery, while still in the fœtal state, fluid continues to be evolved from the lungs, occasionally at an astonishingly high rate. This fluid is identical in osmolarity with plasma and it would appear, therefore, to be simply a transudate from the pulmonary vascular system, its displacement from the blood stream being a natural consequence of the greater blood pressure which exists in the fœtal pulmonary circulation.

Table 1. Osmolarity of Fœtal Plasma, Amniotic Fluid and Tracheal Fluid collected immediately after Delivery (1) and Bronchial Fluid collected after Ligation of Left Main Bronchus (2)

Fœtus No.	Plasma (mosm/l.)	Amniotic fluid (mosm/l.)	Tracheal (1) Bronchial (2) fluid (mosm/l.)	Rate of flow (ml./h/kg)
1364 *A*	339	279	308 (1) 331 (2)	0·85
1344	332	296	315 (1) 334 (2)	6·25
1350	—	258	315 (1) 331 (2)	2·39
1364 *B*	324	276	327 (1) 320 (2)	1·43
1338 *A*	—	302	340 (1)	
1322	—	300	356 (1)	
Average	332	285	327 (1) 329 (2)	

The observations presented here support the view that the lungs may contribute to the formation of amniotic fluid but not as osmotically identical fluid. This finding does not corroborate the observations of Setnikar, Agostoni and Tagglietti[4], who found the osmolarity of the tracheal fluid to be the same as that of the amniotic fluid in goat fœtuses. It may be that their experimental conditions were such as to promote fœtal respiratory movements and aspiration of amniotic fluid just prior to delivery.

Although the initial osmolarity of tracheobronchial fluid is hypotonic to plasma, it would be inappropriate to refer to it as 'amniotic fluid', whether aspirated or secreted, because in every instance the tracheal fluid was hypertonic to the amniotic fluid. The most reasonable inference would be that the pulmonary fluid *in utero* is a filtrate of plasma which is diluted by the diffusive movement of water from the amniotic fluid through the open upper airway or by the occasional bulk movement of amniotic fluid brought about by respiratory movements.

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HÆMATOLOGY

Appearance of Slow α_2-Globulin after Interference with the Liver

Darcy[1,2] first identified a protein in the sera of rats bearing large neoplasms or tight abdominal bandages which was absent in the sera of healthy, non-pregnant adults.

Beaton *et al.*[3] showed the protein to have the mobility of an α_2-globulin in paper electrophoresis but, because it migrates more slowly than β-globulin in vertical starch-gel electrophoresis, it is commonly referred to as slow α_2-globulin (SA_2G).

Beaton *et al.*[4,5], and Heim[6,7] have shown SA_2G to appear in the sera of fœtal, neonatal, young, pregnant and lactating rats, and have confirmed its appearance in rats bearing large neoplasms. SA_2G did not appear in the sera of normal adult or sub-adult, healthy, non-pregnant rats nor in rats treated with œstrogen, progesterone, growth hormone, chorionic gonadotropin, ACTH[8] or thyroxin[8].

Since most of the conditions leading to the appearance of SA_2G in the sera of rats seem to be related to either developmental or neoplastic growth situations, we attempted to determine, in the light of this apparent correlation, whether or not SA_2G would appear during liver regeneration following partial hepatectomy or damage induced by carbon tetrachloride administration.

Sera were obtained, and vertical starch gel electrophoresis was performed by the method of Smithies[9] with minor modifications, as previously described[7]. Partial hepatectomy was performed by the method of Higgins and Anderson[10] with two modifications: (1) a transverse incision rather than a longitudinal one was used; (2) only the central bifurcated lobe, equal to about 26 per cent of the total liver weight, was removed. Despite the removal of a much smaller proportion of the liver, the patterns of regain in weight of liver and changes in rates of mitotic activity were essentially the same as those observed by Higgins and Anderson[10], with the peak of regenerative activity falling between the first and third days after operation.

Sham hepatectomies were performed in the same manner as the partial hepatectomies, including manipulations of the viscera, but without either ligating or excising any portion of the liver.

Carbon tetrachloride was administered intraperitoneally as 40 per cent solution in mineral oil[11], using a dose of 0·834 ml./kg body-weight. The liver showed both gross and histological damage. Contrary to the observations of Leduc[11] on the livers of mice, that damage due to carbon tetrachloride administration is primarily centrolobular, we found the damage in rats to be rather general. The histologically observed repair processes were parallel in time to the regenerative activity observed after partial hepatectomy.

Table 1. Occurrence of Slow α_2-Globulin after Partial Hepatectomy and Carbon Tetrachloride Injection

Treatment	Presence*	No. of animals in each category at indicated No. of days after treatment						
		0	0·5	1	2	4	6	9
Partial hepatectomy	−	—	0	0	0	0	0	9
	+	—	2	0	0	0	4	1
	+ +	—	4	6	8	5	0	0
Sham hepatectomy	−	—	—	—	9	11	—	—
	+	—	—	—	3	1	—	—
	+ +	—	—	—	1†	0	—	—
Carbon tetrachloride injection	−	—	3	3	3	0	5	2
	+	—	4	1	0	0	1	0
	+ +	—	0	3	7	2	0	0
Mineral oil injection	−	—	—	—	9	5	—	—
	+	—	—	—	0	0	—	—
	+ +	—	—	—	0	0	—	—
Untreated control rats	−	26						
	+	2						
	+ +	0						

* −, No slow α_2-globulin demonstrable; +, some slow α_2-globulin demonstrable; + +, much slow α_2-globulin demonstrable.
† Signs of wound infection.

The results obtained are shown in Table 1. SA_2G was observed most frequently and in the highest apparent concentration in those rats in which the greatest regenerative or repair activity was going on following either partial hepatectomy or carbon tetrachloride injection. Untreated control animals, animals injected with mineral oil only and animals subjected to sham hepatectomies generally were negative in respect to the appearance of SA_2G in their sera. As already observed by Beaton *et al.*[5], occasionally a supposedly normal animal will show some SA_2G, but in such cases the animals are usually sick and exhibit signs of diarrhœa.

From these results it would appear that SA_2G can be demonstrated in the sera of rats during regenerative growth and during repair of tissue damage. The occasional failure to observe SA_2G after carbon tetrachloride administration may be due to the possibly indirect mechanism which has been postulated for the activity of carbon tetrachloride in respect to the liver[12]. We are at present investigating whether or not SA_2G appears in the sera after excision of those organs the removal of which involves either only compensatory hyperplasia without regeneration or only wound closure without either of the foregoing.

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PHARMACOLOGY

Enhanced Suppression of the Secondary Immune Response by Combination of 6-Mercaptopurine 'Duazomycin A'

The demonstration that 6-mercaptopurine (6-MP) is capable of inhibiting the immune response[1,2] has aroused much interest and optimism concerning the therapeutic possibilities for organ transplantation and 'auto-immune' diseases. Extension of these studies has shown that immunological tolerance can be obtained by administration of 6-MP during the primary exposure to an antigen[3]. Although initial attempts to modify the secondary response to the antigen were unsuccessful[4], a quantitative rather than qualitative effect seems to have been responsible, since proper timing and vigorous treatment with both antigen and drug will achieve complete suppression of specific antibody production in previously immunized animals[5,6].

The precise mechanism of action of 6-MP is not yet known. Present evidence indicates that, after enzymatic conversion to the ribonucleotide, the analogue interferes with the biosynthesis and inter-conversion of purine nucleotides, but does not replace to a significant degree the normal components of nucleic acids or coenzymes[7]. The biosynthesis *de novo* of normal purine derivatives also can be inhibited effectively by certain reactive diazo-compounds[8,9], including *O*-diazoacetyl-L-serine (azaserine) and 6-diazo-5-oxo-L-norleucine (DON). Marked potentiation is observed when combinations of these agents and 6-thiopurines are used in the chemotherapy of experimental neoplasms[8,9]. Early clinical studies, however, disclosed little or no therapeutic advantage of these combinations in patients with neoplastic disease[10,11]; but such trials utilized the oral route of administration, now recognized to be unsatisfactory, since the diazo-compounds are inactivated by acid-hydrolysis[7]. More recent investigations, utilizing a new glutamine antagonist, 'Duazomycin *A*' (DZM) (*N*-acetyl-DON), in combination with 6-thioguanine (TG), by the intravenous route have shown much more favourable initial results[12].

The work recorded here was conducted to investigate the effects of the combination of DZM and 6-MP on the suppression of the secondary immune response in rabbits previously immunized with a non-living antigen, diphtheria toxoid. The results indicate that DZM, without significant increase in toxicity, potentiates appreciably the ability of 6-MP to block antibody production (Table 1).

Thirty albino rabbits were used in two separate experiments. The animals were inoculated intradermally with 176 *Lf* units of diphtheria toxoid in volumes of 0·1 ml. into the shaved plantar surface of the foot-pad. (Formol toxoid No. 32929-361 was kindly supplied by Mr. W. S. Hammond of Lederle Laboratories.) Using hæmagglutination of sensitized tannic acid-treated sheep erythrocytes as the indicator, a primary antibody response was obtained in every animal. The procedure followed was that described by Boyden[13], as adapted by Stavitsky to the diphtheria toxin–antitoxin system[14,15]. A second identical injection of diphtheria toxoid was given 4–6 weeks later. Drug therapy was initiated on the same day of the challenging injection of antigen and was continued for 6 days, using the dosage schedules shown in Table 1. Sera were titrated blindly and 1+ agglutination[14], which was found to be the most reproducible, was used as the end-point. Solutions of DZM were obtained in sterile ampoules containing 2 per cent disodium phosphate buffer. (We thank Dr. Theodore Medrek of Chas. Pfizer and Co., Inc., for supplies of 'Duazomycin *A*' (DZM).) 6-MP, Burroughs Wellcome and Co. (lot 42596), was dissolved daily in a solution of sodium hydroxide (1 M), diluted to desired concentration with sterile saline, and adjusted to *p*H 9 by titration with dilute hydrochloric acid. Sera wore obtained on days 2, 4 and 6 and antibody titres (Table 1) were determined by the procedure already described.

Table 1. Effect of 6-Mercaptopurine (6-MP) and 'Duazomycin *A*' (DZM) on the Secondary Immune Response to Diphtheria Toxoid

Rabbit No.	Drug (mg/kg)	Hæmagglutination titre Day 2	Day 4	Day 6
	Experiment No. 1			
1	none	0	20,480	163,840
2	none	0	5,120	20,480
3	none	0	10,240	40,960
4	none	80	81,920	163,840
5	6-MP (25)	0	640	20,480
6	6-MP (25)	0	1,280	20,480
7	6-MP (25)	0	80	640
8	DZM (2)	0	2,560	40,960
9	DZM (2)	0	5,120	40,960
10	DZM (2)	320	2,560	40,960
11	6-MP (25) + DZM (2)	0	0	40
12	6-MP (25) + DZM (2)	0	40	640
13	6-MP (25) + DZM (2)	0	40	160
	Experiment No. 2			
15	none	0	5,120	10,240
14	none	0	2,560	10,240
16	none	0	10,240	20,480
17	none	0	40,960	163,840
18	6-MP (20)	0	640	2,560
19	6-MP (20)	160	10,240	10,240
20	6-MP (20)	0	640	5,120
21	6-MP (20)	0	640	1,280
22	6-MP (20)	0	160	1,280
23	DZM (2)	0	2,560	40,960
24	DZM (2)	0	640	5,120
25	DZM (2)	0	160	5,120
26	DZM (2)	0	640	163,840
27	6-MP (20) + DZM (2)	0	0	40
28	6-MP (20) + DZM (2)	0	0	—*
29	6-MP (20) + DZM (2)	0	40	320
30	6-MP (20) + DZM (2)	0	0	320

* Animal died on day 4 subsequent to traumatic cardiac puncture.

This demonstration of enhanced suppression of antibody response to non-living antigen has prompted investigations with homotransplantation, which, although still incomplete, suggest similar results. These findings will be of particular interest in view of reports indicating prolonged survival of renal homotransplants in dogs treated with related agents[16]. Further investigations of glutamine antagonists in combination with purine analogues are clearly indicated, in order to determine their effects on delayed hypersensitivity, γ-globulin-levels and 'auto-immune' disease in experimental animals and man.

The mechanism of action of antimetabolites in suppressing the immune response is not yet clearly understood. Because most agents that possess this property also inhibit cellular proliferation, it has been postulated that this effect is mediated through selective destruction of small clones of antibody-producing cells, particularly if these have been stimulated into mitotic activity by the administration of the appropriate antigen. It is of interest, therefore, that the drug combination used has a well-documented ability to inhibit cellular proliferation and was selected on rational biochemical concepts for its ability to produce a concurrent multi-site blockade of purine biosynthesis. Furthermore, because DZM is characterized by minimal bone marrow toxicity and 6-MP usually has no severe gastrointestinal effects, it is hoped that the anti-immune effects in man will not be accompanied by increased toxicity. In patients with advanced neoplastic disease receiving treatment with combinations of 6-MP, TG or the respective 5-imidazolyl derivatives, and DZM similar immunological studies have been initiated in order to evaluate the effects of these drugs on the immune response in man.

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Effect of Benzquinamide on Blood Pressure

Two substances of the benzoquinolizine group have different effects on depletion of brain catecholamines. While tetrabenazine (intravenous) produces a decrease in the brain amine concentration, benzquinamide (intravenous, supplied by Pfizer Argentina, S.A.) produces a rise in the serotonin- and noradrenaline-level (5 HT: 0·65 γ/g; control: 0·57 and noradrenaline: 0·63 γ/g; control: 0·39).

Benzquinamide is the 2-acetoxy-3-diethylcarbamyl-9,10-dimethoxy-1,2,3,4,6,7,hexahydro-II bH-benzo-a-quinolizine.

Per os, it has a potent tranquillizing and anti-emetic activity, producing little or no sedation. When given intravenously, a significant increase in arterial blood pressure has been observed.

This effect on blood pressure was found while examining its anti-emetic activity during anæsthesia with barbiturate or halothane[1].

This drug has a very weak antihistamine, antiserotonin and anti-cholinergic action.

It was decided to examine its hypertensive experimental effect in rabbits. Ten animals were used, 5 in the control group and 5 under anæsthesia with barbiturates (pentothal). Blood pressure was recorded with an intra-arterial catheter in the popliteral artery, using a mercury manometer with a graphic register[2].

In the control group, an increase in the systemic blood pressure was obtained after the intravenous injection of benzquinamide. It was accompanied by trembling, convulsions, myosis, venous congestion and cyanosis.

The results are summarized in Table 1.

Table 1

Exp. No.	Control blood pressure (mm Hg)	Benzquinamide dosage (mg/kg)	After benzquinamide blood pressure (mm Hg)
1	40	20	70
4	60	20	68
7	45	10	53
8	50	13	78
10	40	20	72

In the animals submitted to barbiturate anæsthesia the following results (as shown in Table 2) were obtained.

Table 2

Exp. No.	Control Blood pressure (mm Hg) Initial	Control Blood pressure (mm Hg) After pentothal	Benzquinamide (mg/kg)	After benzquinamide blood pressure (mm Hg)
5	48	40	20	46
6	50	40	20	50
9	42	38	20	52
12	55	50	20	60
14	42	36	20	52

In all animals an increase in blood pressure was evident. Between 20 and 30 min after the injection of benzquinamide, the blood pressure returned to its initial values. It seems that those animals submitted to anæsthesia have a minor pressure response than the controls. It must be pointed out that no vasoconstriction was observed (as happens with noradrenaline injection)[3]. On the contrary, a marked peripheral vasodilatation was noted.

In a previous paper we examined the blood pressure response to intravenous noradrenaline[2] in animals pretreated with an amino oxidase inhibitor: iproniazid[4,5]. In this case the response seems to be higher than in the controls.

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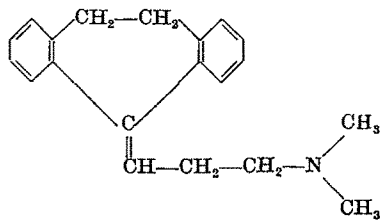
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A Fatal Case of Poisoning with Amitriptyline

NEW psychopharmacological drugs bring relief to many patients. However, their misuse can result in misadventures which may even be fatal. Several reports[1] already document this fact, despite the wide margin of safety these drugs have. This note reports a fatal episode involving amitriptyline, another new drug in the fast growing list.

In the previous 3-year period a 25-year-old man (A. M.) unsuccessfully attempted suicide twice. He was placed under the care of a psychiatrist. His therapy included many different drugs. In the last 6 months only amitriptyline was prescribed. At 6 a.m. one morning A. M. was found dead beside his bed. He was last heard from at 3 a.m. of the same day when he had spoken with a friend. On the table in the same room one tablet ('Laroxyl®', 25 mg) was found. A bottle, now empty, was known to have contained about 50–100 tablets. A. M. was taken to the Institute of Legal Medicine in Basle, where a post-mortem examination was performed. Macroscopic and microscopic pathological examinations revealed no anatomical cause of death. There were no abnormalities except for a full stomach containing 295 gm of undigested food and some white particulate matter.

Chemical analyses were then made. Routine tests indicated opiates, phenothiazine derivates, meprobamate, barbiturates and glutethimide were not present. However, the white particulate matter in the stomach was chemically separated from all other material and was found to be amitriptyline.



'Amitriptyline' (Trade marks: 'Elavil', 'Laroxyl', 'Tryptizol', 'Tryptanol', 'Saroten', 'Sarotex', 'Horizon')

Two different methods were used for the isolation and detection of the drug. These are presented so that others faced with a similar problem may benefit from this experience.

One method was a modified Stas–Otto procedure. A portion of the sample is homogenized with water and ethanol. This mixture is heated under a reflux condenser, for 60 min. The mixture is then cooled, the solid matter separated by filtration and the clear filtrate vacuum distilled to remove the ethanol. The residual aqueous solution is then extracted with ether. These extracts were evaporated to dryness. An aliquot part of the residue was placed on a thin layer chromatogram (silica gel *G*) and run for 30 min in a methanol/acetone/triethanolamine (1 : 1 : 0·03) solvent. The resulting chromatogram was sprayed first with Dragendorff's reagent and then with potassium iodoplatinate. An orange spot at the same R_F as known amitriptyline indicated the unknown was probably this drug. The iodoplatinate spray revealed a second slower running spot which was probably a metabolite of amitriptyline, since it only appeared in the extracts from blood, liver, kidney, urine and not in the stomach content extract.

Table 1

	Stomach	Blood	Liver	Kidney	Brain	Urine
Direct extraction	> 20 mg/ 100g	—	—	—	—	—
Direct extraction after hydrolysis	not done	0·04 mg/ 100 g	1·5 mg/ 100 g	0·5 mg/ 100 g	not done	not done
Extraction after Stas–Otto	100 mg/ 100 g	0·04 mg/ 100 g	—	1·0 mg/ 100 g	5 mg/ 100 g	1·3 mg/ 100 g

The second method was a direct chloroform extraction of the samples using 10 volumes of chloroform to each volume of sample. The extractions were made on homogenized samples of several different tissues, blood, and stomach contents. One series was extracted directly. A second series was first hydrolysed at 100° C for 30 min with 50 per cent hydrochloric acid. Both series were made alkaline by the addition of solid potassium hydroxide. 10–15 ml. aliquots of each sample were successively extracted with 100 ml. chloroform. Each aliquot was discarded and the next added to the same chloroform until the entire sample was extracted. The chloroform was then washed with 20 ml. of 0·5 N sodium hydroxide. The sodium hydroxide was discarded and the chloroform extracted with 5 ml. of 1 N sulphuric acid. The ultraviolet absorption characteristics of this acid extract were recorded. Then the sulphuric acid solution was made alkaline and extracted with ether. The ether was dried over anhydrous sodium sulphate and then evaporated to dryness. The residue was dissolved in methanol and thin layer chromatography, as previously described, was performed.

Both these techniques were applied to samples of blood, stomach content, liver, kidney, brain and urine. The semi-quantitative data recorded in Table 1 are based on the size and density of the thin layer spots compared with standards prepared in an identical manner.

It is interesting to note that when the direct-extraction procedure was applied to unhydrolysed samples, no significant amounts of drug were detected. This is com-

parable with similar recorded data for phenothiazine derivatives[2], imipramine[3] and some tranquillizers[4].

Whether the amount of drug detected in A. M. was enough to cause his death may be debatable. Albeit low, they are comparable with values reported for similar drugs. A better judgement could be made if comparable data were available from living human patients who are being treated with this drug. However, until this kind of data are available from those responsible for the use of this drug, the results in Table 1 may be presumed to be compatible with death, especially in absence of an anatomical cause of death.

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PATHOLOGY

An Organ Culture System for the Examination of Tumour Invasion

For investigations of many of the processes involved in the invasion of normal tissues by malignant cells it is desirable to have an *in vitro* system where the interacting normal and tumour tissues maintain the 3-dimensional tissue structure which they possess *in vivo*. Wolff[1] has used with considerable success a system involving the organ culture of combinations of various tumours with embryonic chick mesonephros, where extensive growth of the tumours accompanies the invasion of the embryonic normal tissue. We wished to develop a system using adult or new-born mammalian organs where the fully differentiated architecture of the normal tissues could be maintained in contact with tumour tissue, with a minimum of cell division.

We have made use of a modification of Trowell's[2] technique, in which we placed small fragments of tissue on a rayon strip laid on a stainless steel grid, the underside of which was in contact with the medium, usually Eagle's HeLa + 10 per cent calf serum. The cultures were maintained in an atmosphere of 95 per cent oxygen + 5 per cent carbon dioxide at 37° for seven days. The normal and tumour tissues, cut to a suitable shape and size, were laid side by side, on top of each other, or overlapping. The normal tissues most commonly used were kidney, liver, lung and skin of young mice, and the mouse tumours were the Harding–Passey melanoma, Sarcoma 180 and a solid transplantable myeloma. The melanoma was used most frequently since the melanin within the cells permitted the identification of individual tumour cells.

Fusion of the normal and tumour tissues occurred within a few days, and examination of sections after seven days in culture revealed the infiltration of melanoma cells into the adjacent organized normal tissues, see Figs. 1 and 2. The infiltration was not in the form of solid columns of tumour cells, but by single or small groups of cells, which were embedded in and surrounded by apparently healthy normal tissue, in which the histological structure was well preserved. No traces of the path of infiltration could be detected. Individual tumour cells

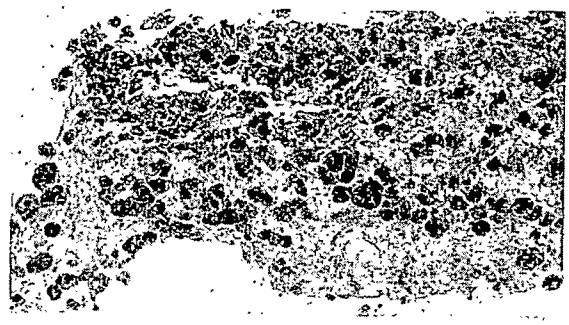


Fig. 1. Section of a 7-day culture of rat liver showing invading pigmented mouse melanoma cells



Section 2. Section of 7-day culture of rat kidney with infiltrating mouse melanoma cells (arrowed)

had penetrated within the normal tissues for distances of up to 400μ in 7 days.

In many sections there were definite indications that the tumour cells had been unable to penetrate connective tissue which was orientated parallel to the tumour–normal tissue interface, an observation previously recorded by Leighton, using sponge matrix cultures[3]. If resistance to infiltration by tumour cells is dependent solely on the relative strengths and stabilities of the intercellular adhesions of normal tissue cells, one would expect that compact tissues such as liver would resist infiltration more successfully than connective tissue. It seems more probable from our work and that of others that the failure of melanoma cells to penetrate the parallel orientated normal connective tissue is due more to the operation of the principle of contact guidance[4] than to the physical barrier which the connective tissue represents. Contact guidance would direct the movements of tumour cells parallel to the overall orientation of the connective tissue. Therefore, tangentially orientated connective tissue would present a barrier, and radially orientated connective tissue would provide a highway for invasion.

The organ culture technique used in this work provides a three-dimensional system for examining the roles of contact guidance, contact inhibition[5,6], nutrition, hormones and other factors on the process of tumour cell infiltration into normal tissues which have maintained, to a large extent, their architectural integrity. The infrequency of mitosis makes possible such investigations in the absence of extensive growth, and the system is in many ways complementary to that of Wolff[1], where considerable growth of the tumour accompanies invasion of the embryonic chick mesonephros.

Many pathologists[7,8] have emphasized the role of pressure, developed by increase in tumour volume, in determining the onset and route of invasion along the

paths of least mechanical resistance. The progressive infiltration of individual tumour cells which has been observed in the organ culture systems cannot be attributed to the development of pressures, as growth of the tumour under the conditions of culture is very small and, moreover, individual tumour cells cannot exert pressures of the type suggested. The mechanism of invasion based on loss of contact inhibition by malignant cells seems to offer the most consistent explanation of these observations. If these cultures can be maintained for increasing lengths of time without increase in tumour cell mass it should be possible to observe progressive stages of invasion dependent mainly on the motilities and contact behaviour of the tumour and normal cells.

One of us (G. C. E.) thanks Dr. I. Lasnitzki for advice in the organ culture technique. This investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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Production of Ultra-structural Glomerular Lesions resembling those of Toxæmia of Pregnancy by Thromboplastin Infusion in Rabbits

The characteristic histological abnormalities of toxæmia of pregnancy (pre-eclampsia) are found in glomeruli and consist mainly of endothelial swelling[1]. Electron microscopic studies of renal biopsies have confirmed that the main change is marked swelling of endothelial and intercapillary cells, and have shown in addition the frequent occurrence of dark, granular material deposited between the swollen endothelial cells or along the basement membranes[2,3].

The hypothesis was advanced that these glomerular changes are the result of a prolonged and very slow state of intravascular clotting, as discussed elsewhere[4]. Evidence for this hypothesis was provided by the results of experiments in which 14 rabbits were perfused with various amounts of tissue thromboplastin ('Difco'). The duration of perfusion varied from 2 h up to about 30 h, and the animals were killed between 1 and 12 h after completion of the perfusion. Ten of these rabbits showed histological abnormalities, consisting of various degrees of endothelial swelling, polymorphonuclear infiltration and fibrin deposits. Glomeruli of 6 of these rabbits were examined by electron microscopy and showed the following features.

In the most severe reactions, large amounts of fibrin were found in the capillary lumens, often entrapped with platelet clumps; platelets appeared to play an autocatalytic part in formation of fibrin, since fibrin threads were often seen to be formed in direct contact with platelets undergoing viscous metamorphosis. Fibrin was also present within intercapillary cells in the same location where injected colloidal materials are known to be deposited; this further substantiates the phagocytic properties of these cells[5], and shows that the fibrin



Fig. 1. Part of a rabbit glomerulus showing swelling of endothelial and intercapillary cells which reduces the capillary lumen

present in lumen can be removed as any colloidal material. The phagocytosis of fibrin was not limited to intercapillary cells; endothelial cells showed progressive swelling and were frequently observed in the process of phagocytosing fibrin present in the lumen. Following its ingestion by endothelial cells, fibrin was observed in vacuoles, undergoing progressive degradation until it was no longer identifiable. Deposits of dark, granular material, similar to those found in toxæmic glomeruli, were frequently observed in the capillary lumens, or sometimes within the intercapillary or endothelial cells.

The following lines of evidence indicate that this material was derived from fibrinogen: (*a*) In some capillary loops materials exhibiting all the transitional aspects between fibrillar fibrin and these deposits were observed. (*b*) The same material was found in experiments in which intravascular clotting was induced by other means, namely, injections of thrombin or of Liquoid[6]. When epsilon-amino-caproic-acid, an inhibitor of fibrinolysis activators, was injected before the induction of intravascular coagulation, this resulted in a marked increase in the amounts of this material within glomeruli[6]. The term fibrinoid, which has sometimes been applied to this material, seems to be appropriate since it appears to be the result of the accumulation of some fibrinogen derivatives in a stage of polymerization different from that of fully polymerized fibrin. It may represent intermediate products, either formed in the lysis of fibrin, or in the conversion of fibrinogen into fibrin. It is known that in slow states of intravascular clotting, circulating fibrin precursors are present in the absence of fully polymerized fibrin[7]. In several glomeruli, the presence of marked intercapillary and endothelial swelling and the deposits of this fibrinoid material, between the swollen cells or along the basement membrane, gave a picture indistinguishable from that observed in toxæmia.

In 2 rabbits, endothelial and intercapillary swelling was observed in the absence of any detectable fibrin formation or fibrinoid deposition (Fig. 1). The swelling started with the formation of blebs of the cellular membrane which protruded into the lumen, the so-called endothelial ballons, and progressed in some loops to complete occlusion of the capillary. The swollen cells often contained numerous vacuoles and vesicles, indicating phagocytic activity.

That all these changes result from fibrin formation and not of thromboplastin itself has been shown in experiments in which intravascular clotting was induced by thrombin and 'Liquoid', and which will be more extensively

described elsewhere[6]. Fibrin and fibrin precursors appear to behave in the circulation like colloidal material, since it has been demonstrated that they are phagocytosed by the reticulo-endothelial system[9]; the observations recorded here provide evidence that derivatives of fibrinogen behave like colloidal materials in that they are arrested in glomeruli and phagocytosed by the intercapillary and endothelial cells[9]. The existence of circulating fibrin or fibrinogen derivatives appears to be responsible for the cell swelling and deposits of dark fibrinoid material, which are the essential changes observed in toxæmia of pregnancy. The cell swelling, which was sometimes observed even in the absence of detectable fibrin formation, also appears to result from the phagocytosis of fibrinogen derivatives. That a mechanism of very slow intravascular clotting, perhaps initiated by the entrance in the maternal circulation of placental thromboplastin, can be responsible for the renal lesions of toxæmia, is substantiated by the recent demonstration by immunofluorescence of large amounts of fibrinogen derivatives in toxæmic glomeruli[4].

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RADIOBIOLOGY

Determination of Carbon-14 in Plant Material by Wafer Counting

A simple method for quantitatively estimating carbon-14 in plant material (for example, carbon-14 tagged herbicides, as in our own case) is to press the material into wafers and to count these directly with a Geiger or proportional counter.

Since any wafer of plant material will almost certainly be at infinite thickness for carbon-14, the count obtained is directly proportional to the specific activity of the sample. If relative measurements only are required these data are sufficient. A quantitative measure of the radioactivity present in a wafer can be obtained from the infinite thickness equation:

$$I_\infty = \frac{sA}{\mu} \text{ (ref. 1)}$$

where I_∞ is relative measured activity at infinite thickness, corrected for background and coincidence loss (c/m); s is relative specific activity of the sample (c/m/mg); A is area of wafer exposed to the sensitive region of the counter (cm²); μ is absorption coefficient for carbon-14 β-particles in the material in question (cm²/mg).

Conversion to absolute units then requires knowledge of the counter efficiency and of the value of μ for the material in question. This information may be difficult to obtain.

A less theoretical method of converting the c/m observed from the wafer to an absolute quantitative measure of the weight of herbicide present is by use of a calibration curve. This is constructed by adding different known weights of the radioactive herbicide to known amounts of the plant material. Wafers are then made and counted in the same counting system as the harvested samples.

This communication reports a comparison of these two absolute quantitization methods of wafer counting for cotton-seed kernels. Also, an attempt was made to determine the absorption coefficient and the self-absorption curve of carbon-14 in cottonseed kernels.

A nuclear measurements *PC-3A* windowless-gas-flow proportional counter was used to count all wafers; the self-absorption curve was determined with a Nuclear Chicago *D-47* Geiger tube with a micromil window (0·150 mg/cm²). All counts were corrected for background; coincidence loss was never significant. In general, at least 2,000 counts were accumulated for each sample, giving an error at the 95 per cent level of 6 per cent; wafers were counted on both sides.

The relative specific activities of the radioactive herbicides were determined from a dilution series in absolute ethanol. Duplicate aliquots of the dilutions were pipetted into 1-in. diameter nickel planchets and dried under an infra-red lamp.

Wafers for the calibration curve were made by placing 1·5 g samples of cotton-seed kernels (hulls removed, oil not extracted), ground to pass a 60-mesh screen, in the steel cylinder (2·90 cm inside diameter) of a wafer-making press. Then a known weight (± 1 per cent) of the radioactive herbicide, dissolved in absolute ethanol, was pipetted into the ground kernels in the press and thoroughly mixed in by hand. The volume of fluid added was kept to 0·10 ml. or less to avoid excessive alteration of the consistency of the kernels. The wafer was pressed out with a minimum of force to avoid loss of the oil. The wafers were weighed to an accuracy of ± 0·1 mg after counting.

Counter-efficiency was determined by counting a disk containing carbon-14, of known absolute radioactivity, using the same geometry as the wafers.

The self-absorption curve was made by weighing out various amounts of ground cotton-seed kernels (with an accuracy of ± 0·1 mg) and suspending them in a few ml. of absolute ethanol and then thoroughly grinding in a Potter homogenizer. Then 2,4-dichlorophenoxyacetic acid-2-carbon-14 (2,4-D-2-carbon-14) of known radioactivity was added to the ethanolic suspension and thoroughly mixed in. The suspension was carefully transferred to a 1·25-in. diameter aluminium planchet and the ethanol evaporated off under an infra-red lamp; care was taken to see that the material was uniformly distributed over the planchet. Samples of 2,4-D-2-carbon-14 in ethanol were dried, counted and left for periods of more than 1 h under the lamp and then recounted. No change in count-rate was found. I_0 (the activity in the absence of any self-absorption) was determined by plating 2,4-D-2-carbon-14 without the addition of kernels.

Determination of μ. If a known amount of carbon-14 is added to a known weight of kernels and the resulting wafer counted, μ can be calculated in the following two ways. The first method involves no knowledge of the counter-efficiency, but it is necessary to measure the relative specific activity of the herbicide by a dilution series in a suitable solvent and then use this value to calculate s. Provided that the dilution series planchets and wafers are counted in the same machine and with similar geometry, there is no need to correct for machine efficiency. The second method requires the use of the counter-efficiency in the calculation, the specific activity given by the manufacturer (or any other absolute measure) being used to calculate s, and I_∞ being converted to an absolute value from the counter-efficiency.

The value of μ was determined from 24 wafers, containing 2,4-D-2-carbon-14 of two specific activities and pentachlorophenol-1-carbon-14 (PCP-1-carbon-14) of one specific activity. With a counter-efficiency of 45 per cent,

which was that calculated by use of the carbon-14 standard, μ was 0·256, and ranged from 0·204 to 0·316. It was unlikely that the counter efficiency was outside the range 40–50 per cent. If the efficiency was 40 per cent the 0·256 value of μ would become 0·227, if 50 per cent, 0·283. The value of μ obtained without bringing the machine efficiency into the calculations was 0·285, with a range from 0·222 to 0·361. The overall mean value using results from both methods of calculation was 0·270.

Comparison of the infinite thickness equation method of quantitization with the calibration curve method of quantitization. Two calibration curves were made, one for 2,4-D-2-carbon-14 and one for PCP-1-carbon-14. In each case, as was expected, a linear relation was found between the weight of radioactive herbicide added to the wafer and the observed c/m (corrected for background). Consequently, a factor could be derived from the curves to convert the observed corrected c/m to parts per million of herbicide. A comparison of the two methods is shown in Table 1. Results were calculated from the infinite thickness equation in the following way. Using the symbols as at the beginning:

$$s = \frac{I_\infty \times \mu}{A}$$

Converting to absolute values, using a machine efficiency of 45 per cent:

$$s = \frac{I_\infty \times \mu \times 100}{A \times 45}$$

From the absolute value of s thus obtained, the herbicide concentration, in parts per million, can be obtained by dividing s by the specific activity of the herbicide, as given by the manufacturer, and then multiplying the result by one million.

Table 1. Comparison of Methods for Calculation of Weight of Herbicide in Cotton-seed Kernels from Measurement of the Amount of Radioactivity present in a Wafer

^{14}C containing compound in wafer	Relative weight of herbicide calculated * from: (1) Calibration curve	(2) Infinite thickness equation, where μ = 0·23	0·24	0·25	0·26	0·27	0·28	0·29	0·30
2,4-D-2-^{14}C	1·00	0·91	0·95	0·99	1·03	1·07	1·11	1·15	1·19
PCP-1-^{14}C	1·00	0·84	0·88	0·92	0·95	0·99	1·03	1·07	1·10

* For full details, see text.

Table 1 shows that if μ is 0·27, which was the mean value found, the weight of herbicide is 107 per cent of the value obtained from a calibration curve for 2,4-D-2-carbon-14, and 99 per cent for PCP-1-carbon-14; if the value of μ for cotton-seed was the same as that usually taken for barium carbonate (0·29) the corresponding figures would be 115 and 107 per cent.

These results indicate that the correct value for μ for cotton-seed kernels is not very far from 0·29. On the basis of these studies, the authors believe that the most accurate way to calculate residue results from measurements of radioactivity at infinite thickness is to use a calibration curve of the type described.

Self-absorption curve. The curve obtained is shown in Fig. 1. All points are a mean of duplicate determinations. The self-absorption curve for barium carbonate (taken from ref. 1 and not determined on our counter) is shown for comparison. The similarity between the two curves indicates that the self-absorption properties of barium carbonate and cotton-seed kernels are similar, though it is known[2] that the shape of the self-absorption curve will vary with the counter used to construct it.

In summary, the value of the absorption coefficient of carbon-14 in cotton-seed kernels was estimated using measurements made at infinite thickness. A self-absorption curve for carbon-14 in kernels was also constructed. Results indicated that the usually accepted value of the coefficient (0·29) is approximately correct. If an absolute quantitative measurement of the amount of carbon-14 present in a wafer of pressed plant material is required, it is probably more accurate to use a calibration curve (in which known weights of the radioactive compound are added to non-radioactive plant material, the resultant wafers being assayed for radioactivity in the same counting system as the actual sample wafers) than to use the equation describing the behaviour of radiation at infinite thickness.

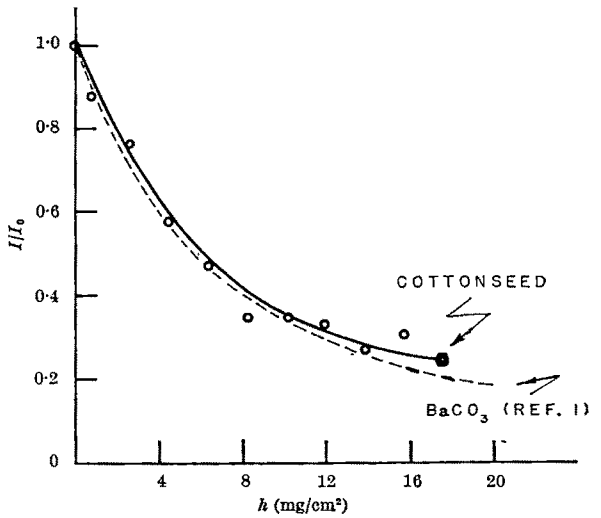


Fig. 1. Self-absorption curve for ^{14}C in cotton-seed kernels. I, Relative measured activity at less than infinite thickness, corrected for background and coincidence loss; I_0, relative activity present in the absence of self-absorption, corrected for background and coincidence loss

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Influence of Calcium Phosphate on the Absorption of Radioactive Strontium

It has been found, in experiments which will be reported more fully elsewhere, that rats which are fasted before and after the intragastric administration of a solution containing strontium-85 absorb about three times as much of the strontium as rats with free access to the stock diet. It has also been found that when the stock diet is restored immediately after the administration of strontium-85 to fasted rats, the fraction of the dose absorbed is no greater than for fed rats. This effect is due mainly to one component of the diet, namely bone-meal. Calcium hydrogen phosphate mixed with cellulose and moistened with a solution of sucrose also reduces absorption when given to fasted rats after the strontium has been administered (Table 1).

Table 1. Effect of allowing rats to eat food containing 10 per cent bone-meal or cellulose containing 10 per cent $CaHPO_4.2H_2O$ on the absorption of ^{85}Sr administered intragastrically to them in the fasted condition

Treatment	Percentage dose absorbed
Fasted before and after dose	21
Stock diet* containing 10 per cent bone-meal fed after dose	5
Cellulose containing 10 per cent $CaHPO_4.2H_2O$ fed after dose	3

* Supplied by North Eastern Agricultural Co-operative Society, Ltd., Aberdeen.

To determine the period during which a protective effect could be obtained, the stock diet, containing 10 per

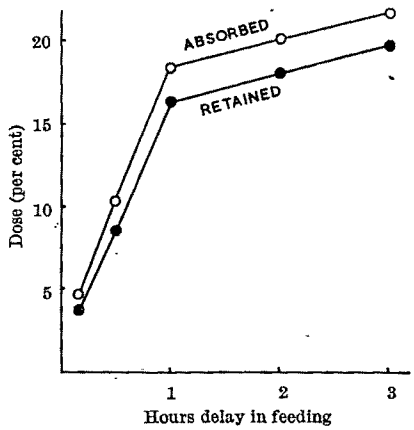


Fig. 1. Influence of food (containing 10 per cent bone-meal) consumed at different times after the intragastric administration of strontium-85 to fasted rats, on the absorption and retention of the strontium after 24 h. Each point is the mean value for 4 rats

cent bone-meal, was restored to fasted rats at various intervals after the administration of strontium-85. The animals each ate between 5 and 8 g of food. Twenty-four hours after receiving the strontium, the rats were killed and the gastrointestinal tract was removed intact. The strontium-85 was assayed in the residual carcass, urine, gut and fæces. Fig. 1 shows that the reduction in absorption (strontium-85 in carcass + urine) and retention (strontium-85 in carcass) after 24 h was greatest for rats which ate within a few minutes of receiving the dose. Absorption increased at further intervals until, at one hour, ingestion of the diet had little influence on the proportion of the dose absorbed. This is in accord with other data on the relationship between absorption and time from administration of strontium.

Macdonald, Spain, Ezmirlian and Rounds[1] found that tricalcium phosphate ($Ca_3(PO_4)_2$) reduced the absorption of strontium-90 when it was given at the same time as the strontium. The present data, however, show that a reduction in absorption may be achieved even if the calcium phosphate is eaten after the radioactive strontium is ingested. Consequently it would seem that calcium phosphate might prove a useful, safe means of reducing absorption after the accidental ingestion of radioactive strontium by man. Experiments are at present in hand to verify this.

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Nuclear Factors involved in the Radiation-induced Depression of DNA Synthesis of Deoxyribonucleic Acid

In experiments described in a previous communication[1], it was found that nuclei isolated from regenerating liver of rats given 800 r. whole body irradiation incorporated less tritiated thymidine when assayed in a DNA-synthesizing system[2] at various times after irradiation than did non-irradiated controls. Somewhat similar results had been obtained by Van Lancker[3], who postulated that the finding might result from direct damage to the DNA template by irradiation. On the other hand, Nygaard[4] has suggested that these findings might result from loss of sodium and potassium from the nucleus due to irradiation damage to the nuclear membrane, such as has been demonstrated by Creasey[5] in the irradiated spleen.

In order to determine the site of radiation damage in the nucleus, experiments have been carried out using nucleoprotein fractions and DNA extracted from nuclei as a source of primer DNA in a DNA-synthesizing system. Nuclei uncontaminated with whole cells or cell debris were isolated in 0·44 M sucrose adjusted to *p*H 5·8 by the addition of citric acid[7]. The nuclei were then extracted in 0·14 M sodium chloride, leaving a residue which is designated DNA-histone consisting of DNA, histone, a non-basic protein which will be referred to as residual protein and some insoluble RNA. This residue was extracted in 0·2 N hydrochloric acid removing the acid-soluble histone, yielding the second fraction termed DNA-residual protein. These two preparations were dissolved in water at *p*H 8 and used as the source of primer DNA in the system previously described[1]. In all experiments, the source of DNA-synthesizing enzyme used was the supernatant from non-irradiated regenerating rat liver homogenates centrifuged at 35,000*g* for 1 h. In each experiment, the amount of DNA in each tube was adjusted to the same level.

Results from various preparations from liver 24 h after irradiation and 48 h after partial hepatectomy are shown in Table 1. In the first group of values obtained by using nuclei as primer, incorporation of tritiated thymidine is lower in the irradiated samples by a factor of roughly three compared with the controls. The second group of values is for DNA-histone; here again, incorporation into irradiated samples is less than into non-irradiated. In the third group, the DNA-residual protein is used as primer and here again incorporation into irradiated preparations is less than that into non-irradiated controls. In all cases, incorporation into the control preparation exceeds that into the irradiated preparation. Lastly, there are the values obtained with purified DNA extracted by the method of Dounce[6] from regenerating liver of irradiated and non-irradiated rats. Here there is little difference in the incorporation of tritiated thymidine into irradiated and non-irradiated preparations.

Table 1

Preparation	Exp. No.	Specific activity (c.p.m./mg DNA) Control	Irradiated	Ratio Irradiated/Control
Nuclei	1	23,545	7,894	0·334
	2	3,216	1,121	0·349
	3	5,047	1,630	0·323
DNA-histone	4	996	535	0·538
	5	53,150	17,990	0·337
	6	29,749	7,489	0·257
DNA-residual protein	7	6,690	1,780	0·266
	8	360	193	0·540
	9	1,894	662	0·350
DNA	10	2,232	2,008	0·90
	11	3,627	4,567	1·26
	12	6,078	8,082	1·33

Assays were performed with regenerating liver 48 h after partial hepatectomy and 24 h after 800 r. 250 kVp X-rays.

These results have a number of implications. First, the observed decrease in thymidine incorporation by the irradiated primer is clearly not related to changes in the nuclear membrane, which breaks down when the nucleoprotein is dispersed in water. Moreover, the suggestion that losses of sodium and potassium from the nucleus are involved is excluded on the basis of these results. Secondly, the nuclear components extractable in 0·14 M sodium chloride, namely, nuclear globulin and some soluble RNA, do not appear to be involved in reducing the ability of irradiated nucleoprotein to incorporate thymidine. Thirdly, the histone component of nucleoprotein, although it may play a part, is certainly not the sole factor involved in promoting the radiation lesion. The fact that the radiation-induced reduction in the ability of DNA to incorporate tritiated thymidine disappears when the DNA is freed of residual protein suggests strongly that the X-ray induced lesion which produces this effect resides in this nucleoprotein complex.

It has been observed in this laboratory that, following irradiation, the ratio of protein to DNA in the nucleus is altered. These changes may be involved in producing the results described here and are at present under investigation.

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Utilization of Radiosulphate by Higher Plant Tissues

THERE is now considerable information concerning the pathway by which sulphate is incorporated into the amino-acids in micro-organisms[1]. In contrast, very little is known about the route for incorporation of sulphate into the amino-acids of higher plants or of the interconversions of these sulphur-containing amino-acids[2].

In the work recorded here, a variety of higher plant materials were examined for the incorporation of radiosulphate into the amino-acid fraction. In particular, the leaves, hypocotyls and cotyledons of radish seedlings were found to show a rapid incorporation of radiosulphate into the amino-acids. Similar patterns of incorporation were found when using cell-free extracts of these tissues supplied with adenosine triphosphate.

In the experiment shown in Table 1, 10-day-old radish seedlings were severed at soil-level and the hypocotyls placed in the buffered radiosulphate solutions as indicated. Samples of the seedlings were placed in the dark and in the light as shown and the uptake and incorporation of radiosulphate into the amino-acids were followed over periods up to 1 h.

At the end of the experimental periods, the seedlings were killed in boiling 80 per cent ethanol, and following homogenization, were extracted using dilute ethanol solutions and water[3]. Sulphate was removed from the soluble fraction by passing through 'Rexyn' columns in the hydrogen form and by ion exchange paper chromatography. The amino-acid fraction, eluted from the 'Rexyn' columns using 2 N ammonium hydroxide, was separated by two-dimensional paper chromatography[3]. The radioactive areas, ascertained by autoradiography, were eluted and the radioactivity assayed using a 'Mylar' window continuous gas flow Geiger–Müller tube. The counts were corrected for background and half-life decay.

It is evident from Table 1 that the uptake of radiosulphate was not appreciably affected by illuminating the seedlings. The rate of accumulation of sulphur-35 by the tissues was approximately linear over the 60 min experimental period. Activity was detected in the amino-acids and in material soluble in anhydrous ether. The latter fraction would presumably contain mustard oils which are abundant in plants related to the radish[4].

After 15 min of sulphate uptake in light or darkness, radioactivity was detected in the cysteine–cysteic acid area on the chromatograms. After 30 min, taurine-^{35}S was present in both treatments and methionine was labelled in the illuminated tissues. With increase in the experimental period to 1 h, the radioactivity in taurine decreased in the light but further increased in the seedlings in the dark. Furthermore, after 1 h, methionine sulphone-^{35}S was present in the tissues, the radioactivity in this compound being greater under conditions of illumination. The results are therefore in agreement with an earlier report[5], where excised mung bean leaves produced cysteine and methionine when infiltrated with radiosulphate.

Table 1. UPTAKE AND INCORPORATION OF RADIOSULPHATE BY EXCISED RADISH SEEDLINGS IN LIGHT AND DARK (EXPRESSED AS COUNTS/MIN)

Fraction	Light*			Dark†		
	15 min	30 min	60 min	15 min	30 min	60 min
Total ^{35}S taken up	115,500	234,000	402,500	106,000	183,000	390,000
Ether solubles	—	550	1,850	—	1,000	900
Amino-acid fraction, cysteic acid and cysteine	360	640	680	1,740	1,920	2,360
Taurine	—	3,240	1,920	—	600	2,100
Methionine	—	200	1,000	—	—	500
Methionine sulphone	—	—	1,450	—	—	400

5 μc. of carrier-free $^{35}SO_4$ in 0·02 M *tris* buffer *p*H 7·4 was fed through the hypocotyls of 0·75-g samples of seedlings.
* Illuminated with 500 ft.-candles in moving air at 25° C.
† Incubated in still air at 25° C.

Similar results were obtained when radiosulphate solutions were fed to slices of radish cotyledons, slices of young radish leaves and intact radish leaves. Tomato and *Coleus* leaves readily produced cysteine-^{35}S from radiosulphate, but methionine-^{35}S was not detected even after a 3-h experimental period.

Cell-free extracts of radish leaves prepared in 0·5 M sucrose, 0·02 M magnesium chloride and 0·025 M *tris* buffer at *p*H 7·4, containing chloroplasts and mitochondria, incorporated $^{35}SO_4$ into the amino-acid fraction.

This incorporation was not markedly stimulated by additions of TPN or by a system producing reduced TPN. Additions of ATP (1 micromole), however, caused a five-fold increase in the sulphur-35 content of the amino-acid fraction as compared to the extracts where cofactor additions were not made. More detailed experiments on sulphate metabolism *in vivo* and *in vitro* by the tissues are now in progress.

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BIOLOGY

Two Components in the Development of Ray Cataract in Frogs

RECENTLY it has been shown in our laboratory that any intra-ocular operation on irradiated tadpoles seemed to result in the development of a ray cataract[1]. Later on, the same results were obtained in adult frogs and mice as well, in which an accelerated development of the aforementioned disease was also brought about by a fine pricking of the irradiated lens[2–4]. At the same time, judging by long-term observations on irradiated tadpoles and frogs, even a very heavy irradiation without subsequent operative intervention affects neither the transparency nor the structure of lenses. The experiments described here suggested to us that a direct relation existed between the degree of irradiation of the eye prior to operation and completeness of the opacity of the lens. Therefore, the question arose as to which doses of ionizing radiation—extreme, minimal, and maximal, combined with operative interference—provoked an accelerated development of the ray cataract.



a *b*

Fig. 1. Eye of a frog irradiated with 15,000 r. 1·5 h after pricking the lens a partial drawing of the lens epithelium to the anterior pole (*a*) can be seen, but no histological changes characteristic of the ray cataract can be found in a lens rendered completely opaque (*b*). (× *c.* 7·5 and × *c.* 15)

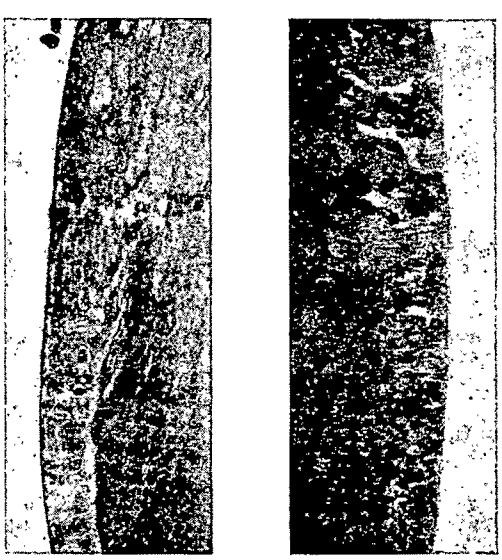
To answer this question, experiments were performed on frogs (*Rana temporaria*), which, like some other lower vertebrates, are distinguished by high resistance to ionizing radiations.

Two days after irradiation of the heads of these animals in a roentgen apparatus (RUD 100 kV/20 m.amp) the centre of one of the lenses was pricked, this time to a depth of no more than $\frac{1}{6}$–$\frac{1}{5}$ of its diameter. The control experiments showed that such a surface prick did not cause an unirradiated lens to become opaque. In all, 62 main and 33 control experiments were carried out.

Regular vital observations on the eyes of all the animals were carried out with the use of a slit lamp. Apart from this, many eyes were histologically examined.

It turned out eventually that the minimal irradiation dose which resulted in the development of a slight ray cataract after pricking was that of 500 r., the maximal being 12,000–15,000 r. The maximal dose was that which produced extremely rapid and strong opacity of the lenses. In the majority of cases this process occurred during 0·5–2·0 h.

After irradiation with the maximal dose and after pricking, a portion of the lens epithelium was drawn to the anterior pole of the lens (Fig. 1). A characteristic cross-shaped figure sometimes appeared in this region thereafter, which was connected to the formation of two crossing cracks running to the depth of the pricking performed. One of them appeared at the site of the anterior, vertical suture of the lens, the other at the site of its horizontal plane.



a *b*

Fig. 2. 1–2 h after pricking of a lens treated with the same heavy dose small vacuoles appear in the transitory zone (*a*), while aggregations of some substance appear in the posterior cortex (*b*). No migration of the cells of the transitory zone and no formation of the pseudoepithelium are found (× 200)

Histological examination of this material, fixed 0·5, 1·5 and 2 h after the operation, revealed the separation of some lens fibres, appearance of several small vacuoles in the transitory zone of the lens and multiple aggregations of some substance in their posterior cortex (Fig. 2). This substance stained similarly to pycnotic nuclei, but, when judged by special histochemical reactions, it was related neither to DNA nor to RNA.

The majority of the nuclei in the ganglion layer and many nuclei in the inner granular layer of the retina were conspicuously pycnotized. This was in good agreement with our previous investigations, which had shown a formative relation between normally functioning, mainly ganglion retinal cells and continuous histogenetic processes in the lens of tadpoles and frogs[5–7]. It may be possible that the ray cataracts in our animals were also formed as a result of a disturbance of the formative equilibrium between the retina and the lens.

Particular attention should be directed to the fact that, after the maximal irradiation and the pricking, no distortion of histogenetic processes characteristic of the ray cataract could be found in the lens rendered opaque—the distortion which many authors regarded as the cause of the opacity of the injured lens. In this case no balloon cells were found in the transitory zone, nor their migration to the posterior pole, and no formation of the pseudoepithelium was related to this latter process. The realization of these cellular processes requires a certain time which is lacking on the extremely rapid development of the provoked cataractal changes. In some cases, when the experimental animals were fixed in 3–5 days after the operation on parts of their eyes, one could see the beginning of pseudoepithelial formation.

Therefore, due to our acute experiments performed on the animals resistant to ionizing radiation, we succeeded in obtaining ray cataracts determined by only physicochemical disturbances in the lenses irradiated, while during the trivial development of the ray cataract in mammals and human beings, the physicochemical component of this disease found by us remained unnoticed, being manifested simultaneously with, and being closely related to, the well-expressed morphological, or better, pathohistological component.

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Mode of Action of Selective Fungistatic Effect of Streptomycin

In the search for selective media for the isolation of *Phytophthora* and *Pythium* species from soil and infected plant tissue, relatively few results have appeared in the literature concerning the fungistatic effect of streptomycin. Eckert and Tsao[1] demonstrated that streptomycin inhibited the *in vitro* growth of *Phytophthora* species. Of five *Pythium* species investigated, *P. arrhenomanes*, *P. graminicolum* and *P. mamillatum* were inhibited by less than 100 p.p.m. streptomycin[2]. A number of investi-

gations have described the ineffectiveness of streptomycin against fungi, including some Oomycetes[4–7].

In this communication results of the fungistatic effect and possible mode of action of streptomycin against two representative Oomycete species are presented.

The *in vitro* fungistatic effect of streptomycin on *Phytophthora infestans* was studied with the agar plate dilution method in the concentration range of 25–200 p.p.m. Diameters of the colonies measured after incubation for 20 days at 15° C are presented in Table 1. Streptomycin at 25 p.p.m. strongly inhibited the growth of *Phytophthora*, whereas a concentration of 200 p.p.m. caused nearly complete inhibition.

Streptomycin also inhibited the *in vitro* growth of an unidentified *Pythium* species isolated from diseased bean roots. The degree of inhibition was estimated by measuring dry weight of mycelia grown in nutrient solutions which contained varying amounts of streptomycin. The dry weight of *Pythium* mycelia developed in the presence of 50 p.p.m. streptomycin was about 50 per cent less than that of the control. 500 p.p.m. streptomycin completely inhibited the growth of the fungus (Table 2).

Concentrations of streptomycin as high as 400 p.p.m. were not fungistatic to species of *Actinomucor*, *Cunninghamella*, *Rhizopus*, *Botrytis*, *Verticillium*, *Aspergillus*, *Trichothecium*, *Fusarium*, *Rhizoctonia*, *Aureobasidium* species and some yeasts.

It is notable that the streptomycin-sensitive Oomycete species have cellulose-containing cell walls, whereas the streptomycin-resistant Eumycetes have chitinous cell walls. It was decided, therefore, to test the hypothesis that variations in streptomycin absorption by the hyphæ of these two fungal groups was responsible for their differences in streptomycin sensitivity.

Table 1. *In vitro* FUNGISTATIC EFFECT OF STREPTOMYCIN ON *Phytophthora infestans*

Streptomycin concentrations (p.p.m.)	Colony diameters (mm)
200	9
100	13
50	22
25	30
0	100

Table 2. *In vitro* FUNGISTATIC EFFECT OF STREPTOMYCIN ON *Pythium* SP.

Streptomycin concentrations (p.p.m.)	Dry weights of mycelia (mg)
500	0
250	44
100	56
50	72
0	133

Table 3. STREPTOMYCIN UPTAKE OF MYCELIA OF VARIOUS FUNGI

Exp. No.	Streptomycin content (μg/g dry weight)				
	Pythium sp.	*Actinomucor repens*	*Cunninghamella echinulata*	*Rhizopus nigricans*	*Monilia sitophila*
1	620	2·5	—	—	—
2	237	0	39	53	—
3	137	2	41	29	14
4	213	24·5	36	65	—

Investigations were conducted on the absorption of streptomycin by hyphæ of *Pythium* sp. and a number of streptomycin insensitive, chitinous fungi: *Actinomucor repens*, *Cunninghamella echinulata*, *Rhizopus nigricans* and *Monilia sitophila*. The fungi were grown on Czapek's agar containing 2 per cent malt extract and 150 p.p.m. streptomycin. The controls contained no antibiotic. After incubation for 6 days the mycelia were carefully removed from the surface of the agar, dried and homogenized in a volume of tap-water 20 times their dry weight. The streptomycin content of mycelial homogenates of *Pythium* sp. and other fungi, estimated by bioassay with *Bacillus subtilis*, are presented in Table 3. Streptomycin contents are expressed on a dry weight basis. The mycelial homogenates of the *Pythium* sp. contained 137–620 p.p.m. streptomycin, whereas the chitinous fungi contained only 2–53 p.p.m. antibiotic.

Mycelial homogenates of both streptomycin sensitive and insensitive fungi were incubated with 50 p.p.m. streptomycin at 37° C, over a *p*H-range of 6–8. No differences in streptomycin content were detected after 72 h incubation. Therefore, the streptomycin-insensitive, chitinous fungi neither absorbed nor inactivated streptomycin to any appreciable degree. These results suggested that streptomycin insensitive fungi do not degrade streptomycin enzymatically in the nutrient media under the conditions of this experiment.

The selective fungistatic activity of streptomycin might be explained by the differences in cell wall composition of the hyphæ of the various fungi studied. The data suggest that cellulosic cell walls are more permeable to streptomycin than chitinous ones.

The *in vivo* antifungal activity of streptomycin against a number of species of *Peronosporales* has been attributed to an effect of the antibiotic on host metabolism rather than the pathogen[8]. The data presented herewith, however, provide evidence that the effect may in fact be a direct one, on the pathogen *per se*.

Additional indirect evidence suggesting that cell wall composition, particularly the cellulose and chitin components, influences antibiotic permeability was presented by Eckert and Tsao[1] and Kinsky[3]. The former investigators noted that the streptomycin-sensitive Oomycetes were insensitive to polyene antibiotics. Kinsky[3], on the other hand, revealed that the mode of action of polyene antibiotic Nystatin was altering the permeability of the chitinous cell membrane of sensitive fungi.

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Physiological Specialization in *Erysiphe graminis* DC. in Oats

PHYSIOLOGICAL specialization has been reported in powdery mildew (*Erysiphe graminis* DC.) with f.sp. *hordeae* attacking *Hordeum* species[1] and f.sp. *tritici* on species of *Triticum*[2], but there is no record of such specialization in *avenae*.

In the course of breeding new varieties of oats at the Welsh Plant Breeding Station, hitherto resistant selections of *A. sativa* L. have succumbed in recent years to attack by *Erysiphe graminis avenae*. In an extensive investigation of the sources of mildew resistance available in *Avena sativa* L. and related species, Griffiths[3] established that there were several genotypes with various degrees of resistance, two of which have been used in the oat breeding work at this Station[4]. First, 01747/10/7 derived from a cross between two selections from (Victory × Red Algerian) and (Red Rustproof × Record) hybrids, and secondly, Cc 4146 derived from a natural cross of *A. sativa* × *A. ludoviciana* Dur. The 01747/10/7 resistance was introduced into Manod, and until recently it has exhibited a high degree of field resistance. Observations in the breeding nursery, however, have indicated that Manod has now become susceptible to attack, and reports from various parts of Britain confirm this. At Aberystwyth the immunity of Cc 4146 to mildew was maintained even when the 01747/10/7 resistance succumbed to attack, but during the past 3 years segregates containing the Cc 4146 resistance have also become infected. Plants of *A. ludoviciana* Dur., Cc 4346 and Cc 4347, originally obtained from Miss J. Thurston, Rothamsted Experimental Station, Harpenden, have,

however, continued to be resistant, showing only a slight development of mycelium followed by necrotic lesions when subjected to heavy infection of mildew. This hypersensitive form of resistance has been maintained in segregates from crosses with *A. sativa*, and these are now being used for breeding mildew-resistant varieties. The immunity of the diploid Cc 3678 has also been maintained throughout this period.

Recent work at the Station has shown that the breakdown in resistance of Manod and Cc 4146 is due to the presence of different physiological races of *Erysiphe graminis avenae*. Single pustules of the pathogen growing on Manod and Cc 4146 in the glasshouse were isolated and multiplied on their respective seedling hosts through seven cycles of selection before testing on the five forms of *Avena* listed in Table 1.

Table 1. Differential Varieties of Oats Showing Source of Mildew Resistance

Differential oat variety	Source of mildew resistance
Milford S.225	—
Manod S.235	01747/10/7 *A. byzantina* Koch.
Cc 4146	Natural hybrid *A. sativa* × *A. ludoviciana*
9065 Cn 6/3/74	Cc4346 *A. ludoviciana* L.
Cc 3678	*A. strigosa* sub-species *hirtula* (Lag.)

Seedlings for multiplication and testing were grown in 5-in. pots and isolated in glass cylinders covered by spore-tight muslin. Table 2 shows the mildew infection on a (0–4) scale as defined by Cherewick[5] on the five differential varieties after inoculation with two of the different isolates, maintained at 15° C under glasshouse conditions, and the observations on their field and glasshouse reaction made by Griffiths[3] during 1948–53 (unpublished results).

Table 2. Mean Infection Types (0–4) Scale produced by Three Physiological Races of *Erysiphe graminis avenae*

Physiological race	Host differential varieties: Milford S.225	Manod S.235	Cc 4146	9065 Cn 6/3/74	Cc 3678
1*	4	1–2	0	0–1†	0
2	4	4	0	0–1†	0
3	4	4	4	0–1†	0

* Varietal reactions observed and reported by Griffiths (ref. 3) under field and glasshouse conditions 1948–53, designated Race 1.
† 0–1, very resistant, only slight development of mycelium followed by necrosis of host tissue.

It is proposed that the differential varieties referred to in Tables 1 and 2 be used as a basis for physiological race differentiation in *Erysiphe graminis* f.sp. *avenae* and that the two physiological races isolated in this investigation be accordingly designated as Races 2 and 3 of *Erysiphe graminis avenae*, and the physiological race to which Manod, Cc 4146, 9065 Cn and Cc 3678 were originally resistant as Race 1.

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MICROBIOLOGY

Influence of Light on the Biosynthesis of Bacteriochlorophyll by *Rhodopseudomonas spheroides*

Many species of the purple bacteria can grow under conditions of aerobic dark metabolism as well as in the light under anaerobic conditions[1]. Cells growing in the dark contain no bacteriochlorophyll and no carotenoids or only traces of these pigments[2,3]. The apparatus for photophosphorylation which is localized in the chromatophores is also absent in aerobic cultures[4,5]. Transfer of aerobically grown organisms to anaerobic conditions in the light results in the adaptation of the cells to form the pigments and the chromatophores[7]. Partial pressure of oxygen and light are the main factors in regulating the formation of the photosynthetic apparatus in the cells[2,3,6,7]; but we did not know the kind of influence of light.

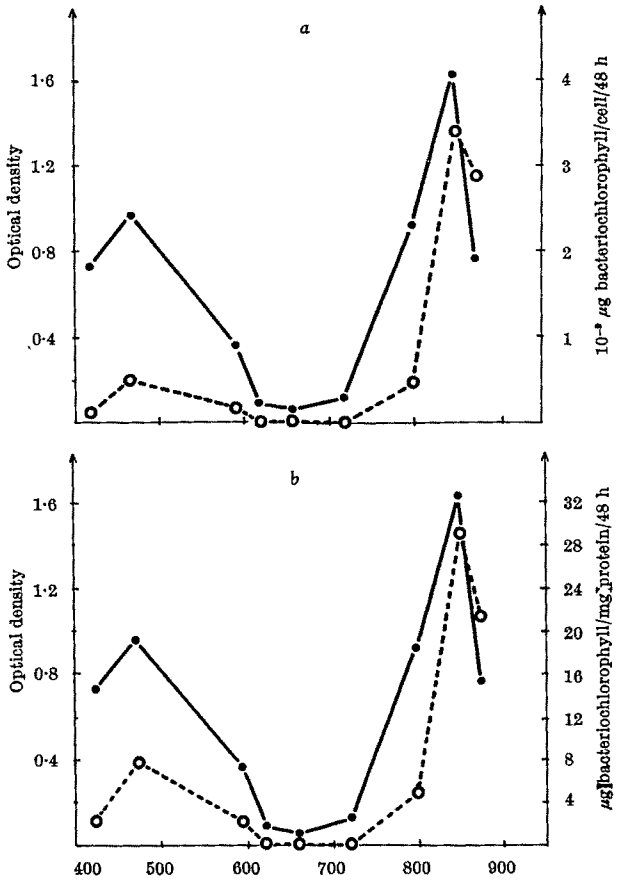


Fig. 1. Action spectrum of synthesis of bacteriochlorophyll in *Rhodopseudomonas spheroides*, irradiated 1,055 × 10^{12} quanta/cm² sec. = 2,630 ergs/cm² sec. as 799 mμ. ●, Absorption spectrum of chromatophores; ○, action spectrum

For this reason we have re-investigated the effect of light in relation to wave-length and intensity of the

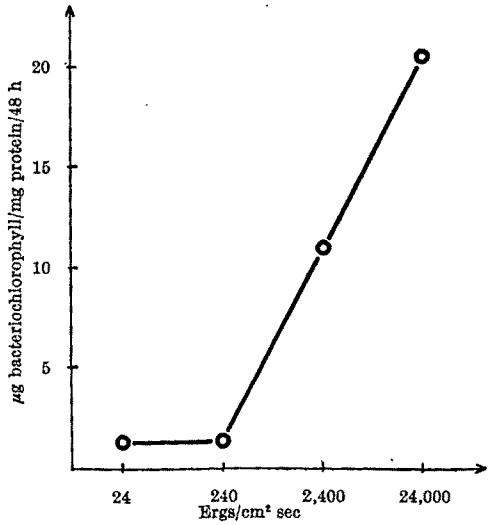
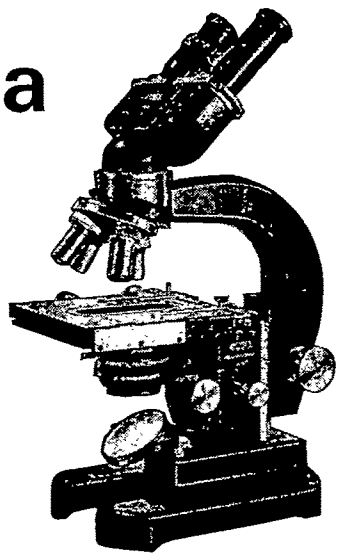


Fig. 2. Content of bacteriochlorophyll in cultures which were transferred from aerobic dark conditions to anaerobic light growth after irradiation with far-red for 48 h at 25° C

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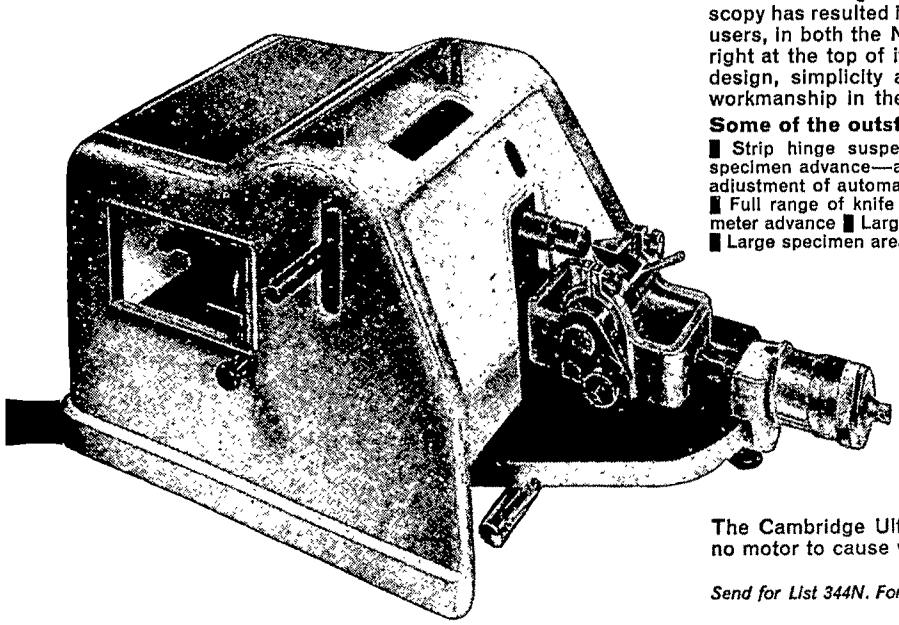


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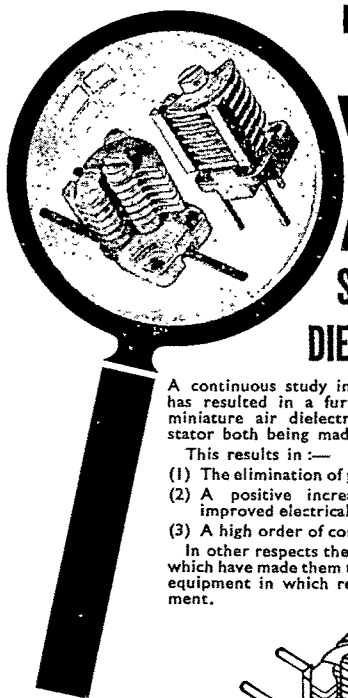
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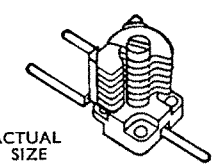
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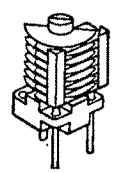
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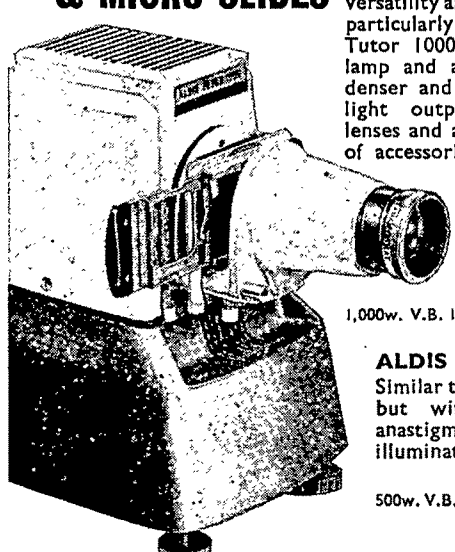


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irradiation on the synthesis of bacteriochlorophyll. Fifty-ml. Esbach dishes covered with a glass plate were used as culture chambers. The other experimental conditions are described elsewhere[7].

If dark-grown cultures are transferred to conditions of anaerobic light-growth, synthesis of chlorophyll starts only after irradiation with far-red or blue or green (Fig. 1). Under the experimental conditions, we have observed a relation between light-intensity and the amount of bacteriochlorophyll synthesized in 48 h (Fig. 2). These two facts suggest that light controls the synthesis of bacteriochlorophyll through photosynthesis. In contrast to the observations of Wolken[8] concerning the synthesis of chlorophyll in *Euglena*, light which is absorbed by *proto*bacteriochlorophyll is not utilized for synthesis of bacteriochlorophyll. Further, we tested the effect of pre-illumination with low light intensities of different wave-lengths; but we could not detect any influence of other pigment systems different from bacteriochlorophyll and carotenoids.

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Similar Effects of Various Neutral Solutes on the Survival of *Aerobacter aerogenes* and of Red Blood Cells after Freezing and Thawing

Aerobacter aerogenes from continuous culture is killed by slow freezing in buffer or by freeze-drying. Rapid freezing in liquid nitrogen results in 50 per cent survival if the organisms are suspended in distilled water, and almost complete survival if they are suspended in 10 per cent glycerol. Ten per cent solutions of some other polyhydroxy-compounds, sugars or of polyethylene glycol protect equally well[1]. The work recorded here extends the investigation to other, mainly non-hydroxylic, compounds. Like the sugars, these had already been tested on human red blood cells subjected to relatively slow freezing (various workers, references below) and it seemed possible that the response of *A. aerogenes* would not be very different from that of red cells.

Table 1. Effect of Aqueous Solutions on Freezing Damage to *Aerobacter aerogenes* from Continuous Culture compared with Their Effect on Freezing Damage to Red Blood Cells

Solute	Conc. (%)	Survival (%)	Protection	Protection (red cells)
Dimethyl sulphoxide	10	97	Complete	Complete (ref. 5)
Dimethyl acetamide	10	95, 96·5	Complete	Complete (ref. 6)
Dimethyl formamide	10	93, 92·5	Good	Complete (ref. 6)
N-Methyl-2-pyrrolidone	2·5	92, 87	Good	Good (ref. 7)
Acetamide	10	93, 90	Good	Good (ref. 8)
Formamide	10	80, 80	Fair	Slight (ref. 8)
2-Pyrrolidone	2·5	51, 52	None	None (ref. 6)
Tetrahydrofuran	4	47, 40	None	None (ref. 6)
Acetone	10	36, 37	None	None (ref. 6)
Pyridine *N*-oxide	2·5	44, 26	None	Partial (ref. 9)
Urea	10	2, 1·3	None (aug.)	None (ref. 6)
Butyrolactone	2	1, 0·5	None (aug.)	None (ref. 6)
Acetonitrile	10	0, 2	None (aug.)	None (ref. 6)
Polyethylene glycol	5	94	Good	Good (ref. 10)
Polyvinyl pyrrolidone	10	89, 96	Good	Good (ref. 11)
Human albumin	10	98, 97, 96	Complete	Good (ref. 12)
Ovalbumin	10	97, 96, 95	Complete	Not tested

(aug.), augmentative.

The experimental procedure for freezing in liquid nitrogen and measuring viability has been described[1]. Apart from the higher rate of freezing, the main difference between the present system and that normally used in experiments on red cells is that distilled water and not saline had to be used, because *A. aerogenes* is very sensitive to salt on freezing. The solutes were at 10 per cent strength (v/v for liquids, w/v for solids) except when this concentration was found to be toxic at room temperature; in such cases the highest tolerated concentration was used.

The results are shown in Table 1; each test includes controls using distilled water which gave viabilities very near to 50 per cent, except for acetone when the controls were a little low. The unfrozen populations were uniformly 95–99 per cent viable. The effect of each solute on the hæmolysis of red cells by freezing and thawing is also given, with a reference; the concentrations used are assumed to be optimal, generally between 10 and 20 per cent for the good protectors. *A. aerogenes* was protected from freezing damage by very much the same compounds as those which protect red cells despite differences in the technique used to determine response of these systems. Another way in which *A. aerogenes* resembles red cells is its sensitivity to cold shock[2], which is, however, observed only with bacterial cells in the logarithmic phase of growth and not with those from continuous culture; with red cells special conditions are also required[3].

It is of particular interest that *A. aerogenes* is protected by the same non-penetrating polymeric solutes which protect red cells, and these results may help in resolving the present controversy regarding the role of intracellular salts in the lethal effects of low temperatures. It should also be noted that the best low-molecular weight protectors are, under other conditions, particularly lethal to gram-positive bacteria such as micrococci, and it is believed that this is connected with the strong solvent action that such compounds can exert on the cell wall, when the solution is sufficiently concentrated[4].

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CYTOLOGY

Multinuclearity of Osteoclasts

The origin and function of the multinuclear osteoclast cell are of continuing interest among contemporary investigators, and have a long history among investigations on amphibian regeneration[1,2].

The reported origin of this cell is varied[3], with reticulocytes, osteoblasts, and osteocytes receiving complete or partial support from Bloom *et al.*[4] and Tonna[5], whereas a hæmatogenic origin from mononuclear leucocytes is recommended by Fishman and Hay[1] and others.

The phagocytic activity of osteoclasts[3,6] lends itself well to this cell, being considered with other macrophages as a



Fig. 1. Of the multinuclear osteoclasts (*Os*) illustrated, one clearly appears to be in the process of ingesting an osteocyte (*O*). The cytoplasm of the osteoclast is vacuolate and contains granules. The cell surface adjacent to the bone (*B*) in a Howship's lacunus appears to be fibrillar (from ref. 6, Fig. 2). (Triple stain, × 200)

member of the reticuloendothelial system of Aschoff[7]. Thus the osteoclast may originate from mesenchymally derived osteoblasts in mammals[5] and in birds[4], or from mononuclear leucocytes in amphibians[1]. Regardless of the exact origin of osteoclasts among the various species studied, these cells acquire a number of nuclei without normally synthesizing deoxyribonucleic acid nor revealing mitotic activity[5]. Therefore one may ask, What is the source of the typically large numbers of nuclei of the osteoclast?

The osteoclast described by Fishman and Hay[1] contains radioactive nuclei (tritiated thymidine) derived from previously labelled mononuclear leucocytes, and numerous unlabelled nuclei. The source of these latter nuclei may be from previously unlabelled mononuclear leucocytes fusing with labelled cells in forming the osteoclast[1].

However, we must consider the ample evidence of phagocytic activity demonstrated by these cells[3,6]. Bloom *et al.*[7], in birds and others in mammals[3], have reported the incorporation of osteocytes into the osteoclast. We have also observed this latter activity (Fig. 1) in the amphibian, *Diemictylus viridescens* (*Triturus* v.)[2]. Therefore we believe that a large share of the nuclei found within the osteoclasts are derived from osteocytes freed from their lacunæ through mineral erosion.

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Meiotic Investigations in Man

It is widely believed that meiotic investigations in man are only possible on freshly fixed testicular biopsies, or on fœtal ovary. As these investigations provide the only method for the detection of such aberrations as paracentric inversions, reciprocal translocations involving pieces of similar size, and small deficiencies of long chromosome arms, and as these may well be very common in man in view of both the frequency of infertility, and of the extreme variability in chromosome morphology evident at mitosis, any method of obtaining relevant data on a population scale would be of great value. Although remarkable progress, largely as a result of the technical improvements following the use of air drying[1] and the extension of the culture techniques evolved for bone-marrow[2] to blood[3], has followed the publication, seven years ago, of a technique for handling cell suspensions from mammals[4], no comparable progress, either in technical development or observational opportunity, has followed the work on human meiosis published by the same authors the same year[5].

Nor have there been any major advances in the past four years which either simplify or accelerate mitotic investigations on post-mortem material; these are still dependent on the skilled and laborious ritual of fibroblast culture. Lymphocyte cultures obtained by briefly dipping an intact thymus in 50 per cent alcohol, rinsing in sterile saline, breaking up in culture medium containing serum and phytohæmagglutinin, and processing after three days, are simple if the thymus is obtained shortly after death; but this is rarely practicable in most countries. Although restricted to adult males, and excluding the very aged and most cases with sex-chromosome anomalies, the disclosure of meiosis, if feasible, would provide a simple post-mortem method of examining the basic caryotype, either routinely or in selected diseases. Such observations may be supplemented by nuclear sexing; although post-mortem buccal smears are unpleasant to take and may be difficult to interpret, excellent preparations showing Barr bodies which are larger and clearer than those in oral epithelium, and present in the great majority of cells in the normal female, may be obtained by making smears from the bladder.

We wish to report our failure to obtain satisfactory preparations of meiosis from post-mortem material, but to point out that numerous cells in zygotene are readily found up to three days after death, provided refrigeration is adequate. We believe that our failures are technical and will be resolved by methods which we have so far failed to elaborate.

Numerous preparations, such as that shown, may be obtained by extruding the contents of a tubule, incubating in hypotonic citrate, fixing by adding excess acetic alcohol, resuspending in acetic alcohol, and air drying. A suspension of cells from inside the tubules may be made by putting a piece of testis into 1 per cent sodium citrate in a plastic bag, cutting into fragments with a scissors, and squeezing between the fingers. Fixation may also be carried out by centrifuging through increasing concentrations of acetic acid in successive layers.

Although inadequate to reveal abnormalities, unless fortuitously well placed, the preparations show nuclear structures of interest. A globular structure which stains



Fig. 1. Meiosis 48 h *post mortem*. The nucleolus is the pale oval structure at bottom right

lightly with methylene blue, and resembles the large nucleolus of the spermatocyte, is not intimately related to the chromosomes: some cells have two smaller nucleoli. A densely staining structure intimately related to several chromosomes is usually evident. Variations in staining of the paired chromosomes appear to be identical in both strands. Satellites are sometimes seen and appear unrelated to any other structure. A small chromosome frequently lies at the periphery and could be the *Y*. If this peripheral localization is found in mitotic cells the possibility that selective loss of the *Y* chromosome might arise after fixation deserves consideration.

Diverse experimentation with this inexhaustible supply should provide techniques from which population investigations comparable with those conducted on *Drosophila* could be carried out.

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GENETICS

Incidence of Pseudocholinesterase Variants in Australian Aborigines

There are now known to be a number of genetically determined variants of the human serum enzyme pseudocholinesterase[1–5], of which the most frequent in Britain are the 'typical' and 'atypical' variants. Homozygotes for the 'typical' variant amount to about 96 per cent of the population, and heterozygotes to about 4 per cent. 'Atypical' homozygotes are only seen once among several thousands. The different pseudocholinesterase types are recognized by determining the degree of inhibition of the enzyme by dibucaine ('Nupercaine') following the method of Kalow and Genest[1], the percentage inhibition being called the dibucaine number. The other pseudocholinesterase variants are extremely rare and they are also discovered in the first instance by their less than normal inhibition by dibucaine, with one exception, the 'silent' variant[4] which in the homozygote results in complete absence of enzyme activity.

Table 1. Incidence of 'Atypical' Serum Pseudocholinesterase in Australian Aboriginals

Place	No. of sera examined*	No. of heterozygotes found
Yarrabah Mission	34 (39)	–
Coen	10	–
Mitchell River Mission	7	–
Normanton	7	–
Hopevale	5	–
Origin unknown	5	–
Lockhardt River	3 (4)	–
Mornington Island	3	–
Aurukun	2	–
Cooktown	2	–
Edward River	2	–
George	2	–
Herbeton	2	–
Kuranda	2	1
Bloomfield	1	–
Croydon	1	–
Georgetown	1	–
Innisfail	1	–
Laura	1	–
Mapoon	1	–
Marreba	1	–
Mona Mona	1	–
Weipa River	1	–
White Rock	1	–
Wrotham Park	1	–
Yorkeys Knob	1	–

* In parentheses: before correction for first degree relationship.

So far, the incidence of the typical/atypical heterozygote has been found remarkably alike in very different populations: Canadian[6], British[7], Greek[7], Portuguese[7], Moroccan (Jewish)[7], Berbers[7], Czechs[8] and Germans[8].

We have recently examined the sera from 104 Aboriginal Australians. Twelve were first-degree relatives and six were therefore taken out to obtain a less-selected sample. In the remaining 98, one heterozygote was found. This does not represent a striking difference from surveys made in other parts of the world, and 'atypical' pseudocholinesterase remains an inherited characteristic with remarkable uniformity of incidence in racially widely different populations.

Harris, Hopkinson, Robson and Whittaker[9] have recently described an isoenzyme of pseudocholinesterase which is recognized by electrophoresis at *p*H 6. It is genetically independent from the 'typical' pseudocholinesterase and its variants. It was found in 5 per cent of 248 unrelated British individuals. We have confirmed the occasional occurrence of this isoenzyme in Europeans, but have not found it in these 104 Australian samples.

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A Case of Somatic Polyploidy

Natural alterations in the number of chromosome sets appear to be uncommon among the somatic tissues of animals. Somatic haploidy has been claimed to occur in the micromeres of sea-urchin embryos[1], and in the tail mesenchyme of tadpoles[2]. The former case was quickly denied[3], while the latter is also better interpreted as the pairing of homologous chromosomes. Polyploidy is well known in mammalian liver and in various tumours, but rarely in other tissues.

In the course of an investigation of the frequency of nucleolar fusion during the development of *Xenopus laevis*[4], the following evidence was found of polyploid nuclei in the notochord. The notochord cells do not divide while differentiating at neurula stages to form a vacuolated syncytium. Mitosis is only resumed shortly before the time of hatching. The resulting interphase nuclei in the notochords of feeding tadpoles are more variable in size than is usual for one tissue. As many as six nucleoli have been seen in these nuclei, although a maximum of two nucleoli per nucleus is found in other tissues, and in the notochord itself of unhatched embryos. Such numbers of nucleoli could be explained theoretically as the result of polyploidy, or as the production of 'accessory nucleoli' by normally latent nucleolar organizers[5]. The nucleolar counts might thus be interpreted as indicating either some tetraploid and octoploid nuclei, or up to four accessory nucleoli. These explanations have been tested by the use of heterozygous mutants which form only one nucleolus in each of their nuclei[6]. The mutants are fully viable and possess the full diploid number of chromosomes[7]. The notochord nuclei of these mutants would be expected to contain up to three nucleoli

Table 1. Frequency Distribution of Notochord Nuclei containing 1–6 Nucleoli

Genotype and specimen	No. of nucleoli 1	2	3	4	5	6	Total count	Minimum percentage of polyploidy
Wild 1	21	48	21	7	2	1	100	31
Wild 2	28	50	16	5	0	1	100	22
Wild 3	29	47	16	5	2	1	100	24
Wild 4	34	55	8	2	1	0	100	11
Mutant 1	69	29	2	0	0	0	100	31
Mutant 2	66	34	0	0	0	0	100	34
Mutant 3	70	29	1	0	0	0	100	30
Mutant 4	67	31	2	0	0	0	100	33

if polyploidy occurs, or up to five nucleoli if some of them were produced by latent organizers. Nucleolar counts from the notochords of four wild-type and four mutant feeding tadpoles are shown in Table 1; their comparison fits the expectation for polyploidy. Some polyploidy is certainly obscured by nucleolar fusion in these counts, as indicated by the odd numbers of nucleoli in wild-type specimens, and by the inequality in size of the nucleoli in some nuclei. Probably well over 30 per cent of the notochord nuclei become polyploid.

The occurrence of polyploidy without hazard to cell-type in this tissue, mammalian liver and experimentally produced polyploid animals, suggests that polyploidy is only an incidental phenomenon of tumours. The common factor in tumours and other cases of spontaneous polyploidy may well be that mitotic activity is resumed after a prolonged interphase of the differentiated cell, when the cellular resources are perhaps incomplete or unbalanced.

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VIROLOGY

Nicotinamide-adenine Dinucleotides, Adenosine Diphosphate and Adenosine Triphosphate Content of Tissues infected by Tobacco Mosaic Virus

The physiology of virus-infected plant tissues is of interest in that an understanding of the processes underlying the development of disease symptoms is dependent on a knowledge of changes in the host tissue metabolism induced by virus-infection. An approach to this problem is a consideration of nucleotide-levels in tissues infected by virus. The formation of tobacco mosaic virus (TMV) may give lower nucleotide-levels in the host tissue as a result of the utilization of nucleotides in virus-ribonucleic acid synthesis. This is of particular significance with regard to the adenosine nucleotides, adenosine diphosphate (ADP), adenosine triphosphate (ATP), nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP), all of which are concerned in the regulation of the intermediary metabolism of the host-tissue[1].

Some possible effects of virus infection on the levels of the compounds in the host-tissue have already been discussed[2]. Moreover, tissues systemically infected by TMV have a lower rate of respiration than comparable healthy tissues[3,4]. This may be the result of changes in nucleotide-levels following TMV-infection[2]. It seems probable that some decrease in nucleotide-levels takes place in TMV-infected tissues as tobacco callus tissue systemically infected by TMV shows a lower concentration of acid-soluble organic phosphorus compounds[5]. In the investigation recorded here the concentrations of NAD, $NADH_2$, NADP, $NADPH_2$, ADP and ATP were determined in tobacco callus tissue cultures systemically infected by TMV.

Table 1. Nicotinamide-Adenine Dinucleotide and Nicotinamide-Adenine Dinucleotide Phosphate Content of Tobacco Callus Tissue Cultures infected by TMV

	μgm/100 mg dry weight	
	Healthy tissue	TMV-infected tissue
NAD	3·8 ± 0·07*	12·0 ± 0·80
NADP	1·6 ± 0·09	0·6 ± 0·002
NADH	0·9 ± 0·01	0·5 ± 0·008
$NADPH_2$	0·5 ± 0·001	0·6 ± 0·006
Total	6·7	13·6

* Mean of four determinations ± *S.E.*

Table 2. ADP and ATP Content of Healthy and TMV-infected Tobacco Callus Tissue Cultures

	μmoles × 10^3/100 mg dry weight					
	Experiment 1		Experiment 2		Experiment 3	
	Healthy	Virus	Healthy	Virus	Healthy	Virus
ATP	5·5	1·4	6·3	1·6	6·4	4·0
ADP	2·3	1·7	3·8	1·7	3·5	1·2
ATP + ADP	7·8	3·1	10·1	3·3	9·9	5·2

Healthy and virus-infected conditioned callus tissue of *Nicotiana tabacum* type White Burley var. Judy's Pride were sub-cultured on a modification of the medium of Morel[6] as suggested by Kassanis[7] containing 0·5 g calcium nitrate, 0·5 g dihydrogen potassium phosphate, 0·125 g potassium nitrate, 0·125 g magnesium sulphate, 20 g glucose, 0·5 ml. Bertholet's solution, 10 mg cysteine hydrochloride, 1 mg thiamine and 5 g of agar per litre. Sub-cultures were made with pieces of tissue approximately 50 mg fresh weight and grown on 15 ml. of media in tubes (15 cm × 3 cm) sealed with polythene and kept at a constant temperature of 25° C with a continuous light intensity of 200 ft.-candles at the level of the culture.

Nicotinamide-adenine dinucleotides were extracted from 1 gm fresh weight samples of tissue. NAD and NADP were extracted by an acid medium of 5 ml. of 0·1 M *tris*-hydrochloric acid buffer *p*H 8·2, 1 ml. N hydrochloric acid and 2 ml. water. $NADH_2$ and $NADPH_2$ were extracted by an alkaline medium, 5 ml. *tris*-hydrochloric acid buffer 1 ml. N sodium hydroxide and 2 ml. of water. The extraction medium was contained in a glass Potter–Elvehjem homogenizer immersed in a bath of boiling water. The tissue was dropped in and heated for 30 sec, homogenized for 1·5 min and then rapidly cooled by immersion in a freezing mixture of dry-ice and ethanol. The extracts were thawed and centrifuged at 20,000*g* for 15 min in a refrigerated centrifuge. The supernatants were decanted; the *p*H of the alkaline extracted adjusted to 8·0 and that of the acid extract to 7·0 by dropwise addition of alkali or acid in the cold. The nicotinamide-adenine dinucleotide content of the extracts was determined immediately by the colorimetric method of Slater and Sawyer[8]. NAD, $NADH_2$, NADP and $NADPH_2$ (Sigma purest grade) were tested for purity by the usual spectrophotometric methods[9] and used for constructing calibration curves for the method of Slater and Sawyer and determining the percentage recovery of nucleotides added to the extracting medium. They were added at ten times the endogenous concentration to 1-g samples of healthy tobacco tissue and the extraction procedure carried out as before. The percentage recovery of added nicotinamide-adenine dinucleotides was NAD 109, NADP 100, NADH. 90, $NADPH_2$ 100.

Adenosine nucleotides were extracted from 1-g fresh weight samples of tissue by homogenizing with 10 ml. o 0·2 N perchloric acid in the cold. A complete extractio was obtained as increasing the acid concentration to 2·0 N failed to extract further ATP. The perchloric acid wa neutralized by N potassium hydroxide in the cold and th

potassium perchlorate precipitate removed by centrifugation. The ATP content of this extract was determined immediately by the luciferin–luciferase method of Strehler and McElroy[10]. The enzyme was extracted from dried firefly tails (Schwartz Bioresearch, Inc.). The light emitted when this enzyme system comes into contact with ATP was measured in a fluorimeter. The reaction mixture contained 0·2 ml. of 0·25 M glycyl–glycine buffer *p*H 7·0, 0·1 ml. of 0·1 M magnesium sulphate, 0·2 ml. of enzyme and 1·0 ml. of the solution for assay rapidly injected and the maximum light flash measured. ATP (Sigma, 99 per cent pure) was used to calibrate the instrument.

ADP was determined in the extracts by conversion to ATP using the enzyme ATP : creatine phosphotransferase which catalyses the reaction $ADP + Cr \sim P \rightleftharpoons ATP + Cr$. The ATP was then determined by the luciferin–luciferase method. 2·6 ml. of the neutralized perchloric acid extract was incubated at 37° C for 1 h with 0·52 ml. of 0·25 M glycyl–glycine buffer *p*H 7·0; 0·26 ml. of 0·1 M magnesium sulphate; 0·52 ml. creatine phosphate solution, stock solution containing 0·3 μmole/ml. in buffer *p*H 7·0; 0·26 ml. of ATP : creatine phosphotransferase (L. Light and Co.), stock solution containing 1 mg/ml. in buffer *p*H 7·0. The enzyme was inactivated by immersion of the incubation mixture in a boiling water bath and 1 ml. aliquots taken for ATP determinations.

The results given in Table 1 show that TMV-infected tobacco callus-tissue contains increased amounts of nicotinamide-adenine dinucleotides. Thus, decreased levels of NAD or NADP will not account for the lower respiration rate in tissues systemically infected by TMV. It also seems probable that formation of TMV does not occur at the expense of nicotinamide-adenine dinucleotide precursors. Although virus infection sometimes results in increased dehydrogenase activity in the tissues[11,12] the increased amount of NAD in the virus-infected tissue actually results in an increase in the NAD/NADH ratio so respiration is not limited by an accumulation of reduced nicotinamide-adenine dinucleotides in the tissue. Occasionally small sections of the TMV-infected tobacco callus tissue turn green. These green tissues showed increased amounts of NADP containing 1·2 μg per 100 mg dry weight.

The results presented in Table 2 show that in tobacco callus tissue infected with TMV there is a decrease in both ADP and ATP, there being less than half of that present in healthy tissue. This decrease may be the result of incorporation of ADP or ATP in virus-ribonucleic formation. The decreased availability of ADP is probably the reason for the lower respiration rate of the virus-infected tissue although further work is required to establish definitely this point.

It is not intended that results obtained with tobacco callus tissue infected by TMV should be taken to apply to other virus host-tissue systems without adequate consideration being given to the physiological state of the tissue and structure of the virus. Even with systemic TMV-infection of *Nicotiana tabacum* leaves the situation is completely different from systemically infected tobacco callus tissue. The presence of chloroplasts in the cell enables a supply of ATP to be generated by photosynthetic phosphorylation but the possibility of compartmentation is greater. Thus, the relationship between ATP as an energy source in virus synthesis and ADP or ATP as a component of virus RNA is certain to be more complex. The different response of different tissues to the same virus is well illustrated by experiments with *Nicotiana glutinosa* leaves showing local lesions induced by inoculation with TMV. These leaves, unlike tobacco callus tissue, show an accumulation of $NADH_2$ and $NADPH_2$ (ref. 13). This result, taken together with the increased respiration rate of these leaves following TMV-induced lesion development[14], may mean that in the tissues the rate of respiration is no longer limited by the availability of ADP but by reoxidation of reduced $NADH_2$ and $NADPH_2$. Further work is in progress to attempt to relate these changes in the intermediary metabolism of the host-tissue to the development of disease symptoms and virus multiplication.

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Interaction of Vaccinia Virus and Cells in Primary and Continuous Culture

Vaccinia virus has a wide host-range, both as regards cell type and animal species. The purpose of the present communication is to describe some differences in the reaction of cells in primary and continuous culture to infection with a vaccine strain of the virus.

Stock virus was prepared from infected chorio-allantoic membranes after three passages of a calf lymph preparation of the LaPine strain No. 472 in embryonate eggs. Details are given elsewhere[1].

Primary cell cultures examined were a fibroblast type, from lung tissue of *ASW/SN* suckling mice 2–3 weeks old, an epithelial type, from kidney tissue of the same animals, and a macrophage type from peritoneal exudate cells of adult animals of the same inbred strain.

Cell lines examined originated from human amnion (*FL*), human carcinoma (*H.Ep.* No. 2) and monkey kidney (*MK*).

Mouse kidney and lung tissues were trypsinized at 4° C, using an active enzyme preparation as suggested by Wallis *et al.*[2]. Fractions taken at 15-min intervals were pooled, and the resulting cell suspension was centrifuged at 800 r.p.m. for 30 min. The cells were then washed by a similar centrifugation in calcium and magnesium-free phosphate buffered saline (*p*H 7·4). The final sediment was diluted 1 : 40 and 1 : 70 for lung and kidney cells, respectively, in a medium consisting of 9 parts by volume of a solution of 0·5 per cent lactalbumin hydrolysate, 0·1 per cent yeast extract and 0·5 per cent glucose in Earle's balanced salt solution, and 1 part of pooled calf serum (*M*). One-ml. aliquots of lung cell suspension, and 1·5-ml. aliquots of kidney cell suspension, were then seeded into 16 × 125 mm Leighton tubes with flat surface dimensions 11 × 37 mm (*LT*). Confluent cell sheets were obtained in 4–7 days.

Macrophage cultures were prepared as described previously[3], with the following modification: the peritoneal exudate was collected by injecting 3 ml. of medium *M* containing 5 units of heparin per ml. into the peritoneal cavity and aspirating the resulting turbid suspension. The cell concentration was adjusted to 4×10^6 per ml., and 1 ml. aliquots were seeded into *LT*. Cultures were ready for inoculation with virus in 2–3 days.

One-week-old cell line stock cultures, grown in milk dilution bottles in medium *M*, were collected by treatment with 'Versene'. After sedimentation, cells were resuspended in medium *M* to a concentration of $1·5 \times 10^5$ cells

per ml., and 1-ml. aliquots of the suspension were seeded into *LT*. Monolayer cultures were obtained in 4–6 days.

In all primary and cell line cultures, the average cell population per tube at the time of inoculation with virus was about 1×10^6.

Table 1. Comparative Yields of Virus in Different Cell Types at Various Time Intervals after Inoculation with Vaccinia Virus (LaPine Strain No. 472) at a Multiplicity of 10 P.F.U./Cell

Cell culture	Average yield in P.F.U./cell at h: 3	8	21	29	53	77
(1) Primary-mouse (*ASW/SN*)						
Kidney-epithelial	0·01	0·01	0·09	0·3	5·3	6·3
Lung-fibroblast	0·2	0·25	6	16	21	12
Perit. exudate-macrophage	0·04	—	0·008	0·005	0·004	—
(2) Continuous						
Human amnion (*FL*)	0·5	2	130	200	225	215
Human carcinoma (*H.Ep.* No. 2)	0·4	8	200	330	280	200
Monkey kidney (*MK*)	0·4	6	210	330	320	150

P.F.U., Plaque-forming units.

Table 2. Comparative Yields of Virus in Different Cell Types inoculated with Vaccinia Virus (LaPine Strain No. 472) at Different Multiplicities

Cell culture	Average yield in P.F.U./cell following infection at a multiplicity of: 4 P.F.U./cell		0·4 P.F.U./cell		0·04 P.F.U./cell	
	15 h	45 h	15 h	45 h	15 h	45 h
(1) Primary mouse (*ASW/SN*)						
Kidney-epithelial	0·04	4·2	0·003	0·9	0·0003	0·07
Lung fibroblast	0·2	30	0·03	9·8	0·001	2·3
Perit. exudate-macrophage	0·03	0·008	0·002	0·0002	0·00005	0·000008
(2) Continuous						
Human amnion (*FL*)	17	300	1·7	280	0·12	60
Human carcinoma (*H.Ep.* No. 2)	46	380	2·3	310	0·19	90
Monkey kidney (*MK*)	54	410	3·3	390	0·23	120

Infectious virus was assayed by the plaque technique in *H.Ep.* No. 2 cells grown in short Leighton tubes (16 × 80 mm) with increased flat surface dimensions (11 × 55 mm), using a liquid medium. The technique, described in detail elsewhere[4], was modified in two respects: plaque assays were routinely carried out using 2-day-old cultures seeded with 1·5 ml. aliquots of a suspension containing $2{\cdot}5 \times 10^5$ cells per ml., and the cultures were maintained in medium *M* with 5 per cent calf serum.

In one series of experiments cultures were exposed for 1 h to 0·5 ml. of a virus suspension, calculated to give a multiplicity of infection of 10 plaque-forming units per cell. After removal of inoculum, cultures were washed five times with phosphate-buffered saline, fed with medium *M*, and incubated at 36° C. They were then examined at various time-intervals for cytopathic effect and infectious virus. For the latter purpose, 2 tubes of each type of culture were withdrawn at each time-interval and the cells were scraped into the medium. The resulting suspension was quick-frozen and stored at − 65° C. Before titration for virus content, samples were thawed in a water bath at 36° C, transferred to cellulose nitrate tubes, and exposed for 5 min to sonic energy generated by a 200-W, 10-kc./s, Raytheon magnetostrictive oscillator. Medium and cells were pooled before assay for infectious virus since, in the cell systems herein studied, as in those described previously[1], most of the virus was found to be cell-bound.

Cell damage was first observed as early as 3 h post-infection in all the cell line cultures, as well as in the macrophage cultures. In the primary fibroblast cultures it was more delayed, first becoming apparent about 5 h later. In all these cultures, damage began simultaneously in all the cells, and became maximal in 24–48 h. In contrast, primary mouse kidney cultures exhibited only focal lesions for several days, complete destruction of the cell sheets taking about a week.

When cultures were examined for virus content, striking differences in yield were observed, as summarized in Table 1. The macrophage cultures reacted in a unique manner by exhibiting a progressive decrease in the titre of infectious virus. This extends a previous observation on the diminishing titre of cell-free virus in macrophage cultures from random-bred mice[3]. Whether these findings point to an abortive infection leading to the formation of immature virus, or reflect the gradual inactivation of the virus originally taken up by the cells, may be resolved by electron and fluorescent microscopic studies now in progress.

All other cultures supported virus proliferation though at different rates and to varying degrees. In the cell line cultures, the latent period was short, first increase in virus being noted in 8 h. Virus yields amounted to 130–330 plaque-forming units per cell after 1 day. Latency was longer, and rate of production of virus slower, in primary cultures of the fibroblast and epithelial types, with maximum yields attained a day earlier in the former type of culture. In both types of primary cultures, virus yields even after 2–3 days of infection were 15–25 times lower than one-day yields in the cell line cultures. Table 2 shows that the pattern of reaction to infection with virus was the same when multiplicities ranging from 4 to 0·04 plaque-forming units per cell were used. Macrophage cultures failed to support virus proliferation at all three multiplicities. Forty-five-hour yields in lung cultures were 7–30 times as high as those in primary kidney cultures, while the yield of cell line cultures inoculated at a multiplicity of 0·04 plaque-forming units per cell was 2–4 times that of lung cultures inoculated at a hundred-fold higher multiplicity.

These differences in the rate and yield of infectious virus production may be a reflexion of one or more factors involved in virus-cell interactions. These include efficiency of adsorption and penetration, of initiation of infection leading to the formation of viral sub-units, and of the assembly of these units into mature viral particles. The absence or presence in varying amounts of aberrant by-products of viral infection, including those of the nature of interferon, may also play a part. Preliminary experiments indicate that there are variations in the efficiency of virus adsorption. These, however, cannot account for the much more marked differences in plaquing efficiency of the virus in the different cell systems. Details of these and other experiments designed to explore the possibilities mentioned here will be published elsewhere.

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PSYCHIATRY

'Dream Time' in Hallucinating and Non-hallucinating Schizophrenic Patients

Observers of human behaviour have long commented on the similarities between the hallucinations of madness and the normal, universal hallucinations of dreaming[1]. Some have postulated that the two kinds of hallucination

share a common mechanism and that a derangement of this mechanism occurs in psychosis. If this were so, one might expect derangement of the mechanism to be present in sleep as well as waking, with a resultant alteration of the amount of dreaming; unfortunately, present theories (whether physiological[2] or psychological[3]) do not explicitly predict the direction of such changes. However, the discoveries of Aserinsky, Dement and Kleitman now permit an empirical approach to this problem. These investigators have demonstrated that in both normal and schizophrenic subjects the low-voltage fast electroencephalogram (EEG) pattern of sleep accompanied by rapid eye movements (REM) is highly correlated with the subjective experience commonly described as dreaming[4]. The present study represents an application of their methods to the estimation of dream time in hallucinating and non-hallucinating schizophrenic subjects.

Table 1. Dream Time in Hallucinating and Non-hallucinating Patients

Values for each subject are means for the data of five nights, except for patient 4 for whom data of only four nights were available. Per cent values are the means of the nightly percentages. Dream time per cent = (dream time/sleep time) × 100; REM time per cent = (sum pages with one or more rapid eye movements/sum pages sleep) × 100

Group	Patient No.	Sex	Age (yr.)	Sleep time (min)	Dream time (min)	Dream time (%)	REM time (%)
Hallucinators	1*	F	23	387	59	15·4	9·5
	2*	M	35	218	41	19·2	16·7
	3	M	20	370	67	17·9	12·4
	4	M	22	397	94	23·7	18·4
	5	M	25	381	75	19·4	13·6
	6*	F	26	383	96	25·1	12·9
	7	M	40	469	163	34·7	14·7
Mean			**27·1**	**372**	**85**	**22·2**	**14·0**
Non-hallucinators	8	F	26	370	79	20·8	12·8
	9	M	21	436	126	29·0	18·5
	10*	F	25	402	87	21·8	13·3
	11	M	22	348	64	18·4	8·7
Mean			**23·6**	**389**	**89**	**22·5**	**13·3**

* For five consecutive days, patient No. 1 received 10 mg of trifluoperazine, No. 2 received 200 mg thioridizine and No. 10 received 20 mg of trifluoperazine and 5 mg of procyclidine. In each case, medication was discontinued two days prior to recording. For thirteen consecutive days patient No. 6 received 20 mg of trifluoperazine and 5 mg of procyclidine, which were discontinued four days prior to recording.

Seven hallucinating and four non-hallucinating patients were examined. None of the patients had a history or findings suggestive of organic brain disease. All hallucinating subjects were experiencing auditory hallucinations, and three of them (Table 1, Nos. 1, 2 and 6) reported visual hallucinations as well. Four subjects were receiving tranquillizing drugs which were stopped at least two days prior to their first recording (Table 1); none of the other patients received medication during the preceding 30 days. Subjects were permitted to retire at their usual bedtimes but, in accordance with the ward schedule, all were awakened at 6·00–6·30 a.m. We usually examined two patients each night and attempted to obtain five consecutive all-night recordings for each subject. Electrode attachments for recording vertical and horizontal REM and a unipolar left occipital EEG were led to an Offner type *T* electroencephalograph which was run continuously at 15 mm/sec.

The usual scoring scheme[5] for estimating dream time from such records takes as the onset of dreaming the emergence of low-voltage fast EEG without sleep spindles or *K*-complexes (Stage 1 of Dement[6], *B*-stage of Loomis[7]), which typically precedes the first rapid eye movement. Correspondingly, the end of the dream period is taken as the end of the emergent Stage 1 activity and the onset of Stage 2 (spindles and *K*-complexes on a low to medium voltage background). Inspection of the records we obtained soon revealed serious obstacles to the use of this scoring system. The EEG tracings of some hallucinating and non-hallucinating patients showed patterns intermediate between the more clear-cut stages found in normal subjects. In these cases there appeared (usually at the end of REM periods) relatively long intervals of low-voltage fast EEG in which eye movements were absent but which resembled Stage 1 except for occasional bursts of α, spindles or *K*-complexes; the spindle bursts elsewhere were less abundant, shorter in duration and of lower amplitude than usual. In addition, the definite REM periods seemed more often interrupted by body movements and by Stage 2 EEG; two patients (hallucinators) showed frequent occurrences of spindles and eye movements within the same 20-sec epoch. These apparently unusual patterns are being studied further. Due to the difficulties they presented in classifying EEG, we adopted the following system for estimating time spent dreaming: each 20-sec interval (one page of record) was scored for the presence of REM, spindles and *K*-complexes. Dream time was taken as the number of pages from first to last REM (inclusive) minus any pages on which a spindle or *K*-complex appeared in the absence of REM.

Table 1 gives results for sleep time, dream time and two related measures for the 7 hallucinating and 4 non-hallucinating subjects. The mean values for the two groups are quite similar and none of the differences, as evaluated by the Mann-Whitney *U* test[8], approaches the 0·05 level of statistical significance (two-tailed). Dream time and per cent dreaming are based on eye-movement and EEG criteria combined, as was indicated above. When eye-movement activity is considered separately (REM time per cent) we again find similar values in the two groups.

Thus, our findings fail to support the hypothesis of a difference in dream time between hallucinating and non-hallucinating schizophrenic patients. There are two reasons for suggesting caution in interpreting these results: first, one can never be certain that a patient who denies hallucinations is not in fact experiencing them; secondly, the drugs administered to four of the subjects shortly before participation in the study may have acted to obscure any true difference. Work is now being carried out which controls these factors more adequately[9].

We thank Dr. W. Overholser, then superintendent of Saint Elizabeths Hospital, and Dr. Mary V. McIndoo, chief of psychiatry at D.C. General Hospital at the time these examinations were carried out, for their co-operation. We also thank Dr. J. G. Lofft and Dr. W. Dobbs of Saint Elizabeths Hospital for recommending patients for examination.

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FORTHCOMING EVENTS

Monday, September 16—Tuesday, September 17

INSTITUTE OF PHYSICS AND PHYSICAL SOCIETY, MATERIALS AND TESTING GROUP (at the University of Bristol)—Conference on "Some Aspects of Surface Behaviour".

Wednesday, September 18—Friday, September 20

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 9.30 a.m. daily—Symposium on "Food Technology in Europe".

Thursday, September 19

BRITISH INTERPLANETARY SOCIETY (at University College, Gower Street, London, W.C.1), from 10 a.m. to 5 p.m.—Symposium on "Satellite Meteorology".

INSTITUTION OF CHEMICAL ENGINEERS (at the Royal Aeronautical Society, Hamilton Place, London, W.1), at 5 p.m.—Mr. T. J. Williams: "Progress in Chemical Process Control in America: Computers, Instrumentation, and Applications".

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

Friday, September 20

BRITISH ASSOCIATION OF CHEMISTS (at the Royal Society of Medicine, Wimpole Street, London, W.1), at 6.30 p.m.—Mr. Henry T. F. Rhodes: "The Progress of Forensic Science" (Hinchley Memorial Lecture).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

HEAD (with good academic qualifications, teaching and/or industrial experience, and able to organize research) OF THE ELECTRICAL ENGINEERING DEPARTMENT—Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (September 19).

RESEARCH ASSISTANT IN APPLIED PHYSICS; and a RESEARCH ASSISTANT IN METALLURGY—The Vice-Principal, Lanchester College of Technology, Priory Street, Coventry (September 20).

LECTURER IN PATHOLOGY at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 25).

LECTURER (with a degree or diploma in the social, biological or physical sciences or technology, and some research and/or teaching experience relevant to ergonomics and/or cybernetics) IN THE DEPARTMENT OF ERGONOMICS AND CYBERNETICS—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire (September 30).

LECTURER (with some experience of hospital laboratory work, and preferably some teaching experience) IN THE DEPARTMENT OF CLINICAL CHEMISTRY—The Secretary, The University, Edinburgh (September 30).

LECTURER (with a special interest in electron theory of alloys and related topics) IN THE DEPARTMENT OF METALLURGY—The Registrar, The University, Liverpool, quoting Ref. CV.406 (September 30).

PROFESSOR OF MATHEMATICS (either Pure or Applied)—The Registrar, The University, Leeds 2 (September 30).

SENIOR LECTURER IN GEOGRAPHY at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

ASSISTANT LECTURER IN SOCIOLOGY IN THE SCHOOL OF SOCIAL STUDIES—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (October 1).

CHAIR OF ENGINEERING SCIENCE—The Registrar and Secretary, University of Durham, 38 North Bailey, Durham (October 7).

TECHNICAL ASSISTANT (with experience in fitting and turning, and preferably a knowledge of instrument making) IN THE DEPARTMENT OF PHYSICS, Rhodes University, Grahamstown, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 10).

LECTURER IN PATHOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (October 11).

LECTURER or ASSISTANT LECTURER (medically or non-medically or veterinary qualified candidate) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. No. CV/403/N (October 11).

CHAIR OF PHYSICS—The Secretary, University of Lancaster, Lancaster (October 12).

CHAIR OF MATHEMATICAL STATISTICS—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (October 15).

LECTURER or ASSISTANT LECTURER IN PHYSIOLOGY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

LECTURER or ASSISTANT LECTURER IN GEOGRAPHY; and a LECTURER or ASSISTANT LECTURER IN THE SUB-DEPARTMENT OF THE HISTORY OF SCIENCE IN THE DEPARTMENT OF PHYSICS, University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

LECTURER or ASSISTANT LECTURER IN PLANT BREEDING AND GENETICS at the University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

B.I.P. RESEARCH SCHOLAR (with a good degree, including chemistry), to work under the direction of Dr. P. H. Plesch, on problems related to cationic polymerization—The Registrar, The University, Keele, Staffordshire (October 21).

RESEARCH OFFICER (with a Ph.D. degree or postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability, and preferably experience in reaction kinetics, surface or solid-state chemistry, and/or high vacuum technique) IN THE DIVISION OF COAL RESEARCH, Commonwealth Scientific and Industrial Research Organization, North Ryde, New South Wales, Australia, to undertake fundamental research on the mechanism of catalysis of the reactions of carbon with oxidizing gases—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 480/428 (October 26).

CHAIR OF MORAL PHILOSOPHY—Secretary to the Curators of Patronage, University of Edinburgh, Old College, South Bridge, Edinburgh 8 (October 31).

CHAIR OF PHYSICAL GEOGRAPHY—The Registrar, University College of Wales, Aberystwyth (October 31).

DIRECTOR (with high academic qualifications and extensive experience of research and of the administration of research) OF THE TEA RESEARCH INSTITUTE OF CEYLON—The Chairman of the Tea Research Institute of Ceylon, "Lynwood", 50 Edinburgh Crescent, Colombo, Ceylon; and Dr. F. R. Tubbs, C.B.E., Director, East Malling Research Station, East Malling, Maidstone, Kent (November 11).

GENETICIST (with a good honours degree and a background of Drosophila genetics and biochemistry)—The Secretary, Poultry Research Centre, Agricultural Research Council, King's Buildings, West Mains Road, Edinburgh 9.

LECTURER (well-qualified physicist, with suitable research, industrial or teaching experience, and prepared to join in the development in teaching and research taking place in the Physics Department) IN PHYSICS—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

PART-TIME RESEARCH ASSISTANT (with an honours degree in botany) IN THE BIOLOGY DEPARTMENT, for an investigation of the anatomy of fruits and seeds—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

POST-DOCTORAL RESEARCH ASSISTANT (with a Ph.D. in physics or physical metallurgy), to work on a three-year D.S.I.R. sponsored project: superconducting properties of lead–bismuth alloys—Drs. R. L. Bell and H. W. King, Metallurgy Department, Imperial College of Science and Technology, London, S.W.7.

PRINCIPAL LECTURER (graduate of a British university or equivalent qualifications, and preferably experience in teaching heavy-current electrical engineering subjects) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire.

RESEARCH ASSISTANT (graduate with biological qualifications) IN THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY AND CANCER RESEARCH, for work on immunological investigations with emphasis on fluorescence microscopy—Dr. J. O. Laws, Department of Experimental Pathology and Cancer Research, School of Medicine, The University, Leeds 2.

RESEARCH ASSISTANT, to work on turbine blade vibration—Head of the Department of Mechanical Engineering, Battersea College of Technology, London, S.W.11.

SENIOR TECHNICIAN (with considerable experience in a botanical laboratory and in technical photography including photomicrography) IN THE DEPARTMENT OF CRYPTOGAMIC BOTANY—Prof. J. Colhoun, Department of Cryptogamic Botany, University of Manchester, Manchester 13.

TECHNICIAN (recent graduate) IN THE UNIT OF EXPERIMENTAL ECOLOGY, DEPARTMENT OF BOTANY, to assist in nutritional and climatic studies—The Bursar (B.71), The University, Sheffield 10.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Building Research Station Digest (Second Series), No. 36: Jointing with Mastics and Gaskets—1. Pp. 4. (London: H.M. Stationery Office, 1963.) 4*d*. [177

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Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1063-F: Geology of the Burdock Quadrangle, Fall River and Custer Counties, South Dakota. By Robert W. Schnabel. Pp. iv+191–215+plates 17–19. Bulletin 1089-C: Geology of the Willow Springs and Rosamond Quadrangles, California. By T. W. Dibblee, Jr. Pp. v+141–253+plates 10 and 11. Bulletin 1122-D: Geology of the Anlauf and Drain Quadrangles, Douglas and Lane Counties, Oregon. By Linn Hoover. Pp. iv+62+plates 1 and 2. Water-Supply Paper 1528: Geology and Ground-Water Resources of the Scottsville Area, Kentucky. By William B. Hopkins. Pp. vi+333+plates 1–3. Water-Supply Paper 1646: Ground-Water Geology of Grayson County, Texas. By E. T. Baker, Jr. Pp. v+61+plates 1–6. (Washington, D.C. Government Printing Office, 1963.) [127

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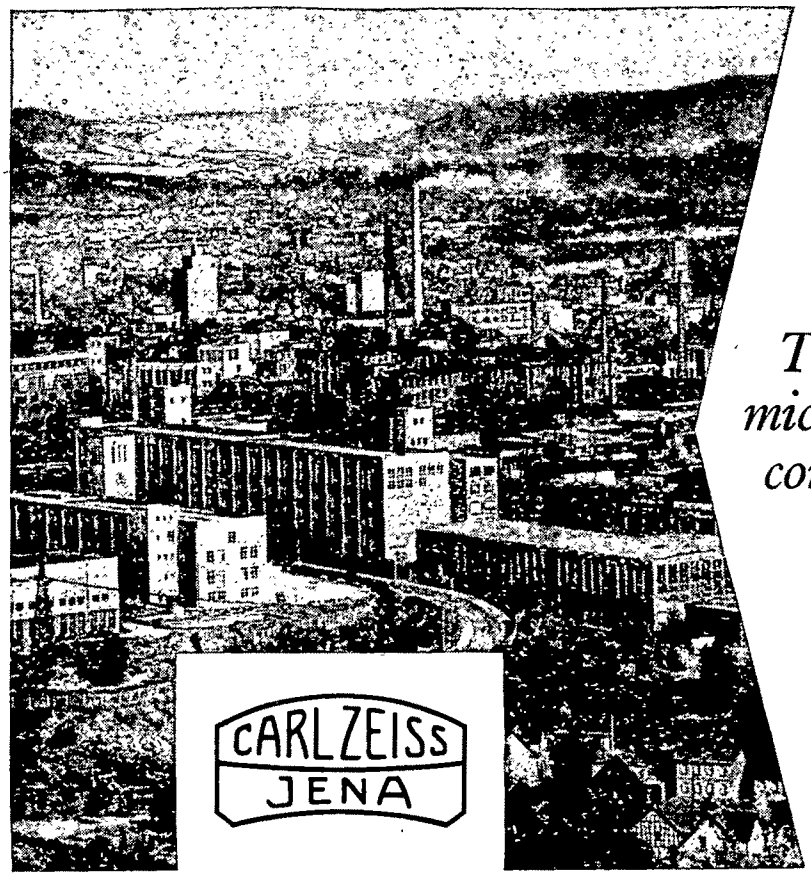
Bulletin of the Florida State Museum, Biological Sciences. Vol. 7, No. 2: Fossil Testudinine Turtles of Florida, Genera *Geochelone* and *Floridemys*. By Walter Auffenberg. Pp. 53–98. 60 cents. Vol. 7, No. 3: The Colubrid Snake Genus *Thamnophis*—a Revision of the Sauritus Group. By Douglas A. Rossman. Pp. 99–178. (Gainesville, Fla.: Florida State Museum, 1963.) [127

Smithsonian Institution, United States National Museum, Washington. Bulletin 233: Host Relations of the Parasitic Cowbirds. By Herbert Friedmann. Pp. ix+276. (Washington, D.C.: Government Printing Office, 1963.) 1.25 dollars. [127

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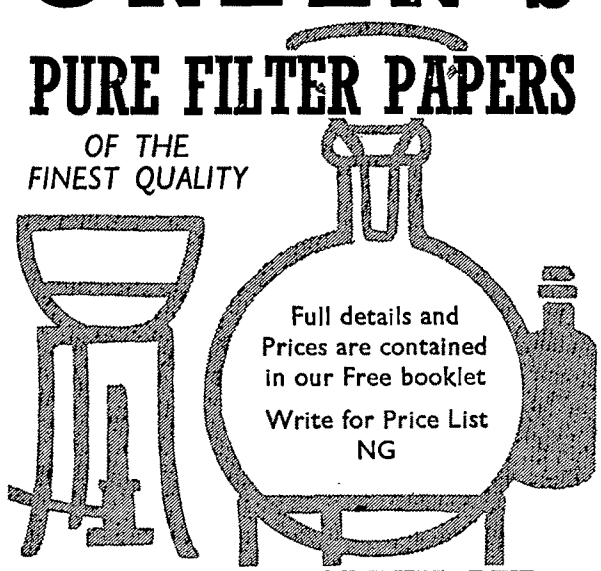
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APPOINTMENTS VACANT

UNIVERSITY OF EDINBURGH
RESEARCH FELLOWSHIP IN SEISMOLOGY

Applications are invited for a D.S.I.R. Research Fellowship in Seismology, tenable in the Department of Astronomy of the University of Edinburgh. Applicants should be prepared to undertake field and laboratory studies of seismic waves and to relate their observations to earth structure and tectonics. Advanced equipment is available for recording on magnetic tape and for high-speed playback in analogue or digital form. Qualifications must include Ph.D. or equivalent postgraduate experience in experimental geophysics. Experience in the maintenance or development of electronic equipment is desirable but not essential. The Fellowship will initially be available until April 1, 1965, with good prospects of extension. Salary in the range £900 to £2,000 per annum, according to qualifications and experience.

Applications, including the names of two referees, should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, by September 30, 1963.

PILKINGTON BROTHERS LIMITED
Glass Manufacturers, require a

PHYSICIST

at their Research Laboratories to carry out basic research work involving model studies of viscous and non-viscous flow and heat transfer problems.

A good honours degree in physics is essential, with some experience of university or industrial research.

The position is in a salary range which can rise to £2,000 per annum.

Please quote RES./J3 in applying to L. P. Laidlaw, Group Staff Department,

PILKINGTON BROTHERS LIMITED,
Grove Street, St. Helens, Lancashire.

UNIVERSITY OF MANCHESTER

Applications are invited for two posts of Lecturer in Radio-astronomy in the Department of Physics of the Faculty of Science. The successful applicants will be expected to pursue research at the Nuffield Radio-Astronomy Laboratories, Jodrell Bank, where the Mark I 250 ft. Radio Telescope is in operation and the Mark II instrument is under construction. A specialized knowledge of radio-astronomy is not essential and applicants with interests or experience in electronics, astronomy or astro-physics are encouraged to apply. Salary scale from £1,250 to £2,150 per annum; initial salary according to qualifications and experience. Duties to commence as soon as possible. Membership of the F.S.S.U. and Children's Allowance Scheme.

Applications should be sent not later than September 30, 1963, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained.

AGRICULTURAL RESEARCH COUNCIL

Geneticist with background of Drosophila genetics and biochemistry required for the staff of the Poultry Research Centre, Edinburgh. Good honours degree essential. Appointment as Scientific Officer in scale £791 to £1,309 according to experience. F.S.S.U. Applications, with names of three referees, to Secretary, Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh 9.

COMMONWEALTH OF AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF FISHERIES AND OCEANOGRAPHY

BIOLOGIST

Applications are invited for appointment to a position of Biologist to be stationed at the Organization's Regional Laboratory in Perth, Western Australia.

The Division is engaged in a programme of study of populations of sperm whales whilst continuing to make observations on humpback whales. The appointee will be expected to assume responsibility for these programmes with a view to making assessment of sperm whales and maintaining watch on the condition of the stocks of humpback whales. The applicant should be able to carry out studies into the biology of whales and to perform the computations required for stock assessments.

Applicants should preferably possess a Ph.D. degree in biology or have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability. Applicants should also have had training in modern methods of population research.

Salary: Dependent upon qualifications and experience within the ranges:
£A1,674 to £2,511 per annum (but not less than £A1,979 per annum) or
£A2,655 to £3,015 per annum.

Promotion within C.S.I.R.O. is by merit and may go beyond the upper limit of the scale within which the original appointment is made.

By mutual arrangement the appointment may be for an indefinite period or for a fixed term of three years.

Fares paid for the appointee and his dependent family and return fares to country of origin will be paid for an appointee who accepts appointment for a fixed term. Further particulars supplied on application to:

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 320/274) should be addressed by October 12, 1963.

Plant Breeding Assistant

There is a vacancy in the Horticulture Section of the Unilever Research Laboratory, Colworth House, for an assistant to the Plant Breeder to work on the production of new varieties of vegetables for processing. Applicants should possess an ordinary degree, or equivalent, in Botany, Agriculture or similar subjects, with some knowledge of genetics.

The Laboratory (total staff 750) offers attractive working conditions, and there are good prospects for advancement.

Please write for an application form to:
THE STAFF OFFICER (REF: FRR 240A),
UNILEVER RESEARCH LABORATORY,
COLWORTH HOUSE, SHARNBROOK, BEDFORD.

UNILEVER RESEARCH

MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY
(Faculty of Technology in the University of Manchester)

Applications are invited for a temporary post of Experimental Officer in the Department of Chemistry to carry out research and development work on the controlled oxidation of aromatic compounds and of more complex materials. The initial tenure will be for two years and there is the possibility that a suitable candidate would be assimilated into the permanent staff of the department in due course. Candidates should have distinct skill in experimental organic chemistry, and a sound knowledge of chemical theory. Salary range, etc., £880 by £40 to £1,200 per annum. Family allowances. Superannuation Scheme.

Conditions of appointment and forms of application may be obtained from the Registrar, The Manchester College of Science and Technology, Sackville Street, Manchester 1, to whom applications must be returned as soon as possible.

CITY OF LIVERPOOL EDUCATION COMMITTEE
COLLEGE OF TECHNOLOGY
Principal: S. A. J. Parsons, B.Sc.(Econ.), M.I.Mech.E., M.I.Prod.E., M.B.I.M.

Applications are invited for the following appointments. (1) Lecturer in Mathematics. Applicants should be prepared to teach to graduate level. They should have teaching and industrial or research experience, and be prepared to undertake research. (2) Lecturer in Physics. Applicants should be suitably qualified, able to teach up to degree and Diploma in Technology level and be prepared to undertake research. A special interest in Acoustics is desirable. Teaching and industrial or research experience is essential. Salaries: Lecturer: £1,670 by £45 to £1,895 per annum.

Application forms (returnable by September 23, 1963) and further particulars from Director of Education, 14 Sir Thomas Street, Liverpool, 1.

THOMAS ALKER,
Town Clerk.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF ANIMAL HEALTH

VETERINARY PARASITOLOGIST

Applications are invited for appointment to a position of a Veterinary Parasitologist with the Organization's McMaster Animal Health Laboratory, situated in the grounds of the University of Sydney, with which a close association is maintained.

The appointee will be required to participate in specific aspects of the programme of research concerned with the epidemiology and control of helminth infections of sheep.

Applicants should possess a degree in Veterinary Science, or its equivalent, together with a Ph.D. degree or research training of an equivalent standard and duration, and must present satisfactory evidence of postgraduate experience in a relevant field of parasitology.

SALARY : Dependent upon qualifications and experience within the range :

£A1,674 to £A2,511 per annum (but not less than £A1,979 per annum).

Salary for a woman will be £A188 per annum less than corresponding rates for men.

Promotion within C.S.I.R.O. is by merit and may go beyond the upper limit of the scale within which the original appointment is made.

Fares paid for the appointee and his dependent family. Further particulars supplied on application to :

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, W.C.2, London, to whom applications (quoting Appointment No. 202/200) should be addressed by October 12, 1963.

Soil Scientist
for research opportunity

COMINCO invites applications for a responsible position in its soil-fertilizer chemistry research programme.

Candidates should hold or soon obtain a Ph.D. degree in either Soil Chemistry, Soil Science, or in Soil Fertility and Plant Nutrition. Research experience in the fields of minor element soil-chemistry or minor elements in plant nutrition is desirable. Duties include developing an active research programme concerned with minor fertilizers; preparation of reports and publications; and maintaining liaison with external research organizations.

Interviews with qualifying applicants will be arranged

Please address inquiries to :

T. McEwan, Personnel Division,
The Consolidated Mining and Smelting Company of Canada Ltd.
Trail, B.C., Canada

THE UNIVERSITY OF LEEDS: SENIOR Technologist required in Medical Research Unit. Must have sound training and experience in electron microscopy (RCA, EMU 3, and Philips EM 100 available) and ultramicrotomy. Possession of science degree or diploma desirable but not essential. Salary between £900 and £1,650 per annum according to training and experience.—Applications and inquiries to Professor C. E. Lumsden, Department of Pathology, School of Medicine, Leeds, 2, not later than September 28.

HISTOLOGY. STUDENT OR JUNIOR Technician required at Toxicology Research Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey.—Apply, with particulars of education and experience, to the Director.

THE QUEEN'S UNIVERSITY OF BELFAST

DEPARTMENT OF BOTANY

Applications are invited for the post of Research Assistant in the Department of Botany, to commence October 1, 1963. The salary, depending on qualifications and experience, will be in the range £450 to £525. Applicants should be honours graduates in botany or biochemistry and should be interested in working towards a higher degree.

Applications, giving the name and address of a person to whom reference can be made, should be submitted to Professor Carr, Department of Botany, David Keir Building, Stranmillis Road, Belfast, 9, by September 21, 1963.

HORTICULTURE GRADUATES

required by

AN FORAS TALUNTAIS (The Agricultural Institute) for its Horticulture Division. Level of entry to Research Staff depending on qualifications and experience. ESSENTIAL—Honours Degree in Horticulture or equivalent. Marriage and children's allowances. Superannuation Scheme including F.S.S.U. arrangement. Applications also considered from 1963 Degree candidates.

Further particulars and application forms from Director, An Foras Taluntais, 33 Merrion Road, Dublin 4. Latest date for receipt of completed form—October 10, 1963.

BRISTOL COLLEGE OF SCIENCE AND TECHNOLOGY
ASHLEY DOWN, BRISTOL, 7

Department of Physics

The Governors invite applications for the post of :

READER IN SOLID STATE PHYSICS

Applicants should have a good honours degree in physics and appropriate research experience.

The Reader will be expected to play a leading part in the development of the research of the Department, and to pursue actively his own research in some aspect of solid state physics.

Salary (under review) within the range £2,250 to £2,700 per annum.

Please quote reference CST 63/47 (re-advertisement).

ALSO

PRINCIPAL LECTURER IN THE DEPARTMENT OF PHYSICS

Applicants should possess an Honours Degree in Physics or equivalent qualification, appropriate teaching and research experience and administrative ability. Industrial experience would be an advantage.

Salary within the range £2,150 to £2,375 per annum.

Please quote reference CST 63/67.

Further particulars of both posts and application forms from the Secretary and Registrar.

UNIVERSITY OF SYDNEY

TEACHING FELLOWSHIP IN ANIMAL NUTRITION

Applications are invited for the position of Teaching Fellow in Animal Nutrition in the Department of Animal Husbandry. Candidates should be graduates in Agricultural or Rural Science, Veterinary Science or Science. The successful applicant will be required to assist with practical work and lectures in Animal Nutrition and will be encouraged to register for a higher degree. Salary for a Teaching Fellow is within the range £A1,295 by £A60 to £A1,535 per annum, plus cost of living adjustments (at present £A10 per annum). The appointment will terminate on December 31, 1964, in the first instance but may be renewed on an annual basis. Salary will commence from the date of arrival in Sydney.

Applications giving details of qualifications and experience and names of two referees, should be forwarded to reach the Registrar, University of Sydney, Sydney, New South Wales, Australia (from whom further information may be obtained) not later than **October 28, 1963.** In addition, overseas candidates are requested to forward two copies of the application to the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

DIRECTOR OF ANIMAL NUTRITION

- This is a new appointment involving, amongst other responsibilities, the extension of existing facilities so as to provide a national farm advisory service appropriate to the Group's activities in animal feedingstuffs, together with the formulation of animal feeds, the co-ordination of feeding trials and nutritional research.
Applicants should be aged 40–50 and have obtained a position of eminence in the field of animal nutrition, coupled with practical administrative experience at a high level.
The appointment will involve building up an organization, with appropriate scientific backing, to provide the services required; the successful applicant will require considerable energy and determination, as well as the ability to work well with colleagues, to select staff, and to build and lead his own team.
This is a senior position in a large organization; an appropriate salary is envisaged and the appointment is pensionable. The appointment will be based in London or the South of England, but considerable travelling is required and a car will be provided.
- *Write briefly and in strict confidence, quoting Ref.* 14Q/63, *to:*

The Group Staff Manager,

Ranks Hovis McDougall Limited,

53, Eastcheap, London, E.C.3

The British Food Manufacturing Industries Research Association

has the following vacancies for Scientists of graduate standing:

PLANT BIOCHEMIST or BOTANIST

to undertake investigations into the fundamental and technological causes of some defects arising in fruit and vegetable products.

BACTERIOLOGIST

to work on the microbiology of bacon, with particular reference to the microbiological aspects of prepackaging in vacuum film wraps.

Both posts carry Life Assurance and Superannuation benefits. The salary will be commensurate with age and experience. Applications should be sent to the Director of Research, The British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

THE HYDRO-ELECTRIC COMMISSION, TASMANIA

GEOLOGIST **Salary £A1,474 to £A2,496 per annum.**

Duties will include regional mapping, logging and interpretation of drill cores and detailed investigations of dam sites, conduit routes, etc.

GEOPHYSICIST **Salary £A1,474 to £A2,496 per annum.**

Required to carry out geophysical surveys in connection with geological investigations and hydro-electric construction.

Applicants for either position must hold a degree in Science or Engineering with Geology as a major subject.

Headquarters—Hobart.

Travelling expenses payable and general information from the Agent General for Tasmania, 457 Strand, London, W.C.2, to whom applications, in duplicate, supported by copies of references or names of referees, should be addressed.

EAST AFRICAN COMMON SERVICES ORGANIZATION

Research Officer (Entomologist) required to plan and carry out research on the biology of malaria carrying mosquitoes in East Africa, and to direct field and laboratory work of an established entomological research team. Candidates must possess an honours degree in biology, with appropriate postgraduate experience, and be nationals of and permanently resident in Great Britain or the Republic of Ireland. Appointment on contract for one tour of two years with gratuity (taxable) of 25 per cent of emoluments on satisfactory completion of contract. Salary within the scale £2,172 to £2,808 a year. Free return passages for officer, wife and up to four dependent children. Education allowances. Mid-tour passages for children being educated in the United Kingdom. Furnished quarters available at low rental. Approximately four months home leave on full salary on satisfactory completion of contract. Local leave of sixteen days a tour also allowed.

Application forms from Director of Recruitment, Eland House, Stag Place, Victoria, London, S.W.1. (Please quote RC.318/145/02 and state full name.)

AUSTRALIA

UNIVERSITY OF NEW SOUTH WALES

invites applications for appointment to the positions of

LECTURER/SENIOR LECTURER
DEPARTMENT OF BOTANY
SCHOOL OF BIOLOGICAL SCIENCES

Salary: Lecturer, £A1,905 range £A2,605 per annum. Senior Lecturer, £A2,755 range £A3,255 per annum. Commencing salary according to qualifications and experience. Applicants must hold an honours degree with research qualifications in botany or a related field of biology, equivalent to the Ph.D. Qualifications in genetics (including cyto-genetics, biometrical genetics or fungal genetics) would be advantageous for one position, whilst experience in plant physiology (including growth analysis) and/or biochemistry would be desirable for the other position. The Department of Botany under Professor H. N. Barber, F.R.S., is housed in a new building together with the Departments of Biochemistry, Microbiology and Zoology. The School is equipped with advanced biochemical apparatus, glass houses, constant environment facilities, and field stations. The successful applicants will lecture at both undergraduate and postgraduate level and there will be opportunities for research.

Details of conditions of appointment, including superannuation, study leave, housing scheme and travel arrangements, may be obtained from the Agent-General for New South Wales, 56-57 Strand, London, W.C.2, with whom four copies of applications including the names of two referees should be lodged before October 30, 1963. A copy of the application should be forwarded by airmail to the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., to reach there before the above mentioned date.

BIRKENHEAD EDUCATION COMMITTEE

BIRKENHEAD TECHNICAL COLLEGE

Principal: C. V. Vinten Fenton, M.Sc., A.M.I.Mech.E., A.M.I.E.E.

Applications are invited for the undermentioned full-time teaching staff:

SCIENCE DEPARTMENT

Lecturer in Physical Chemistry.

Assistant Lecturer Grade "A"—Chemistry.

Salaries in accordance with the Burnham Technical Scales, 1961, as amended by the Remuneration of Teachers (F.E.) Order, 1963.

Further particulars and forms of application may be obtained from the undersigned, to whom completed applications should be returned by September 24, 1963.

H. GLYN WILKINSON,
Director of Education.

Education Offices,
63 Hamilton Square, Birkenhead.

KINGSTON UPON HULL EDUCATION COMMITTEE

COLLEGE OF TECHNOLOGY

Principal: Emlyn Jones, M.Sc., F.R.I.C.

Applications are invited for the post of Senior Lecturer in Theory of Machines and Machine Design to commence duty as soon as possible. Applicants must be honours graduates with wide industrial and teaching experience able to undertake research. Salary: Burnham Technical Scale, £1,895 by £55 (4) to £2,115 per annum.

Application forms and further particulars, to be returned within 14 days of the date of this issue, may be obtained from the Chief Education Officer, Guildhall, Kingston upon Hull.

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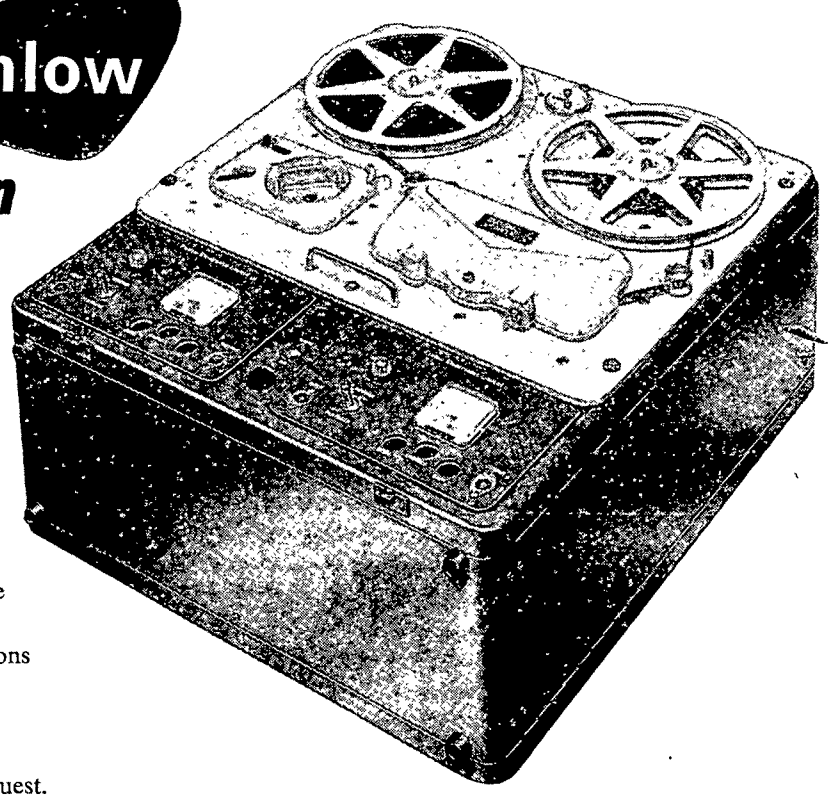
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NUCLEAR POWER POLITICS IN BRITAIN

IT is obvious from the figures for expenditure and man-power given in the ninth annual report of the United Kingdom Atomic Energy Authority* that the pre-occupation of the Authority is overwhelmingly with the reactor programme and the research on which any such development must be based. It is scarcely surprising that when the content and scale of Britain's nuclear power programme is being criticized the future position of the Authority should also be the subject of comment. Beyond a reference to the work on two possible reactor systems for the merchant marine field (*Vulcain* and the integral boiling reactor), on which attention was concentrated during the year, and to the probability that in the long term substantial quantities of enriched uranium will be required for the civil nuclear power programme, the report does not attempt to consider the future prospects for nuclear power. However, they were the subject of some discussion in a debate in the House of Lords on July 10, although that debate was largely concerned with the merits of a Government decision regarding a tender for the new Wylfa power station. The competitivity of nuclear power at January 1, 1963, however, is considered in a paper recently published by the Comitato Nazionale Energia Nucleare†, which takes the view that nuclear power is on the threshold of economic competitiveness. It does not accept entirely without question the statement that the advanced gas reactor in the United Kingdom could produce electric power at 0·45*d.*/kWh, since the statement is based on suppositions that are open to challenge. These include the distribution of overheads, design and construction costs over a programme calling for sufficient such stations to supply at least 6,000 MWh to cover the Central Electricity Generating Board's requirements by 1968.

By 1970, however, at least 10 per cent of Britain's electric power capacity will be represented by nuclear plant, at a cost for magnox gas-graphite reactors of 0·6*d.*/kWh, and lower still for the advanced gas reactor. It was argued by Lord Aldington in the House of Lords that, if the right methods of calculation of costs were used, nuclear power would prove broadly comparable with the best sources of conventional power during 1965–66; if the same output was programmed in future from each design of nuclear power station as for each design of conventional plant, the costs of nuclear power would prove substantially lower. Further, failure to calculate costs correctly had led to false ideas as to the competitiveness of nuclear power, which had discouraged the building of such stations. He believed that the earlier hopes held out for nuclear power were fully justified, but national policy on nuclear power should be guided throughout by a firm belief in its competitiveness and in its economics. It was not the technical efficiency of nuclear power plant or the capability of its designers or anything inherent in nuclear energy itself that appeared to keep its cost above those of conventional power.

The debate had opened on a motion of Lord Coleraine asking for a committee of enquiry into the present arrangements and prospects of the nuclear energy industry. He had been particularly disturbed at the break-up of teams of skilled designers and others under the present arrangements, and in this he was fully supported by Lord Aldington and Lord Stonham. Lord Aldington had also challenged the acceptance of a depreciation period of twenty years for nuclear plants as too pessimistic; although it was possibly justified in 1955, to-day it was out of date, and one of twenty-five or thirty years should be substituted. Lord Mills, however, pointed out that the Powell Committee, on which the Atomic Energy Authority, the Central Electricity Generating Board, the Ministry of Power, the Treasury and the Board of Trade were represented, was already examining the position. In view, too, of the comments of the Select Committee on Nationalized Industries on the electricity supply industry in a report published last May, he thought that all the facts were sufficiently well known, and that a radical review of the commercial thinking of the industry had already been put in train.

In replying for the Government on the debate, the First Lord of the Admiralty, Lord Carrington, began by sketching briefly the history of nuclear power programmes since the first orders for such power stations were placed in 1956. In March 1957, it was decided to adopt a much expanded construction programme amounting to the installation by the end of 1965 of 5,000–6,000 MW of nuclear generating capacity, in view of an expected serious shortage of fuels. By October of that year, however, it was decided to stretch the programme over a longer period and to reduce the rate of installations. The four industrial Consortia formed in 1954 also obtained two overseas contracts, in Italy and in Japan. When the strain on Britain's coal supplies had eased and prospects for oil supplies improved in October 1960, the Government again revised the programme and it was decided to order nuclear power stations at the rate of about one a year. In this situation, and in the absence of further export orders, the Consortia re-grouped themselves into three organizations.

Lord Carrington emphasized that the nuclear power policy was part of the wider energy policy, and pointed out that for 1960–68 the programme concentrated on the development of nuclear technology and construction capacity against the needs of many years hence. The scale and pattern of the nuclear programme after 1968 was the subject of a more detailed study of the economic and technical factors and prospects, which was put in hand last year in co-operation with the electricity supply industry and the Atomic Energy Authority. Basic research and development of reactor systems were the responsibility of the Atomic Energy Authority, which had a statutory responsibility to develop nuclear energy. However, neither Lord Carrington nor the Minister for Science, to whom the Authority is responsible, when he spoke later, said anything to remove the current uneasiness as to the continuing magnitude of the Authority's effort or its relation to the civil research and development effort generally. Lord Carrington did, however, point out that the Authority's relations with the Consortia covered more than the present constructional programme, and associated them from the beginning in new designs of reactors developed by the Authority. As to costs, he

* United Kingdom Atomic Energy Authority. Ninth Annual Report for the period 1st April, 1962–31st March, 1963. Pp. ix + 87 + 7 plates. (London: H.M.S.O., 1963.) 6*s.* 6*d* net. (See also p. 1141 of this issue of *Nature*.)

† Competitivity of Nuclear Energy at 1 January, 1963. Pp. 25. (Rome: Comitato Nazionale Energia Nucleare, 1963.)

pointed out that these depended on whether more or less favourable assumptions were adopted regarding various factors, operators of such stations tending to take the more conservative, and organizations responsible for research and development, the more optimistic assumptions.

Lord Carrington vigorously repudiated the suggestion that the Generating Board and not the Government was the arbiter of the nuclear power programme, but he held out no hope of an early decision by the Government which would make a final apportionment of costs between the taxpayer and the consumer in the development of nuclear power. Without public funds, however, it was unlikely that development of nuclear power would have occurred at all. Lord Hailsham affirmed his complete confidence in the future of nuclear power, but fully accepted the need for an enquiry into the economics of nuclear power. He did not disclose, however, the terms of reference of the Powell Committee, but indicated that, broadly speaking, it was designed to reconcile the differences of view which might legitimately be held about the economics of nuclear and of conventional power, and, to some extent, about the economics of particular types of reactor.

Accordingly, Lord Hailsham suggested that judgment on these issues should be postponed until the Powell Committee had reported on the general situation. Dounreay, he admitted, would have a very marked bearing on the economics of nuclear power in the future, both because of the high hopes of the breeder factor in the fast reactor, and also because of the possibility of the reactor burning plutonium. That, however, lay in the future. He also declined at that stage to commit the Government to overriding any decision of the Central Electricity Generating Board affecting the future of the Consortia but agreed that any suitable representation should be considered.

Lord Hailsham was apologetic for his slender contribution, which certainly seemed to emphasize the excessive extent of his responsibilities outside those of science and technology, particularly in view of the rarity of debates on nuclear power in the House of Lords. Concern regarding the future of the Atomic Energy Authority may well be unjustified. The extent of the resources which Britain can commit to this field, however, requires continued appraisal in the light of her whole research and development effort. The Authority's report is not a discouraging document, however; but it is disappointing to find that in such a debate the responsible Minister could neither comment on the report of the Atomic Energy Authority itself nor on the importance of that effort in civil research and development as a whole.

APPROACHES TO ATOMIC PHYSICS

THE Vanuxem Lectures delivered by Prof. C. N. Yang in Princeton University during 1959 have recently formed the basis of a book*, the essential purpose of which is to give an account of the historical development of our ideas of the fundamental constitution of matter for an audience of educated people with an interest in science but not technically expert in the subject. This it does by a lucid description of the most crucial experiments and new conceptions, illustrated by diagrams and photographs. The topics discussed include the discovery of the electron and of the nuclear atom; the development of quantum mechanics; the discovery of the neutron, the mesons and hyperons; and the idea of parity with the demonstration of its non-conservation in weak interactions.

Prof. Yang emphasizes the important part in the progress of discovery played by technical innovation, and the essential contribution made by the interplay between theory and experiment. The work is enriched by a number of quotations from eminent physicists bearing on the main theme.

Such a book, written by a mature and competent authority, is a valuable contribution to the wider dissemination of scientific culture; but it also serves a second purpose scarcely less important. There is a growing tendency in teaching physics to abandon the traditional treatment which roughly follows the historical development of the subject. In the interests of economy of time and simplicity of presentation, it is being replaced by a logical treatment in which the historical sequence is ignored. There is much to be said for such an approach when the aim is to give instruction to students of other disciplines for whom a knowledge of physics is an essential piece of technical equipment; or when the content of an honours course in physics has become so formidable that there are advantages in removing all material which appears not strictly essential. But for those who are going to be serious investigators in physics, such an unhistorical approach sacrifices much of importance, and books like Prof. Yang's do something to restore a balance.

There are two serious deficiencies in a strictly utilitarian and unhistoric syllabus. In the first place, it can lead to graduates who are well instructed and soundly indoctrinated, but who have no acquaintance with the spirit of enquiry and how discoveries are made. Discovery to them appears as a kind of magic, the result of the sudden insight of a man of genius. There is as much to be learned about the nature of discovery from a knowledge of those experiments which failed as from those which succeeded. To stress only the latter gives little insight into the great and exciting human activity which we call research.

It is probable that most university students in physics are shown J. J. Thomson's experiment on the measurement of the ratio of the charge to the mass of the electron; but how many are told of Hertz's earlier experiments of a similar nature, to which Prof. Yang refers, and which failed to show any deflexion so that it was concluded that the cathode-rays are uncharged ? Or of the assumptions made earlier by Schuster which led him to interpret similar experiments as indicating that the cathode-rays are charged atoms ?

A familiarity with the main lines of development of a scientific discipline is not to be seen as a kind of embellishment of knowledge, but as an essential part of a mature understanding. It can be especially important in providing some sense of direction in those difficult decisions which are sometimes involved in deciding general lines of policy for a whole future period of investigation. History, including the history of science, is not 'bunk', even although it never provides ready-made solutions of present problems which allow us to escape the exercise of judgment.

* *Elementary Particles: a Short History of some Discoveries in Atomic Physics. (The Vanuxem Lectures, 1959.)* By Dr. Chen Ning Yang. Pp. 68. (Princeton, N.J.: Princeton Univ. Press; London: Oxford Univ. Press, 1961.) 22*s.* net.

At the present time elementary particle physics presents us with just such difficult decisions. For the sixty years which Prof. Yang brings under review there has been a ceaseless flow of discovery to which Europe has made a unique contribution, and to-day the subject appears to be full of life and vitality. But in the next few years we shall have to decide whether to build a great new accelerator for the technical equipment of Europe in ten or twenty years time. The cost of a proton-synchrotron for an energy of about 300 GeV is in the range of £150 million. Are we justified in committing such large resources when it seems unlikely that we can hope for any early return in the form of practical applications such as those which followed from the advances in nuclear physics which culminated with the discovery of the fission process ? When such a machine comes into operation, in ten or fifteen years time, what will be the state of the subject ? Moreover, in particular, will it still be attracting a large fraction of the most gifted of the youth of the world who devote themselves to science ? Will it perhaps be superseded before it comes into operation through unexpected technological innovations which will allow the production of high-energy beams of protons, in high intensity, at a greatly reduced cost ?

It seems very unlikely that we shall be able to find clear-cut and well-founded answers to all these problems before it is necessary to make a decision. Some scientists are therefore opposed to a new generation of accelerators for Europe, and are willing to accept the consequence that, in this field, Western Europe would decline into an entirely secondary position. It has even been said, if with irony, that the only consequence of Rutherford's discoveries is that the present cost of electricity in Britain is more than it would otherwise have been.

The proponents of such a view would get much benefit from reading Prof. Yang's book. He quotes a passage from the 1930 Faraday Lecture in which Bohr speaks of the new prospects for the whole of physical and chemical science opened up by the discovery of the atomic nucleus. The main fruits of the great developments of nuclear physics in the first half of the twentieth century are not so much the technological triumphs in nuclear energy, but rather that our whole concept of matter has by them been radically transformed so that all our science and technology are infused with the ideas which were born in that epoch.

Our present position is not so clear as in 1912 when the consequences for many sciences of the discovery of the nuclear atom could be enthusiastically discussed, but it is a reasonable speculation in the light of the history of the subject that a great increase in our understanding of Nature will indeed follow from the advances in elementary particle physics in the next two or three decades. The subject is advancing with explosive rapidity; there are many profound questions which we can ask and for which at the moment we see neither the solution nor even a serious approach. But, as Prof. Yang remarks, in discussing recent progress in our new understanding of the remarkable symmetries which Nature displays, "one learns to hope that Nature possesses an order that one may aspire to comprehend". All our history suggests that a great development in our understanding will again in the long run infuse all our thinking, with profound consequences for philosophy and practice.

Confidence that Nature is indeed an ordered and logical system which man can therefore aspire to understand was of crucial importance in the development of modern science. The notion is embodied in the idea of the 'Laws of Nature'—a phrase which gained widespread currency only quite late in the Renaissance and which evidently owes much to theological ideas; as Needham remarks†, "Just as earthly imperial law-givers enacted codes of positive law to be obeyed by men, so also the celestial and supreme rational creative deity has laid down a series of laws which must be obeyed by minerals, crystals, plants, animals and the stars in their courses". Believing the world to be an ordered system, men were encouraged to attempt to unravel it. This was an idea unacceptable to classical Chinese thought, and it is suggested by Needham that its disposition to regard Nature as inscrutable, as too complex to be unravelled is one reason why Chinese science made little headway in a period when Western science was making spectacular advances. In this sense, Chinese thought at this period was too subtle; it was too impressed by the immense complexity of phenomena so that in Needham's phrase, "it was groping after an Einsteinian world-picture without having laid the foundations for a Newtonian one".

Our present belief that there is a great future field of discovery in elementary particle physics, crucial for our decision as to what resources should be devoted to it, is again an expression of our confidence of order in Nature. We know, for example, of four principal forces, gravitational, electro-magnetic, nuclear, and the weak forces responsible for such processes as the β-decay. At the present time they appear entirely unrelated; there is no serious approach to a unified field theory embracing them all. But we are confident that one day the relations between them will be established and that our present ignorance is a guarantee that rich fields of discovery lie ahead.

Or again, the facts about the elementary particles and their excited states or resonances appear at the moment as an empirical body of knowledge and their deeper significance escapes us. But we believe that there must be an underlying order which we can discover, just as was the case with the excited states of atoms. No doubt the order is of a different kind from that which prevails in atomic systems; but our problems ought not to appear more forbidding to us than did the task of unravelling atomic spectra to physicists a hundred years ago.

Against this background, Prof. Yang's remark is particularly interesting. It is remarkable that two of the most subtle and profound contributions to elementary particle physics in the past thirty years, by Yukawa, and by Lee and Yang, respectively, have been made by representatives of oriental cultures who have made themselves masters of Western scientific thought. It shows the great benefits to science which have followed from its international dissemination. This has ensured that the representatives of different cultures, who cannot but be deeply influenced by the particular climate of ideas in which they have been fostered, have been enabled to contribute to its development. And it is a promise for the future when this process will have progressed even further.

Prof. Yang's book serves not only as a valuable contribution to the dissemination of scientific culture but also to emphasize the place of the history of science in our thinking, and we should feel most indebted to him that he found the time to write it.

† "Human Laws and the Laws of Nature", by Dr. J. Needham. A lecture delivered at Hatfield College of Technology in April 1961.

INTRACELLULAR 'FREE' AMINO-ACIDS

Amino Acid Pools

Distribution, Formation and Function of Free Amino Acids: Proceedings of a Symposium on Free Amino Acids held at the City of Hope Medical Center, Duarte, California, May 1961. Pp. xi+815. Edited by Joseph T. Holden. (Amsterdam, London, New York: Elsevier Publishing Company, 1962.) 120*s*.

IT is now a commonplace of biochemistry that treatment of living cells with boiling water, detergent solutions, alcohols or mild acids in the cold, etc., releases mixtures of free amino-acids from within the cells. Therefore the cells contain amino-acids either in the free state or in some form from which they are readily released in the free state. Such intracellular amino-acids constitute the 'amino-acid pool' of the cell, and pools have now been described in cells ranging from micro-organisms to mammalian, plant and insect tissues. This volume is the first comprehensive collection of data and theories about the nature, distribution, function and variation of amino-acid pools, and presents an expanded account of the proceedings of a symposium held at the City of Hope Medical Center, Duarte, California, in 1961. The volume is edited by J. T. Holden, who was chairman of the Organizing Committee and contributes two major articles on amino-acid pools in micro-organisms. The contributions are set out in detail with many illustrations and accompanied by edited accounts of discussions which followed presentation of the papers at the meeting. Originally the title of the symposium included the term 'free amino-acids', but, since some of the participants doubted whether intracellular amino-acids are truly free, the title of the book has been restricted to the non-committal *Amino Acid Pools*. More than seventy authors contribute to the book, and it would be invidious to single out individuals for specific mention.

Amino-acid pools are relatively easy to study (perhaps deceptively so) and, when quantitative data can be obtained, provide an indication of changes in the metabolic state of the cells. It is essential to establish accurate analytical methods for the extraction, identification and estimation of the amino-acids and to relate the results to the pools in the cells before extraction. When extracts are subjected to examination by paper chromatography, questions arise concerning the nature of unknowns as well as the proper identification of probably knowns. Appropriately, therefore, the volume begins with discussion of analytical techniques, accuracy of identification, production of artefacts and recognition of errors. The first part of the book then contains some thirty papers describing the amino-acids found in pools extracted from plants, micro-organisms, insects and vertebrates and includes six papers on amino-acids of the blood of man and animals in various normal and pathological states and after treatment with drugs. Participants were "encouraged to submit as much chromatographic evidence as they felt was required to document their conclusions". The result is that there are many pages of photographs of chromatograms (a rough count gives 900) which add more to the weight (nearly 4 lb.) of the book than to its scientific value. There is a mass of factual material in these 500 pages which provides a fund of information for anyone contemplating this type of investigation. The particular problems arising from extraction of specific tissues provide many valuable contributions to the methodology of pool investigations.

The second part of the volume contains nineteen contributions on dynamic aspects of amino-acid pools and sets out more fundamental attempts to elucidate the properties and functions of internal free amino-acids. Amino-acid pools can accumulate as a result of endosynthesis or transport of preformed amino-acids from the external medium, and both phenomena raise problems of membrane permeability and transport processes. Various theories of membrane passage are discussed by a number of speakers and the nature, localization and metabolic character of the pool debated. The relationship of free amino-acids to protein metabolism is clearly of importance, and the last group of papers in the symposium deals with protein turnover and its control, the role of free amino-acids as obligatory intermediates in protein synthesis, and the metabolic significance of lipo-amino-acid complexes. The book is completed by a report of a round-table discussion on the state of the intracellular amino-acids.

Protein synthesis occurs much more efficiently in cells than in test-tubes because the cell interior is a highly controlled and integrated metabolic system. The free amino-acid pool provides a delicate measure of the balance of the metabolic processes forming this system and its study should throw much light on cell physiology. This symposium is invaluable for anyone entering the field of amino-acid metabolism in any living tissue and provides, within the compass of one volume, an assembly of information nowhere else available. E. F. Gale

CARBOHYDRATE CHEMISTRY AND BIOCHEMISTRY

Advances in Carbohydrate Chemistry

Vol. 17. Edited by Melville L. Wolfrom. Pp. xi+503. (New York and London: Academic Press, 1962.) 114*s*. 6*d*.

VOLUME 17 of this now venerable series contains rather fewer, but on average longer, articles than its predecessors. There are reviews of higher carbon sugars (J. M. Webber), sugars of the cardiac glycosides (T. Reichstein and E. Weiss), oligosaccharides (R. W. Bailey and J. B. Pridham), platinum-catalysed oxidations (K. Heyns and H. Paulsen), dicarbonyl carbohydrates (O. Theander), purine nucleosides (J. A. Montgomery and H. J. Thomas), and enzymatic synthesis and degradation of starch and glycogen (D. J. Manners). Most of the articles bring earlier reviews up to date, as well as covering new ground; the chapter on oligosaccharides provides the first comprehensive compilation of these sugars in this series. About 200 are listed, and a measure of the rapidity of development in this field is that an unpublished list prepared by one of the authors now totals about 350. The volume also contains a review of the late H. O. L. Fischer's life and work, by J. C. Sowden.

Publications of this type do not warrant the usual type of review. Each article is a survey by an expert in the particular field, and as such is warmly welcomed by carbohydrate chemists in general. The articles are of a style that dates very little and because, when it is possible, they contain compilations of compounds with their properties, they have come to be the major source of reference in the field. Anyone with serious pretensions to be a carbohydrate chemist would do well sooner or later to spend the £70 required to purchase the complete series. The editors, M. L. Wolfrom, now editing his fifteenth volume, and R. S. Tipson, his ninth, deserve the largest share of the credit, for their zeal in enlisting contributors, in ensuring that all aspects of the subject are eventually covered, and in imposing their own high standards to ensure clarity and attractiveness of presentation. This laudable drive for high standards of presentation has, however, led them in the preface to describe as "deplorable" the "babel of initials employed for the designation of many of the starch hydrolases, in contrast to the more-meaningful names utilized for the glycogen hydrolases". In truth only two starch hydrolases, *R*-enzyme and *Z*-enzyme have been so designated. Others, which are not hydrases, also carry this type of name, namely, *D*-, *P*

Q-and *T*-enzymes. These names were deliberately initiated by Prof. Stanley Peat and the late Sir Norman Haworth to apply to enzyme preparations the precise function and purity of which it was thought unwise to speculate on by coining a specific name. The names were always intended to be replaced by explicit nomenclature when the purity and, particularly, the function of the enzyme preparations had been clearly characterized. The course of events has shown the wisdom of the use of 'initial' names. Thus *R*-enzyme has proved to be a mixture of two enzymes performing chemically identical functions, but on different substrates[1]; *Z*-enzyme proved ultimately to be identical with a well-known enzyme, α-amylase[2]. In the glycogen field, by contrast, one name for an enzyme has survived only because it became abbreviated to an 'initial' name, that is, *PR* enzyme. Speculation on the nature of its action has led it variously to be called *p*rosthetic-group *r*emoving enzyme, *p*rotein-*r*upturing enzyme and *p*hosphate-*r*emoving enzyme[1]. Similarly a glycogen hydrolase preparation, amylo-1, 6-glucosidase, was recently reported in *Nature* to depend for its action on two enzymes, a hydrolase and a transferase[3]. The best-intentioned of names "gang aft a-gley", and a policy of caution will impose less of a burden on the literature than one which provides names that later prove not to mean what they say. To me this policy of caution provides the most prudent way of tempering the advice attributed to one of the first editors of this *Advances* series, the late C. S. Hudson: "If you can't name it, don't make it!"

W. J. WHELAN

[1] References to the original literature are given in a review by Whelan, W. J., *Nature*, **190**, 954 (1961).
[2] Manners, D. J., *Adv. Carbohydr. Chem.*, **17**, 371 (1962).
[3] Abdullah, M., and Whelan, W. J., *Nature*, **197**, 979 (1963). Brown, D. H., Illingworth, B., and Cori, C. F., *ibid.*, **197**, 980 (1963).

SPACE DYNAMICS

Space Flight

Vol. 2: Dynamics. By Krafft A. Ehricke. (Principles of Guided Missile Design.) Pp. xxi+1,210. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1962.) 230*s*.

THIS massive text-book could have been published as six 200-page monographs, each an important and pioneering contribution to the dynamics of space flight. Most authors would be well content to have written one of these six contributions. But Mr. Ehricke is cast in the heroic mould, and has accomplished single-handed what would normally occupy a large team of writers, each with a different point of view and style.

The first section of the book (Chapters 1–2) covers the dynamics of sub-orbital ballistic flight (including orbit-to-ground trajectories) and satellite orbits, including ascent paths and perturbations. It is a valuable feature of this and other sections of the book that Mr. Ehricke keeps the mathematics as elementary as possible, is clear in his exposition and gives a wealth of illustrations (more than 300 in all) in the form of diagrams and graphs of close grid: consequently, his book should be readily intelligible not only to mathematicians but also to physicists and engineers. Chapter 2 is, however, the least satisfactory part of the book because it is rather outdated: the discussions of the effects of the Earth's gravitational field and atmosphere seem to have been written about 1958 and, despite the addition of more recent titbits, are defective.

Chapter 3 deals with transfer between orbits about the same centre of attraction, from the solved problem of transfer between circular coplanar orbits to the recalcitrant one of transfer between non-coplanar ellipses. Mr. Ehricke's bold and pragmatic attack on the problem, using elementary concepts, is in refreshing contrast to the usual calculus-of-variations approach, which consists in writing down innumerable insoluble equations. Mr. Ehricke can perhaps be criticized for not making it clear that the rules-of-thumb he propounds do not always provide the answers; but it is far better to have some rules than none. He completes the subject in Chapter 4, which covers transfers between one central force field and another, and is largely concerned with hyperbolic orbits.

The next section (Chapters 5 and 6) discusses powered ascent paths and their optimization, taking into account thrust, lift, drag and the influence of gravity.

Chapter 7 is on low-thrust flight, which is important because in future interplanetary journeys the power unit is likely to be an ion or plasma rocket, with a much higher efficiency than a chemical rocket but a much lower thrust. This chapter runs to 233 pages and could stand as a book on its own, for it treats the subject more thoroughly than existing texts. Chapter 8 discusses lunar flights, including Earth–Moon flight paths, lunar circumnavigation, impact and landing, and lunar satellites. Again it is an impressively thorough survey, illuminated by much original work. The book ends with a long and systematic treatment of the dynamics of interplanetary flight, covering practical aspects of flights to the various planets—the best departure times for flights, the most convenient orbits, etc.

The book is excellently printed on good-quality paper, includes examples at the ends of chapters and lists of references (which are, however, almost all pre-1960). There is some vacillation about units of length, with nautical miles, feet and kilometres all making an appearance, but this is a minor defect. Misprints are very rare, and it is unfortunate that one of them affects the date given for the launching of *Sputnik* 1, which is in error by a year.

This book will remain as one of the enduring monuments of astronautics when most of the multi-million-dollar satellite launchings have been forgotten, and any organization which spends large sums on space activities will find it difficult to refute the conclusion that a copy should be bought for every appropriate member of its staff.

D. G. KING-HELE

THE EARTH SCIENCES

Mining for Metals in Wales

By F. J. North. Pp. viii+112+8 plates. (Cardiff: National Museum of Wales, 1962.) 12*s*. 6*d*.

The System of Mineralogy

By James Dwight Dana and Edward Salisbury Dana. Vol. 3: Silica Minerals. Seventh edition entirely rewritten and greatly enlarged by Prof. Clifford Frondel. Pp. xii+334. (New York and London: John Wiley and Sons, Inc., 1962.) 60*s*.

Volcanoes and their Activity

By A. Rittmann. Translated from the Second German Edition by E. A. Vincent. Pp. xiv+305. (New York and London: Interscience Publishers, a division of John Wiley and Sons, Inc., 1962.) 90*s*.

Introduction to Physical Geology

By Prof. Chester R. Longwell and Prof. Richard F. Flint. Second edition. Pp. 504. (New York and London: John Wiley and Sons, Inc., 1962.) 60*s*.

DR. NORTH'S *Mining for Metals in Wales* contains an account of the history of metalliferous mining in the Principality; together with a description of the geological mode of occurrence of the many varieties of ore that have been worked there. Metalliferous mining has now almost ceased in Wales, either for economic reasons, or through exhaustion of the sources. Attempts to revive the mining of lead and zinc were made a few years ago,

but none is now produced. The only mine still working is one in Glamorganshire, which produces iron ore (hæmatite). Yet, at one time or another in the past, gold, silver, copper, lead, zinc, iron and manganese ores were extracted in considerable quantities; and for a year or two there was even a small output of cobalt oxide. Indeed, the famous Parys Mountain mine in Anglesey is said to have been the largest producer of copper in Europe in the mid-eighteenth century; and the history of the mining of argentiferous lead ore goes back to Roman times. With the passage of time, mining techniques, too, have changed considerably, and Dr. North has something to say on this aspect of his subject. He has, in fact, condensed a very great deal of information drawn from many sources, often not easily accessible, into a short but very readable book with a number of attractive illustrations. The bibliography, which lists 164 references, is particularly valuable. Although the book has been produced by the National Museum of Wales primarily as a museum guide, for its newly established Department of Industries, it is, nevertheless, an important, authoritative, and much-needed contribution to the history of mining in Great Britain.

In 1915 a decision was made in the United States to prepare a completely new, enlarged and thoroughly revised (seventh) edition of Dana's classic *System of Mineralogy*. This was a major undertaking on which work has been proceeding ever since. Volume 1 of this edition, covering the elements, sulphides, sulpho-salts and oxides, was published in 1944; and Volume 2, which included the halides, carbonates, phosphates and other salts, in 1951. Volume 3, on the silica minerals, is the latest to appear. It has been written by Prof. C. Frondel of Harvard, who was a co-editor of the earlier volumes. It describes the properties and modes of occurrence of all the known polymorphs of silica, not only specific forms such as quartz, tridymite, cristobalite and more recently discovered species such as coesite and stishovite, but also the less well-defined varieties such as chalcedony, agate, opal, jasper, flint and so on. The text is well illustrated by line and half-tone illustrations and, as in earlier editions, much interesting historical matter has been included. This latest addition to the new 'Dana' will doubtless be welcomed by students and research workers in mineralogy and geology and by crystallographers and mineral technologists. Two further volumes are stated to be in the course of preparation, to complete the work. These will deal exclusively with the silicate minerals.

Volcanoes and their Activity is a translation of the second, revised and enlarged edition of Prof. A. Rittmann's *Vulkane und ihre Tätigkeit* (1960), which has been made by Prof. E. A. Vincent, now professor of geology at the University of Manchester. The book covers a wide field. The earlier chapters discuss volcanic activity in general, the products of volcanoes, and their structure and geographical distribution. In succeeding chapters the author discusses the physicochemical properties of magmas, the nature of magma chambers, and the mechanism of volcanic eruptions. In the last two chapters he elaborates a geological theory embracing vulcanicity, mountain building, magmatism, the Earth's interior, and the origin and development of the Earth. The theoretical views put forward in the latter part of the book are personal to the author and, admittedly, controversial. For this reason he states that his book should not be regarded as a text-book. Prof. Rittmann is, however, generally acknowledged as an authority on his subject. His book summarizes the results of many years of observation in the field; and it is on this work that his theoretical conclusions are based. His book, therefore, should be welcomed by all students of igneous geology, and volcanology in particular, especially because there is no equivalent book in the English language. The numerous figures illustrating the text add to its interest, many being reproductions from actual photographs of volcanoes and their products.

The second edition of Longwell and Flint's *Introduction to Physical Geology* is a thoroughly revised, enlarged, and largely re-written version of the original edition of 1955. A feature of this elementary text-book is its richness in line and half-tone illustrations drawn from many sources, including air photographs. Their number has been substantially increased, thereby adding to the value of the book for teaching purposes. Other changes include the introduction into the text of definitions of geological terms whenever they are first used, rather than in a separate glossary; and the addition of a brief summary of contents at the end of each chapter. The book should succeed very well in its main object; but the series of appendixes on the identification of common rocks and minerals, and on the properties of the latter, and on the use of maps and map symbols, are so short that their value is questionable.

ULTRA-PURIFICATION OF SEMICONDUCTOR MATERIALS

Ultrapurification of Semiconductor Materials: Proceedings of the Conference, Boston, Massachusetts, April 11–13, 1961. Edited by M. S. Brooks and J. K. Kennedy. Pp. 655. (New York and London: The Macmillan Company New York, a Division of The Crowell-Collier Publishing Company, 1962.) 12.50 dollars; 94*s*.

THIS volume is a report of the Boston conference held in April 1961 and contains forty-nine papers. The title is perhaps a little misleading, in that many of the materials considered are not themselves semiconductors, but are used in the preparation of semiconductors or semiconductor devices. The papers are divided into three groups dealing with chemical preparation and purification analysis, and the physical methods of preparation. The first group of papers contains information on the purification of silicon, boron, bismuth, tellurium, gallium, rare earths, indium, antimony, and arsenic. It begins with an excellent review paper by Fuller of Bell Telephone Laboratories detailing progress so far on the purification of elements and compounds. The paper also discusses analytical methods for trace impurities and contains a most useful bibliography of fifty-one papers. The first three papers of the group deal with the purification of silicon from silicon tetraiodide and silicon hydride. The fourth paper deals with the preparation of boron from boron trichloride. A paper on organometallic compounds in the preparation of high purity metals is very complete and has 151 references. The last two papers in the section deal with chromatographic methods for purification, the starting point being organometallic compounds.

The second group of papers starts with a review by G. H. Morrison dealing with activation analysis, emission spectroscopy, and mass spectra. In this section there are eight papers dealing with emission and mass spectroscopy to detect impurities in elements and compounds, mainly of the III/V class. One is left with the impression that when materials are pure enough to be interesting as semiconductors these analytical techniques are already stretched to limit of sensitivity.

The third group of papers, which deals with physical methods of preparation, starts with a section on zone-purification processes, mainly from the point of view of the physics of the zone-refining process. The second section on crystal growing, deals mainly with silicon and III/V compounds, while the final part contains miscellaneous papers mostly concerned with semiconductor materials.

This book will be most useful to the physicist or chemist working in the field of material preparation, both semiconductor and other related materials. The bibliographies attached to many papers are in themselves most valuable.

A. A. Shepherd

COMMONWEALTH UNIVERSITIES CONGRESS, 1963

By Sir JAMES COOK, F.R.S.
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J. S. FULTON
Vice-Chancellor of the University of Sussex
AND
Dr. D. G. JAMES
Vice-Chancellor of the University of Southampton

THE Ninth Quinquennial Congress of the Universities of the Commonwealth was held in London during July 15–19. The first Congress of the Universities of the Empire had been convened in 1912 by the University of London, and at that Congress a decision was taken to establish a bureau of information for the Universities of the Empire. The office of the Universities Bureau of the British Empire was opened in London the following year. In 1948 the name was changed to the Association of Universities of the British Commonwealth, which was incorporated under the Companies Act. A major function of the Association has been the organization of further congresses which the Congress of 1912 recommended should be held at five-yearly intervals. Congresses were, in fact, held in 1921, 1926, 1931, 1936, 1948, 1953 and 1958.

It was with such circumstances in mind that London was chosen as the venue of the recent Congress, a gala occasion which marked the jubilee of the Association. It was more than fitting that the University of London played the part of host and did so very worthily. The president of the Congress was the chancellor of the University, H.M. Queen Elizabeth the Queen Mother, and the deputy president was the vice-chancellor, Dr. P. S. Noble. The chairman of the Association in its jubilee year was the indefatigable principal of the University of London, Sir Douglas Logan. To him is due in no small measure credit for the outstanding success which marked every phase of the Congress.

Shortly before the Congress, H.M. The Queen had approved the grant of a Royal Charter to the Association, to be known henceforth as the Association of Commonwealth Universities. The charter was formally handed over to the chairman by the Queen Mother at the Congress dinner on July 17. During the period of the Congress the necessary steps were taken to wind up the Association of Universities of the British Commonwealth.

Among the numerous illustrious vice-presidents of the Congress were four royal chancellors of universities, three of whom (H.R.H. The Duke of Edinburgh, H.R.H. Princess Margaret and H.R.H. the Princess Royal) honoured working sessions of the Congress with their presence. The Queen gave a garden party for members of the Congress and their ladies. Other social highlights included receptions by the Government, by the Corporation of London in Guildhall, and by the Twelve Great City Companies of London, as well as a reception by the University of London. So there were many indications of the unique character of this gathering of about 1,000 people. They included 380 delegates from 110 member university institutions of the Association supported by numerous observers and guests, among them representatives of kindred organizations such as the Association of American Universities, the International Association of Universities and the National Committees of University Rectors in Western Europe.

At the general meeting of the Association held on the last day of the Congress an invitation to hold the next Congress in Australia in 1968 was accepted with gratitude. This conforms with the principle agreed at the Eighth Congress in Montreal in 1958 (*Nature*, 182, 1283; 1958) that alternate congresses should be held in Commonwealth countries other than the United Kingdom.

An innovation at the recent congress was the splitting-up of its members into three discussion groups (designated *A*, *B* and *C*) which met simultaneously during three sessions. These three group discussion sessions supplemented the three plenary sessions. In this way it was possible to cover a larger range of topics than in previous congresses. It was found, as was expected, that the more intimate conditions of the group discussions facilitated free exchange of views.

Diversity of Universities

At the first plenary session on July 15, Sir Eric Ashby gave the inaugural address on "The Diversity of Universities in the Commonwealth". In his characteristic and inimitable way he painted a vivid and graphic picture, from an ecological point of view, of the evolutionary pattern of Commonwealth university development which had led from a very few prototypes to a wide variety of forms of institution—universities which had adapted themselves to their several environments. The process of change is far from complete, he thought, and in the new African universities is likely to be far-reaching. Universities do change and become adapted to the communities they serve, in spite of their common origin and their desire to remain on an international 'gold standard' of learning. London and Manchester, Michigan and Cornell, all in their origin based to some extent on German universities, are now very different from German universities as they are from each other. So, too, the universities of India and Australia have diverged from one another as they have from their common English ancestors. Moreover, the universities of Canada, diverse among themselves, have moved away from the French and American systems on which they were based. "The constitutions exported to Ibadan, Makerere and Salisbury were designed for nineteenth-century Manchester . . . nothing is more certain than that they will change."

Leaving diversity in the structure of universities, Sir Eric turned his attention to diversity in the student body. He suggested that of three children born in 1945, in Toronto, in Sydney and in London, the chance that the Canadian is now a full-time student is about 1 in 6, for the Australian it is 1 in 9, and for the Londoner 1 in 20. These differences represent, he claimed, differences in the social philosophy of education between the three countries. Higher education can be regarded as a national investment, as a kind of 'consumer-goods' conferring status, like television sets and cars, or as a civic right. There is a mixture of all three points of view in any one country, but with varying proportions of the ingredients. In Britain higher education is not yet conceded as a right, but is sought as a 'consumer good'. This, or the notion of a civic right, would be dangerous in the new States where, Sir Eric inferred, investment in high-level manpower is the most important present requirement. "However

diverse our universities are it is our common task to create in them an environment for higher education which promotes continuous innovation and renewal. If universities fail to do this, Western civilization will decay."

United Kingdom University Developments

In the other two plenary sessions members of the Congress discussed "Significant University Developments in the Commonwealth, 1958–63". One session was devoted to the United Kingdom and the other to Nigeria. The first speaker on the United Kingdom universities, Sir William Mansfield Cooper, reviewed the changing scene at home since the Eighth Congress, from the point of view of a vice-chancellor who had become chairman of the Vice-Chancellors' Committee within the review period. Sir William's address was read for him by Mr. V. Knowles, registrar of the University of Manchester, Sir William having been summoned to Buckingham Palace to receive the accolade. In his rather gloomy and somewhat cynical survey of the quinquennium with its frustrations, he referred to the mounting pressure for university places which had stimulated the establishment of the Universities Central Council on Admissions. On student quality during a period of rapid growth, Sir William thought that the pure scientist has few complaints, the technologist has more, while the arts man is most likely to be depressed. He suggested reasons for these faculty variations and then recounted the familiar story of inadequate financial provision to meet the needs of an expanding university programme. In spite of the anxious period through which the universities had passed during the past two years, Sir William felt that the University Grants Committee and the system which it represents are now more strongly established than at the beginning of the quinquennial review period.

Sir Nevill Mott spoke about research in the universities and repudiated the view that university research is just an expensive luxury to stimulate the dons and keep them up to date. He thought that both pure and applied research should be put in universities whenever possible and commended developments such as the links between Oxford and Harwell and the recent appointment of five members of the staff of the National Engineering Laboratory to be visiting professors at the Royal College of Science and Technology in Glasgow (see *Nature*, **198**, 940; **199**, 843 (1963)). See also p. 1137 of this issue. While welcoming the central service for universities provided by the National Institute for Research in Nuclear Science, Sir Nevill questioned the assumption that in some fields of research the necessary facilities are too big for a university. The implication that concentration of major facilities in selected universities necessitates more direction from the top did not greatly alarm him. Academic freedom means the right to argue, and, provided universities retain this right, it is perfectly consistent with their character that they should welcome suggestions from Government about lines of research which the national interest requires.

The third opening speaker on the United Kingdom universities, Dr. C. H. Wilson, principal of the University of Glasgow, gave an interesting survey of the establishment of seven new universities and outlined the many respects in which they contrast with the earlier model of British university. From the Government's point of view, they represent a national investment, designed to produce an additional 21,000 university places in the mid-1970's. Dr. Wilson suggested that they represent just as much a demand in the older universities for innovation in the university scene. The Academic Planning Boards which have fashioned their character have sought to diversify their degree systems and to diversify their government. However, it is in middle-age rather than in youth that the most inventive ideas are available and Dr. Wilson thought that it is from those already established in universities that new ideas have to come. He had been impressed by the vastly greater conservatism on the part of the scientists than was the case with non-scientists. The first-degree systems in science are more intractable to change than in arts and social sciences. Dr. Wilson ended by reference to the proposed largely residential character of the new universities. "I ask myself, when I look at the cost of this and begin to quantify it for a large number of foundations, how long can it go on in quite this way? I ask myself, too, whether the demand by undergraduates for residence will be for the kind of residences we have built."

It is not possible here to do more than touch on a few of the many interesting points brought out in the ensuing lively discussion. Mr. J. S. Fulton (Sussex) referred to the plans of his University and the stimulating effect on brilliant young staff of participating in such an experiment. Sir Malcolm Knox (St. Andrews) deplored the increasing threat to the distinctive character of universities which he thought must end in impoverishment of our academic life. He suggested that many young people needed training of a kind given admirably at technical institutions of various kinds. They were not academically minded, and should not be brought into the universities. Sir Malcolm was also disturbed about the way in which the distinction between a university and other forms of education is being blurred and by the way in which universities are being thrown into the political arena. This view of the "voice from the past" was contested with varying degrees of heat by several subsequent speakers who acknowledged the designation of expansionists. Dr. J. P. Baxter (New South Wales) wondered why we had established a large number of small new institutions instead of increasing the size of existing universities to a more economic level and also why we placed these new institutions in cathedral cities "away from the throbbing heart of industry". Dr. Brynmor Jones (Hull) directed attention to the administrative demands which rapid expansion makes on academic staff to the detriment of their opportunities for research. Prof. R. F. Treharne (Aberystwyth) would like the universities to set up their own machinery for deciding how schemes of teaching and research should be distributed among them—perhaps an inter-university committee to advise the Government. Present arrangements tend to concentrate resources in a few places and the smaller institutions are overlooked. Dr. M. R. Siddiqi (Sind, Pakistan) directed attention to some disadvantages of the system of affiliated colleges in India and Pakistan and thought that ultimately these colleges should be given university status. The Ven. H. Ratanasara (Vidalankara, Ceylon) was anxious that the new universities should be protected against external pressures.

Nigerian University Developments

The fascinating story of university development in Nigeria during the past five years was unfolded at the third plenary session on July 19 by three opening speakers —Sir John Lockwood (London), Dr. Davidson Nicol (Sierra Leone) and Dr. E. Njoku (Lagos). As a member of the Ashby Commission, Sir John had had some share in promoting this development. In 1958, he reminded us, there was a single university institution—University College, Ibadan. Now there are five independent universities. The student population has increased two and a half times, and the rate of growth promises to accelerate. Sir John described in outline the character of these new universities and referred to the decisive part played by the University of Nigeria at Nsukka in fostering re-thinking about the nature and functions of African universities. He mentioned the striking fact that, in two regions at least, 40 per cent of the national budget is spent on education. This illustrates Nigerian determination not to neglect or waste the talent of its people. Training of teachers is in the forefront of the university programmes and all the universities are showing determination to

become genuinely Nigerian institutions. This is exemplified by proposals for the establishment of Institutes of African Studies.

Dr. Nicol reminded the Congress of the part played by the Inter-University Council for Higher Education Overseas and by the Universities of Durham and London in promoting university development in Africa. He discussed the need for a new philosophy of higher education in Africa and the ways in which fruitful co-operation between African and other Commonwealth universities might develop. Staff secondment will be needed, and facilities should be provided for African staff to participate fully in the work of the departments of universities in other countries as part of their training as university teachers. The African universities must develop their graduate schools, and here, too, co-operation with Commonwealth universities will assist. They must adapt and extend their curricula to suit their environments. Dr. Nicol reiterated the need to develop African studies, in which a promising start has already been made.

After commenting with approval on the fact that he had been preceded by two non-Nigerians, Dr. Njoku directed attention to the very rapid rate of change in the Nigerian university scene. The time-scale seemed different from that of the rest of the world. Although Ibadan's rate of growth had been the fastest of the institutions in the British post-War overseas territories, in Nigeria itself the rate was considered far too slow. Last session's enrolment of nearly 1,800 will increase rapidly. A measure of the new time-scale is given by the University of Lagos. Its Act of Incorporation was passed in April 1962. The inaugural meeting of the Provisional Council, at which Dr. Njoku was appointed vice-chancellor, was held in June, and the first students were admitted four months later. The Nigerian target is 10,000 students by 1970. The establishment of a Manpower Board and a National Universities Commission have been important developments and a very significant one has been the great interest aroused outside Nigeria in its problems of higher education.

Prof. C. T. Ingold (London) referred to the part played by the London University Senate Committee on Colleges Overseas in Special Relation, and directed attention to the continuing great need for the Commonwealth universities to second and lend teachers to Nigeria. Dr. N. S. Alexander (Ahmadu Bello University) stressed how much Nigeria has to offer the expatriate—immediate results to be seen; surplus of research problems. In reply to Dr. R. Craig (Rhodesia and Nyasaland), Dr. Alexander suggested that both research workers and teachers are required; there is a place for the young man, recently graduated, and also for older people approaching retirement. Dr. H. M. Taylor mentioned an informal agreement recently made for interchange of staff between Keele and the University of Nigeria.

Group Discussions

Three general themes were chosen for the three group discussions. In Group *A*, the role of the layman in university government was discussed in three aspects: in educational policy, in finance and in public relations. The background to all these discussions was described by Dr. H. C. Coombs, governor of the Reserve Bank of Australia, and pro-chancellor of the Australian National University, when he said at the outset that universities must ultimately be accountable to the society of which they form part: the social isolation of universities is undesirable, and because of their dependence on public funds impossible. He went on to say that the layman's role had two sides to it. "Laymen", he said, "should see themselves within the University as representatives of the community, observing the University in action and seeking, within limits, to ensure that, allowing for the special character of the University as an institution, its social purposes are performed adequately and at reasonable cost. Outside the University, their role, as I see it, is to stand as an interpreter of the University to the community and to its government, which cannot adequately hope to know its work or grasp its problems, and as a guarantor that, consistent with its special role and ways of work, the University recognizes and accepts its role in society and its ultimate accountability for it." It would scarcely be possible to find a better short statement of the value of the layman to universities.

Dr. Coombs spoke with particular reference to the role of the layman in the fashioning of the university's educational policy. He was very conscious that in this field the role of the layman must be limited; but even here, he said that the layman may well contribute by "asking questions and inviting more detailed examination" of educational policy; and the President of Toronto spoke of the need of maintaining "a dialogue" between layman and academic, if the universities were to have their proper place in society.

Dr. John Deutsch, formerly secretary of the Treasury Board of the Government of Canada, and now vice-principal of Queen's University, Kingston, introduced a discussion on the importance of the layman for the fashioning of universities' financial policies. If the role of the layman in educational policy is to ask questions and compel discussion, his role in financial policy is more positive: he has a range of experience of the utmost value to university administrators; and Dr. Deutsch had no difficulty in illustrating this. But he went on to speak of "partnership" between "professional and layman": "the role of the layman", he said, "is not to govern or impose his will . . . but to help to create conditions in which academic purposes of the institution can be achieved more fully and its integrity can be defended more surely". The discussion on the layman in public relations was introduced by Sir Douglas Robb, chancellor of the University of Auckland, who brought out, in greater detail, the importance of the layman in interpreting, as Dr. Coombs had said, the university to the layman.

It may be said that, in general, the role of the layman in university affairs was so wisely expounded by Dr. Coombs, Dr. Deutsch and Sir Douglas Robb that in the long and full discussions which followed their talks, no difference of view between layman and academic was expressed. It became more clear than ever that throughout the Commonwealth the services rendered to universities by laymen is incalculably great.

The general subject of discussion in Group *B* was the university student. In the three sessions the topics covered were: (*a*) selection and admission; (*b*) postgraduate training; (*c*) amenities, housing and community life. The second of these—given a comprehensive and challenging send-off by Mr. Nöel Annan (Cambridge) and Sir Patrick Linstead (London)—was perhaps not surprisingly the most cohesive. Whatever their differences in other respects, universities everywhere share together a concern for the pursuit of truth. Thus the successful training of the postgraduate in the spirit and the methods of research, his apprenticeship to the higher scholarship, is a vital interest to them all. The outside world needs, no doubt, men and women trained in schools of research at universities; but the universities most of all need them for their own survival and fertility in the future. Inevitably there arose in discussion the sharply divisive and perennial issue, whether the Ph.D. can be fully effective as a means of training if its subject is, apparently, "trivial" or of "no importance". Otherwise, the discussion revealed the similarity of the problems raised by the rapid growth in the number of postgraduate students in the universities of the Commonwealth and the United States; the part that should be played by the postgraduate in teaching; the need for adding to the traditional thesis other forms of training in research methods; the effect of the increase in the number of postgraduates on the

development of university research, particularly if they attract growing support from foundations and Government research agencies; the language barrier to the free movement of postgraduate students. A number of practical suggestions—chiefly for the improvement of information about the facilities available for research—promised greater co-operation between different parts of the Commonwealth in the future.

The first and third discussions revealed greater divergencies. Again, this was perhaps to be expected since neither selection and admission nor what comes under the heading of community life can avoid raising explicitly or by implication the fundamental issues about the function and purpose of a university. Should qualifications for admission and methods of selection be directed solely to the choice of those whose interests are 'academic', an intellectual *élite*; or designed to include within a university's membership a much wider range of gifts and qualities. The answer to these questions rests on the view taken about the nature of the university itself and of its place in modern society. Is its primary aim the production of the scholar, the Fellow of the Royal Society ? Is it to build up a bank of professionally qualified persons needed by an underdeveloped country if it is to reach the point of economic 'take-off' ? No such stark opposition of view was made explicit in the discussion, but implicit assumptions, such as these, coloured much of the discussion about the examination as a test for entry; its value in predicting success at the university; the merits and demerits of the open-entry system of the United States; and the social consequence of failure under the rigorously selective system as practised, for example, at 'Oxbridge'.

The third and last discussion ranged widest of all. There were those who took for granted, so it seemed, that the modern undergraduate at entry was fundamentally perverse and irresponsible, overdeveloped intellectually and socially as well as emotionally immature; those who defended him warmly against the criticisms of his elders; those who felt that he (especially the 'new student') was too much 'of the world' already and needed to be withdrawn during his university years from the pressure and hurly-burly of ordinary existence; for them the hall of residence on the university campus was the right answer; those, on the contrary, who felt that segregation —whether from family or other social ties—and an artificially high standard of living were wholly bad; these approved of the civic university whose students live with their own families. These differing views reflected in some degree the difference between old and developing countries; but they divided also even those from the same countries and environments.

But all—segregationists and integrationists alike—united in one aim—to prevent, if possible, the university from turning out a lop-sided individual, intellectually precocious but humanly underdeveloped. There was a general welcome for the plea—put forward at more than one session by Dr. Furneaux of the University of London —for the universities to take seriously the task of 'knowing themselves', in particular for research and still more research into the psychological forces that mould, and the stresses that beset, the contemporary student, and into his special needs if he is to make the best possible use of his opportunities and of society's investment in him.

Group *C* discussed co-operation among universities of the Commonwealth and consideration was given to: (*a*) regional studies as an academic discipline; (*b*) intercommunication between Commonwealth universities; (*c*) the Commonwealth Scholarship and Fellowship Plan. Regional study, according to Dr. J. H. Parry (Swansea), who introduced the discussion, does not define a subject-matter very precisely. The Commonwealth is a region, from the point of view of a historian, a political scientist or a constitutional lawyer, but not from the point of view of a geographer or an anthropologist. The U.S.S.R. looks a much tidier subject from a geographer's point of view, but not from that of a historian. Considerable controversy was aroused by Dr. Parry's claim that regional studies are not for undergraduates because of their superficiality and lack of precision, with the concomitant danger of turning the ill-informed into the misinformed. Prof. R. J. M. Wight (Sussex), the second opening speaker, challenged this view and described the development of European studies at Sussex. In the subsequent discussion illustrations were drawn from Islamic studies, Far Eastern studies, and African studies, and while there was general agreement about the value of regional studies there was a sharp cleavage of opinion about the level at which they should be undertaken. Dr. Siddiqi (Sind, Pakistan) made the interesting, if obvious, point that regional studies are applicable only to such a range of subjects as languages and the social sciences, and not to such university disciplines as mathematics and the sciences. The main value of regional studies was held to reside in their ability to co-ordinate a range of different disciplines and to break down subject barriers.

In the second session, Prof. P. M. S. Blackett (London) opened the discussion by stressing the need for the supply of expatriate staff for overseas universities, which now have some 700 British-born teachers, representing about 6 per cent of our total complement of university teachers. He referred to the Committee on University Secondment, to the problems to which reference was afterwards made by its chairman, Sir Charles Morris, and one of its secretaries, Mr. F. P. Dunnill, of the Department of Technical Co-operation. Reference was made to the 15 prestige awards announced in the House of Commons on the previous day, to the need to inculcate the idea that a period of overseas service will count as a virtue and to the need to deal with such problems as the education of children of seconded staff. In referring to the need for research funds for staff on secondment, Prof. Blackett asserted that the Department of Scientific and Industrial Research has the right, which has not been exercised, to provide grants for this purpose. He also suggested that greater initiative should have been taken by the Government in promoting staffing of overseas universities. Inter-communication by means of persons was also the theme of the second opener, Sir Robert Aitken (Birmingham), who gave as a prescription for a university staff: (*a*) a majority from the home country; (*b*) some from other Commonwealth countries; (*c*) a few from other countries. Complete nationalization of a university staff is undesirable, especially in a developing country.

An authoritative account of the working of the Commonwealth Scholarship and Fellowship Plan in the United Kingdom was given by the Earl of Scarbrough (chancellor of the University of Durham), the first chairman of the Commonwealth Scholarship Commission. The awards so far made totalled 1,102, of which 584 (from 43 countries) have been taken up in Britain and 295 in Canada. The number of current awards in the past academic session was 820 of which 467 were held in Britain. So the plan is in sight of reaching its objective in numbers, 1,000 scholarships at any one time—500 in Britain and 250 in Canada. Of the 142 scholars whose awards in the U.K. ended last session, 43 have taken up academic posts. Lord Scarbrough thought it important to maintain the essential character of the Plan—educational opportunity at the highest level. He also stressed the importance of flexibility, to meet the varying needs of the Commonwealth countries. The Oxford Conference in 1959, at which the Plan was formulated, envisaged some awards to junior members of teaching staffs who would undertake teaching as well as research. This part of the scheme has not been implemented. Is it attractive to universities ? Some speakers urged that funds should be made available for such awards and also for awards to assist the building up of research schools. It was pointed out, however that the object of the scheme is to benefit individual

rather than universities and that there are other schemes to provide teachers. The view was expressed by critics that the Plan has tended to impoverish research schools in less advanced countries and to enrich research schools already well provided with postgraduate students of high quality. Although extraneous needs should not be allowed to dilute the Plan, which has been widely welcomed in Commonwealth countries, it was recognized that funds are needed to supply other needs, including the support of research schools in new universities overseas.

In the four days following the London Congress the executive heads of the Commonwealth universities, accompanied by the American university presidents, held further meetings in the University of St. Andrews, where the topics for discussion were "Secondment of Staff", "Governing Bodies of Universities", "Development of University Staff Associations" and "Aspects of University Expansion". It was not the purpose of either this meeting or the larger Congress to pass resolutions or to make recommendations. There was general agreement, nevertheless, that the exchanges of views and experiences had been of great value. A full report of the proceedings of the London Congress will be published by the Association of Commonwealth Universities.

COLLEGES OF ADVANCED TECHNOLOGY IN BRITAIN

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THIS article is being written a few weeks before the appearance of the report of the Robbins Committee. The new master plan for higher education which we expect to emerge will, *inter alia*, re-define the role of the Colleges of Advanced Technology and bring their future into sharper focus. This is, perhaps, the last occasion when it is possible to survey the progress and perspectives of these Colleges in the relative calm which follows the initial phase of emergence and rapid growth and precedes a new period of intense activity and development.

It is still difficult to understand the long induction period between the Percy Report of 1945, which made the basic recommendations for the new strata of colleges and their designation as Colleges of Advanced Technology in 1956. The need was obvious and universally acknowledged, and was not being filled. Britain emerged from the Second World War into a world where its survival depended on creating a technologically advanced industry. Successive surveys of scientific and technological manpower revealed not merely the acute shortages, but, more seriously, the continued underestimation of our future needs[1]. However, the problem was not only one of quantity, but even more of quality. The basic engineering industries of the country, on which the modernization of all our industries depends, were particularly slow in increasing their proportion of professional engineers and technologists[2]. Equally serious is the fact that, of all the industrial countries, we are the only one in which the majority of our professional engineers are still trained by part-time and evening study[3] with all its attendant scientific limitations and cultural narrowness.

Finally, our existing higher educational system and the structure of secondary education which serves it has remained seriously restrictive in two ways particularly significant to the present theme. First, it has failed to bring into higher education more than a minority of those with the latent ability, particularly from the families of manual workers[4], and, secondly, it has failed to break down the cultural and social barriers between talent and technology[5]. For the majority of people, education, including higher education, is a prelude to practical activity. They will spend the greater part of their working lives in industry in its widest sense, including commerce and social organization. Technological education is concerned with serving two related social purposes: first, to enable its students to make the most fruitful and creative contribution to the industry they serve and, secondly, to enable industry to make the fullest and most creative contribution to the life of society.

We are thus concerned with a dynamic concept of industry with the continuous evolution of new methods and social purposes. It is clear that technological education cannot be restricted to some narrowly vocational role of fitting a human cog into a pre-existing and unchanging machine. The men we train are going to change industry and interpret its function within society. It is by these standards that we need to assess the past and present shortcomings in our education of technologists and the present attempts and future perspectives in developing new methods.

The Colleges of Advanced Technology were originally a group of the larger technical colleges. Most of these were founded at the turn of the century, and for the first fifty years were largely concerned with training engineers and other technologists by the methods prevailing in Great Britain, those of evening and, latterly, part-time day courses for students apprenticed in industry. This had the advantage of bringing the Colleges into close contact with industry. Their staffs were largely recruited from industrial technologists. The dichotomy between science and its industrial application which has so seriously affected full-time education and research was largely absent. On the other hand, the narrowness of the part-time system, its scientific limitations and the high wastage imposed by the severely intense nature of the courses weakened and restricted not only the Colleges but also the whole of our technological development. At the beginning of the post-war period, the Colleges had developed a supplementary stream of full-time students, often studying for external degrees or professional qualifications. Research developed more slowly and unevenly, mainly concentrated in the science departments. Nevertheless, the public image of even the largest of the Colleges was that of a night school of higher education, devoted on one hand to the vocational training of engineers and, on the other, to reclaiming in all fields talent that failed to find its way into the universities.

This was the background which the Colleges had to re-assess after their designation as Colleges of Advanced Technology in 1956. The main immediate objectives were: to retain and extend their interaction with industry while removing the limitations of previous methods of education; to develop new forms of undergraduate education appropriate to the wider needs of industry; to expand their staff as rapidly as possible and to improve their quality while continuing to emphasize the value of responsible industrial experience; to expand their research activities to a comparable level, but with a different emphasis, to that of the larger faculties of science and technology within universities; to widen the range of their studies and researches to cover all those mainly concerned with their central objective of providing a future leadership for industry.

In the rest of this article an examination is made of the progress which has been made in the attainment of these objectives and the new problems and perspectives that are

emerging as a result. During the period since designation the eight original Colleges, later joined by two others, have multiplied their full-time undergraduate population from about 3,500 to more than 10,000; their full-time teaching staff has almost doubled to a total of about 1,800, and the majority of the new staff have had both industrial and significant research experience. Research which was originally concentrated in certain subjects has now spread to virtually all the departments in all the Colleges. A substantially new form of undergraduate education, the 'sandwich' course for the Diploma in Technology, involving a close interaction of industrial and academic experience, has been soundly established during the period, the numbers increasing from less than 200 to nearly 6,000.

It would now be useful to examine some of the features of this development in greater detail. Consider first the question of co-operation with industry. The new type of sandwich course has been fashioned largely in the Colleges of Advanced Technology, where 75 per cent of the students are enrolled, although the overall approval of the courses has been under the ægis of the National Council for Technological Awards (N.C.T.A.). While such courses have existed in some form or another for many years, the development of the Diploma in Technology in the past seven years has been new both in scope and in nature. It originated from a general attempt by individual colleges and industry to attract a better level of student into the professions concerned with industrial engineering. Indeed, during the initial period perhaps one of the most significant problems has been the methods of selection for such courses. The general requirement is equivalent to university entrance level; but over and beyond this the objective is to find some method of both attracting and selecting the kind of students who are going to benefit most from the blend of industrial and academic training involved. As the investigations in ref. 5 have shown, the grammar schools have tended to send only their second- or third-best students into engineering technology at the universities and, in some schools, the initial reaction to the new type of course was to place it even further down the list of priorities. Fortunately, increasing contact between the Colleges and the schools has helped to disperse this illusion, and the proportion of good students who put down the Diploma in Technology course as first choice is rising significantly. Nevertheless, the problem of selection has a number of special features to it. In the first place, frequently both industry and College have to be satisfied with regard to the likely suitability of the student. This is certainly the case of works-based students, but is advisable even in the case of college-based students. Otherwise, the College may accept students and find it not possible to get them past the personnel and training officers in industry for the purposes of industrial training. Fortunately, this does not necessarily involve an elaborate apparatus of dual interviewing; it does require an assessment by the College or industrial staff concerned of the qualities likely to be required by the other partner in the enterprise. The College tutor interviewing students has to assess the likelihood of the student being capable of making a success of industrial training and a subsequent industrial career, as well as having the qualities of intelligence, critical ability and imagination required by the disciplines of the academic course. Up to the present, these methods of selection have been evolved and could only be developed by empirical means. As might be expected, the first few years tend to show a correlation between performance and initial examination successes in those courses, such as mathematics, where one would expect a strong influence of innate ability. On the other hand, in the case of engineering and some branches of applied science, no such simple correlation obtains, for example, between marks in a General Certificate of Education (Advanced Level) examination and performance in the course. However, there is empirical evidence that success in the course, if not distinction, is frequently bound up with the early indications of the degree of committal to an industrial career.

A feature of the Diploma in Technology course is that it permits the entrance of Ordinary National Certificate students as well as grammar school students. A precaution here is that it is normally required that the Ordinary National Certificate students must have a certain minimum mark in their final examinations. It is significant that at the Birmingham College of Advanced Technology in the first seven years of some Diploma in Technology courses in engineering, the proportion of first-class honours was very markedly higher among Ordinary National Certificate students than those recruited from the grammar schools, in spite of the fact that the latter would have had a much stronger basis for the scientific and mathematical studies which are ultimately a very strong feature of the course.

These first experiences with different streams of entrants demonstrate the previously suspected existence of reserves of ability for higher education which could escape the normal processes of university selection. Nevertheless, the large majority of future students will inevitably be recruited from secondary schools at the age of eighteen or more. It is therefore desirable that some methods be devised of projecting into the schools themselves the capacity to cultivate and detect future ability in technology. Part of the answer may lie in the development of new methods of teaching mathematics and science in such a way as to avoid an excessive pre-occupation with them as things in themselves, in favour of the concept of them as tools for application to real situations. The research being initiated by such foundations as the Nuffield Trust is of considerable significance here and should be greatly extended. A longer term contribution may lie in the recruitment into the schools of science and mathematics teachers with industrial and technological experience and the potential role of the Colleges of Advanced Technology in this field will be mentioned later.

The sandwich course system of undergraduate training, though not the only method used by the Colleges of Advanced Technology, is a major feature of the work of most of them. It is therefore necessary to re-examine briefly the concepts on which this mode is based and the factors likely to affect its expansion. Most scientists and engineers are employed in industry (in its widest sense) and the large majority of these are concerned directly with the application of science and technology in practice. Hence a method of education deliberately conceived to serve these objectives could, if successful, eventually become dominant and the Colleges face the ultimate role of educating the majority of industrial engineers and applied scientists. This could mean the multiplication of their present output by a large factor, possibly as much as ten.

The viability of the sandwich method depends on a degree of co-operation and interaction with industry which is at present only in its initial stages. To survive in the long term, it must prove to be qualitatively sound and economically justifiable. The qualitative factors depend on the devising of an educational system which produces a man who is committed to (or involved with) the idea of serving the community through the application of science in industry. He must, nevertheless, have a sufficiently independent standpoint and critical and imaginative ability to be an initiator of change whenever it is desirable or necessary. No single system of interaction of industrial and college experience will serve all technologies. The main problem is to interact the two experiences in such a way that they reinforce each other to the maximum extent. Individual Colleges are accumulating experience on this through the continuous contact between their staffs and their industrial counterparts, particularly during the industrial training periods. It

begins to emerge that an exact correspondence of experiences is both impracticable and unnecessary. What is necessary is that each part should engage the initiative of the student and that the industrial experience in particular should involve him in making a real contribution to a team engaged on a significant project. The inculcation of techniques, though necessary in certain technologies, is secondary to the main purpose of the maturation of a concept of science in its application and a comprehension of the many-sided co-operative effort needed for its prosecution. The viability of the sandwich course depends on the assumption that this process can take place concurrently with scientific education rather than exclusively after it. An important feature is that, even though the industrial and academic periods form a single course of training, they are nevertheless separated into significantly long individual periods (six months or one year). Research is needed into the effect of this alternation of different though related experience. Preliminary investigation shows that in many cases it can lead to an enhancement of the critical outlook to both sets of experience. At the same time, it can provide an immunization against the kind of disillusion which afflicts so many graduates who enter industry for the first time at the end of their university study.

The question of the viability of the high-level sandwich course from an economic point of view is of the highest significance. During the past year, there have been distinct signs of a decrease in the expansion of industrial places for students, and the reason given in nearly every case is economic—the charge on industry for providing the maintenance expenses of the student. From the national point of view, there is no reason why the sandwich method of training should be more expensive than a full-time education followed by some kind of postgraduate apprenticeship or induction period. Indeed, in so far as there are some initial parts of industrial experience which are inevitably routine in character, it is clearly more economical to finance them at the level of a student maintenance grant than at the salary of a graduate technologist. However, the present method of finance of sandwich students, which throws the burden of maintenance of students on firms, who, in many cases, may never employ the students concerned, may now become a major obstacle to expansion. If university students are supported wholly by the State, it must be willing to give the same support to sandwich students and perhaps to participate in the cost of industrial training. The simple immediate measure would be to make annual maintenance grants for sandwich course students from public funds to cover the period of the full twelve months for which they are in training, instead of a maximum of six months as at present. The industrial contribution to the cost should either come from taxation or a levy covering the whole industry.

The sandwich course has been dealt with in some detail to show the extent to which co-operation with industry is an intrinsic part of the life of the Colleges. Nevertheless, whatever evolution the methods of education will undergo, the same need will be evident though it may be expressed in different forms. Mention has been made of the extent to which the Colleges draw their staff from industry. During this emergent period, they have been relatively successful in attracting young men of high quality, and this has partly arisen from the fact that the particular method used for establishing salary scales in technical colleges has led to very favourable conditions for young men of lecturer level in the age-range 26–32, as Prof. Irving has already pointed out[6]. The average age of the staff of Colleges of Advanced Technology is, as a consequence, at the moment probably the youngest for any period in their history and younger than they are likely to be at any future date. This has its positive side; such people are, in most cases, approaching their peak in initiative and creative ability. Their contact with industry is fresh and the system of organization, particularly in sandwich courses, enables them to keep it so.

On the other hand, the recruitment of senior staff, whose salaries and conditions, as Prof. Irving has noted, lag behind those in other professions and industries, has naturally proved difficult. It is not surprising that there are no Fellows of the Royal Society at the Colleges of Advanced Technology, where, until recently, there has not been a single position with a salary equal to that of a university professor in science and technology. Neither are the Colleges likely at the moment to attract into their own leading positions people who have, say, held research managerships or are at director level on the management side in important industries. Clearly they need to do all of these things. The young staff they have are the ultimate guarantee of the future, but, in the meantime, as the size of departments grows, there is a pressing need for a more mature leadership. Heads of department are extremely hard-pressed at the moment; there needs to be a rapidly developed multiple professoriate in each department in order that the burden of initiative and leadership can be appropriately distributed. This is partly a problem of raising the material prospects at the higher level in the Colleges, bearing in mind that the quality of leadership they need is not merely of academic distinction, but, in many cases, of proved ability to manage and lead large enterprises. However, whatever the attractions, it is neither possible, nor even desirable, that all the people who can make a major contribution from industry should have to leave industry to do so.

The Colleges will need arrangements that permit extra-mural appointments of the highest level. Already some of these have been provided in the individual Colleges in the form of industrial consultant lecturers. However, it needs the establishment of extra-mural professors with a full status in the Colleges, as well as in industry, including a participation in the policy decisions of both, to secure the contribution which is really needed. The contribution of such people needs to be distinguished sharply from the older contribution of part-time lecturers, which is likely, if anything, to diminish rather than increase as evening classes disappear. The part-time lecturer, although giving valuable service, was not sufficiently an organic part of the College for which he worked. He rarely saw the whole picture, and his contribution was minimized by the fact that it consisted only of teaching. Unless the industrial technologists can be drawn into the policy-making, the planning and carrying out of research, into staff colloquia and seminars, as well as into the teaching of undergraduate and postgraduate students, they cannot form an integral and co-ordinated part of the life of the Colleges. This poses entirely new problems for co-operation with industry, problems which must be faced if we are to optimize the use of our most talented scientists and technologists, whether their main pre-occupation is in College or industry. It need scarcely be said that, reciprocally, there is needed an extension of the consultative and other methods by which College staff are drawn into the life and work, particularly research, of industrial units. These problems will tend to emphasise the regional significance of the Colleges, since such close co-operation will tend to take place predominantly on a regional scale. Nevertheless, there will be certain industrial connexions for each College which will tend to be of a national character, for example, metallurgy in the case of Birmingham College of Advanced Technology, material science, including fibre science, at Bradford, computer technology at Northampton College of Advanced Technology, production engineering at Loughborough, and mechanical engineering at Salford.

The industrial influence of the Colleges is particularly important in the case of research and postgraduate courses. Research has two significances for any college of higher education; first, to train research workers and, second, to provide a staff who are advancing the knowledge of the subjects they teach. For both these purposes it is

proper that research in Colleges of Advanced Technology should frequently be in close co-operation with research in industry. The Membership of the College of Technologists, devised by the N.C.T.A., has already set a new pattern for the training of the postgraduate research student, in which part of his work must be undertaken either within industry or under industrial supervision. More generally, the research in the Colleges of Advanced Technology is already tending to take the form of group rather than individual projects, with the concept of building up viable teams of workers for defined areas of research. Such a structure is making possible a closer alignment of the research of the Colleges with industrial needs. Undoubtedly, the major problem inhibiting all research in the Colleges at the moment, however, is the absence of a suitable higher award, to which reference will be made later. The same inhibition affects the development of postgraduate courses, which are another feature in which the closest co-operation with industry is desirable. The number of individual postgraduate courses is probably at present multiplying more rapidly than any other feature of the Colleges of Advanced Technology. While many of these are of a part-time character or of a relatively short full-time duration, they are a means of drawing into close co-operation people of the highest level in industry.

Of particular importance from the point of view of regional influence is the prosecution of management studies at postgraduate-level. Here we are dealing with a new discipline, about which, although there is a very widespread concern for its extension, there is considerable controversy concerning its nature and method. One thing which is reasonably certain is that at this stage it is almost impossible to disentangle the education of the manager from the management situation itself. This means that management studies are or should be an outstanding example of the close interaction between industry, business and college, about which we have been speaking. In this field, above all, it would seem necessary for the College and senior industrial staff to co-operate in producing an educational component to the whole preparation of managers, whether this takes place in industry or in College. It follows that this demands a strong regional influence by the College and its capacity to engage the direct co-operation of significant industrial enterprises. The importance of this is to be seen in contrast with the concept that the solution of our management education problems is to create a sort of national superschool of management on the lines of the Harvard School of Business Studies. The danger of this approach is that it could denude the growing points in the main industrial regions, whether in universities or Colleges of Advanced Technology, of their leading staff, and could postpone the close interaction of industry and College essential to success. Naturally it is necessary that the industrial co-operation should be supported in this field by strong College departments with an adequate backing of research: such examples as the work of Jahoda at Brunel and the school of research under Lupton at Birmingham have been points of initiative that have now been repeated in several other Colleges of Advanced Technology. In concluding this brief reference to the regional influence of the Colleges of Advanced Technology attention should be directed to the question of their relation to the next strata of colleges, the Regional Colleges of Technology. There are some 24 of these colleges, each of which undertakes a proportion of university-level work; they share between them the remaining 25 per cent of the Diploma in Technology students. A pressing question is the future of this work when the Colleges of Advanced Technology are given the power to grant their own degrees and hence withdraw from the ægis of the N.C.T.A. It seems desirable that future administration should permit the closest links between the Colleges of Advanced Technology and the Regional Colleges near to them. This could be obtained by a system of affiliation in which work in Regional Colleges would receive recognition in part or in full for awards under the overall academic supervision of the College of Advanced Technology.

The concept of a university is sometimes taken to involve a more or less complete coverage of all departments of human knowledge and research. For any single institution of education in the modern world such an objective is illusory. Nevertheless, the close link between the Colleges of Advanced Technology and industry compels them to widen their range of studies far beyond the purely technological or vocational. Such a range includes fundamental science in all its main branches, the social sciences and applied science and engineering. Indeed, it includes any subject which can be studied in the context of its possible application. There has always been a strong stream of the fundamental physical and biological sciences in certain of the Colleges. For example, Chelsea has developed strong schools in biochemistry and physiology, Salford and Battersea in chemistry, Bradford in polymer science. More recent has been the growth in all the Colleges to a greater or lesser extent of departments or schools of social science. At the undergraduate-level these are developing partly by the cultivation of traditional disciplines such as economics and partly by the devising of new courses, such as the sandwich Diploma in Sociology at Bristol, which lays emphasis on the sociology of an industrial society. New methods of studying languages in the context of problems of communication in the modern technological world are planned in some Colleges, while the Welsh College is unique in having a postgraduate diploma in the teaching of English as a foreign language. Brunel College is pioneering a sandwich course in industrial psychology, and Loughborough College is experimenting on a new synthesis of engineering and social science. Most Colleges are seriously considering the introduction of departments of education, partly because of the outstanding need to introduce a stream of teachers with industrial and technological experience, and partly in order to prosecute research into their own objectives, the nature of higher technological education itself. Both Birmingham and Brunel have pioneered in this field with research on the educational problems arising in sandwich courses.

Apart from this necessary extension of the overall range of interests of the Colleges, two developing concepts of the breadth of higher education and research are cardinal to their work. The first concerns the balance between specialization and breadth in the undergraduate course. Here the Colleges are tending to develop what might be called a concentric rather than a parallelistic approach to the problem of broadening the education of the specialist. This approach recognizes that development in depth, specialization to the point of independent work, is inescapable and desirable, but that fertile specialization in technology interacts organically, not merely with contiguous sciences, but with the social background in which it operates. Stated simply, this is not an attempt to add a gloss of wider culture to the technologist, but to develop in him a sense of his own contribution to society and its culture. This is why general and social studies are regarded as an integral part of his education as a specialist. This attempt calls for a high degree of interpenetration of departmental interests on the part of the staff and may lead to an abandoning of the rigid departmental structure in favour of organizing as teams all teachers who contribute to the education of a particular stream of undergraduates. The second development, which is at a still more nascent stage, is the creation of inter-disciplinary teams for research and postgraduate instruction. The tendency for research in the Colleges to become objective-based again breaks down departmental barriers and may require a new form of organization with the necessary flexibility to assemble and re-allocate research effort in the direction shown to be most fruitful. Examples of these beginning

are the formation of teams on such subjects as powder technology and materials science at Bradford, engineering design at Bristol, computer technology at Northampton, metal deformation and high-temperature technology at Birmingham.

This brief study of the Colleges has been bound to emphasize the general features that they have in common and to neglect their diversity of interests. Inevitably, no single pattern will obtain in the future, and even the problems that they have in common will probably permit several different solutions and organizational forms. However, the very fact that to some extent the Colleges are all deliberately orientated to the production of people who will solve practical problems and create new industries leads one to believe that they must develop a highly integrated approach in their philosophy of education, corresponding to the complex interaction of science and practice in the industrial world. It follows that, to fulfil this function, the Colleges cannot be small colleges; they must be large enough to include creative workers in the many disciplines that must interact in modern industrial technology. The actual size of an individual College will depend on the range of applied sciences and technologies, including the social sciences, that it includes: no College can contain the whole range; but it is necessary that each should contain a range sufficiently broad to be reasonably viable as a self-evolving unit. The Colleges' own preliminary estimate of the minimum immediate target to make this possible would provide for the education of some 40,000 full-time students, distributed in units of between 3,000 and 6,000 among the ten existing Colleges. It seems probable that in the light of more mature reflexion these figures will have to be revised upwards. As Prof. Bowden has pointed out, a typical Continental or American college of technology frequently contains 10,000 students. Naturally it is the number of staff in each complex of specialists that is critical rather than the number of students, which indeed can be calculated from some overall staff-student ratio, although this latter is clearly subject to modification in the light of more economical methods of teaching that may result from research in this subject.

The method of organization of these large units of science and technology poses some interesting problems. As mentioned already, it is likely that several Colleges will experiment in new forms of organization which involve the breaking down of departmental barriers and the creation of a much smaller number of large schools, each with a multiple professoriate and other staff, with directors of studies responsible for the whole team concerned with an undergraduate or postgraduate course, and with inter-disciplinary research teams flexible enough to be formed and re-formed according to the fertile growth of the research concerned. In some centres, such as Birmingham, considerable thought has been given to a system of federated colleges. Such a federation might include a college of technology and science, a college of commerce, a school of education, a college of industrial fine arts and a college of architecture and building.

The foregoing considerations refer to the organization of staff and facilities: the planning of the student community poses another set of problems, some of which are common to all institutions of higher education. However, the stress that Lord Fleck and many other leading industrialists have laid on the all-round development of the student as a future leader of industry makes the provision of residential accommodation, preferably on the campus, a key question for the Colleges of Advanced Technology. In this connexion, the development of the student village at Loughborough is being carefully studied, although other solutions will probably be necessary on urban sites. The main problem here is not that of imposing some kind of ready-made ethos or even moral outlook on the coming generations of students, who might indeed, with some reason, question the capacity or qualifications of the academic staff to undertake such a role; it is rather to create a new kind of community which, while on the campus, is open to the maximum opportunities for self-education, including the undertaking of responsibility, the formation of values and purposes, but, in another sense, is an open community in that a large proportion of the students, during their periods of industrial experience, will be deliberately breaking away from time to time from the college atmosphere and immersing themselves in the world of industry and commerce.

As mentioned at the beginning, this article is being written immediately before the Robbins Committee report. If, nevertheless, the venture that has been outlined is considered to be soundly based, it is necessary to consider what are the pre-conditions for its development and success. Five of these are of sufficient importance to be mentioned briefly.

The first is that, although the Colleges are to some extent committed to educating a man who will serve industry, and although they form an open community with a considerable interpenetration of industrial staff and influence, nevertheless they need, above all, the full autonomy associated with a university if they are to formulate and solve the many complex problems both of an academic and organizational nature that their purposes impose on them. The question of the issue of degrees both of a first and higher nature, including the doctorate, is not therefore merely a question of status: it is a vital organizational pre-requisite to the bold educational experimentation that must be undertaken in this field of higher technological education. This is particularly true in the case of higher degrees such as the doctorate. The doctorate is not only a national but an international symbol of maturity in research and creative work. The Colleges must be able to grant their own doctorates to attract and retain the outstanding leaders of research and the students of ability who are necessary to develop these fields in a fruitful way.

The material pre-requisites are the second pre-condition, and these are likely to be expensive, since science and technology are inevitably the most expensive to study and research. There has been a continuous underestimation nationally of worth-while expenditure in this field, both in universities and in other forms of higher education. It is thought in some quarters that the starving of university finance has been compensated by the lavish treatment of the Colleges of Advanced Technology. It is paradoxically true that, while under the local authorities, they were in fact generously treated, although their demands for capital equipment on the largest scale were somewhat limited by the restrictions on the research already noted. It must be stated that their first year under the direct finance status of the Ministry of Education has brought many of them disappointments in their plans for the immediate expansion of their material facilities. It would be unkind and perhaps unjustified to judge the future by the experience of this first year, which may have resulted from the problems involved in estimating a new venture, and there are signs that the scope of capital equipment required is now receiving attention, for example, in the plans to equip the Northampton College of Advanced Technology with a computer centre costing about £250,000. Whatever Minister is to be responsible for the future direction of higher education, must, so far as science and technology are concerned, be served by a body which can represent to him with the highest authority the proper and adequate allocation of funds for the development of the highest level of technological education and research.

The third pre-condition for success is the expanded co-operation with industry, and these aspects have been touched on already. Paradoxically again, this co-operation, although intrinsically between individual firms and the Colleges, may well depend on the creation

of new overall mechanisms for apportioning nationally the cost of industrial training and large-scale research projects. This is particularly true if co-operation is to be extended with the medium- and smaller-sized firms, the finances of which will not permit an altruistic attitude towards their profit and loss accounts. The fourth prerequisite is the recruitment of staff, both of the quality and in the quantity required. In order to reach the target of 40,000 students, we may estimate that an additional 3,500 teachers are required. A proportion of these need to be men of very great ability either drawn from industry or sharing their activities between industry and College. Some of these will inevitably be in short supply in the initial period until their work in industry can be supplemented by the new supply of people coming from the Colleges. Nevertheless, the financial factors, particularly salaries and facilities for research, must be faced realistically. The point is that if the top staff of Colleges of Advanced Technology are to be responsible for training future leaders of industry, they must themselves be men of the highest calibre. The fifth condition for sucess has already been dealt with to some extent when we were considering the problems of entrants and selection. It is that of co-operation with the schools. During the past few years this has developed to an unprecedented extent and a good many of the barriers of misunderstanding have begun to be broken down. In one College, for example, the staff make perhaps something like one hundred visits per year to the schools in the region, and several thousands of school boys and girls come in contact with the College. Increasingly, the staff at the schools are visiting the Colleges, taking part in symposia and conferences and special courses, and are beginning to appreciate something of the requirements and objectives. Nevertheless, it remains, from the long term point of view, highly desirable, if an adequate stream of our talented young people are to find their places in the Colleges of Advanced Technology as well as in university departments of applied science and technology, that new methods of teaching science should be experimented with and that a stream of industrially experienced teachers should find their way into the schools.

After a long induction period, higher technological education in Britain, through the medium of the Colleges of Advanced Technology, has taken the initiative in a new experiment, the construction of a fully viable and self-supporting spectrum of study and research, deliberately harnessed to supplying industrial and social needs. The impetus of their designation has carried them forward with a rate of expansion creating many problems demanding complete re-appraisal of our whole philosophy of scientific and technological education. Much of their work carries the inevitable marks of haste and improvisation. It is now essential that the administrative and organizational measures necessary for them to develop autonomously and with full momentum should be put into operation without delay.

[1] Edwards, E. G., *Technology*, **6**, No. 2, 39 (1962).
[2] *Industrial Research in Manufacturing Industry*, 24 (Federation of British Industries, 1961).
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[4] *Rep. Central Adv. Counc. for Education* (England), 2, 116 (H.M.S.O., 1960).
[5] *Technology and the Sixth Form Boy* (Oxford University Department of Education, 1963). See also, *Nature*, **199**, 958 (1963).
[6] Irving, H., *The Three Cultures* (Leeds Univ. Press, 1963). [See also *Nature*, **199**, 627; 1963.]

OBITUARIES

Prof. J. J. Sudborough

Dr. John Joseph Sudborough died at his home in Torquay on July 25 at the age of ninety-three.

Born in Birmingham in 1869, he was educated at the Camp Hill Grammar School and in 1886 entered Mason's College with School and Entrance scholarships. He studied mainly under Profs. Tilden and Lapworth and graduated B.Sc.(London) in 1889 with double first-class honours in chemistry and geology.

Two years research work under Prof. Tilden followed, the subject being the additive reactions of nitrosyl chloride with olefines. In 1891 he obtained one of the first scholarships offered by the Commissioners of the 1851 Exhibition and proceeded to the University of Heidelberg, where he worked under Prof. Victor Meyer on stilbene derivatives. He graduated Ph.D. in 1893 and continued for a further year as private assistant to Victor Meyer, working on the subject of 'steric hindrance' as shown by diortho-substituted benzoic acids.

Returning to Britain, he worked for a period at Owens College, Manchester, and obtained a D.Sc.(London) degree. He was then appointed lecturer in organic chemistry at University College, Nottingham, under Profs. Clowes and Kipping, and there continued research, with student assistance, on 'steric hindrance' and on the additive compounds of trinitro-derivatives of benzene.

In 1901 he succeeded Dr. Lloyd Snape as professor of chemistry at University College of Wales, Aberystwyth. It was a time of great activity in Wales, for the University had been established in 1896 and the new intermediate schools were sending their matriculated pupils to the Colleges. Sudborough quickly realized his opportunity, organized an honours degree course and a research school and secured increased accommodation for his rapidly expanding department. A few years later, the generosity of Mr. David Davies (afterwards Lord Davies) and his Llandinam family enabled the College, in 1907, to erect the Edward Davies Memorial Laboratories, the equipment of which the professor supervised with meticulous care and of which he became the first director. During his decade of service at Aberystwyth, Sudborough taught and trained in research many men who later occupied important positions in the scientific world. He also took a leading part in College administration, served as dean of the Faculty of Science and as an officer in the Officers' Training Corps Contingent.

In 1911, after the death of his wife, he accepted the appointment as professor of organic chemistry at the Indian Institute of Science, Bangalore. Here, in association with the director, Dr. M. W. Travers, he inaugurated the work of the Chemistry Departments and developed a sound and thorough system of training in organic chemistry. In 1914 Dr. Travers retired, and Sudborough became head of the Departments of General and Organic Chemistry with Dr. H. E. Watson as assistant professor. Their output of papers, many of technical importance, was afterwards considerable.

After retirement in 1926 he lived at Ermington, South Devon, where he took much interest in local affairs and served on the Plympton Rural District Council. Later, moving to Torquay, he became honorary secretary for many years of the Torquay Natural History Society. He became a life governor of the University College of Wales and served for many years on its Council.

He was twice married, first to Miss J. Hunter, of Belfast, and secondly to Miss Elsie Bean, daughter of Mr. A. Bean, sometime postmaster-general of the Punjab, who survives him. He leaves no children.

T. Campbell James

Dr. B. Lambert, O.B.E.

Dr. Bertram Lambert died on July 1, aged eighty one. For half a century, as undergraduate, demonstrator

and finally as administrative head, he was associated with the laboratory first called the Chemistry Department, University Museum, Oxford, then the Old Chemistry Department, and later the Inorganic Chemistry Laboratory. He went up to Oxford from Giggleswick School as exhibitioner at Merton College, and obtained a first class in chemistry in the Final Honours School of 1903. The head of the Department, Prof. Odling, appointed him to the small body of demonstrators whose duties were to lecture and to teach, but with no encouragement or example towards research. Lambert, however, as an undergraduate had received a taste of the new physical chemical methods of measurement which had just been introduced from Ostwald's Laboratory in Germany into the undergraduate course at the Daubeny Laboratory belonging to Magdalen College. Single-handed he began to prepare himself for original work, which in those days was concentrated on the development of techniques for achieving the highest accuracy of physical measurements on chemical substances. Everything needed to be done the hard way. By intensive and methodical late-night practice, Lambert made himself equal to a professional in glass-blowing, and highly skilled in the laboratory arts of apparatus construction. Having no technical assistance, he made his own vacuum pumps, traps, manometers, internal heating devices, and often his own taps (all, in early days, of soda glass). This manipulative skill culminated in the construction of a quartz microbalance for the measurement of gaseous densities (*Phil. Trans. Roy. Soc.*, 1950), whereby the atomic weights of carbon and nitrogen were determined with an accuracy of 5 parts in 10^5.

His first published work related to the rusting of iron, and involved lengthy experiments on the preparation of highly purified water, oxygen and iron, from which he concluded that the rusting process was essentially of an electrolytic nature, a view which was only to find general acceptance many years later. After the First World War, he took up the study of adsorption of gases with a long series of carefully planned experiments on the palladium–hydrogen system and on the equilibria of vapours and gases with ferric-oxide and silica gels. This work brought out the difficulties of the subject associated with hysteresis effects. A paper published in 1939 showed how, by the most careful attention to detail, chemical methods of gas analysis could be brought to an accuracy of 0·02 per cent on a total volume of only 10 c.c. During all this work he was continually perfecting matters of technique and methods of achieving the highest purity of the gases used; particular reference may be made to his preparation of pure nitrogen. The elaborate care he constantly took meant that his output of publications was not large; his involvement in the planning and technique of measurement often seemed to exceed his interest in the significance of the results. His sound instruction in method, however, was invaluable to students who worked under his supervision.

Dr. Lambert is perhaps best known for his initiative in the First World War towards the production of respirators. Immediately after the first gas attack, the Army set up a defence organization under Col. Horrocks, assisted by Col. Harrison and Major Sadd, and early trials were made of respirators of impregnated cloth of Balaclava helmet shape. As early as June 1915 Lambert, while on leave from the Royal Engineers, himself constructed a box-type respirator with soda–lime–permanganate granules as absorbing agent. He took this to the War Office, and his principle was quickly recognized as superior to all other proposals. In the following year box-respirators with Lambert's granules, together with charcoal, were issued to the troops, and continued in use throughout the War. This contribution was recognized later by a substantial monetary award from a War Inventions Board of scientists and by an O.B.E. from the Government.

As soon as the First World War was over, Lambert returned to Oxford and was elected Fellow of Merton College. Additional responsibilities came easily to him. Besides carrying a full load of tutorial teaching, laboratory demonstration and research, he was called on to act as mediator and peace-maker in disagreements during the early part of Prof. Soddy's tenure of the chair. When Soddy retired he effectively administered the Inorganic Chemical Laboratory until his own retirement in 1947. He was active in all affairs and committees relating to chemistry at Oxford, and served helpfully as a member of the University Chest during the Second World War.

Lambert possessed the virtues of a true Yorkshireman: cheerfulness, sociability, self-reliance, moderation, appreciation of honest, hard work, and wise judgment of men and things. He enjoyed playing cricket when young, and bowls and golf in later years. His marriage to Sylvia Dewe in 1908 was a happy one; she survives him. His daughter, Peggy, is married to Group Captain Peter Broad, and his son, James Dewe Lambert, is Fellow of Trinity College and University lecturer in chemistry at Oxford.

E. J. Bowen

NEWS and VIEWS

The University of Strathclyde

It has recently been announced that the Royal College of Science and Technology, Glasgow, has received its charter as the fifth university in Scotland. This important development in university education in Scotland was described by Dr. S. C. Curran, principal of the College, in *Nature* of August 31, p. 843. It has now been announced that the new name of the Royal College is to be University of Strathclyde. Lord Todd, professor of organic chemistry in the University of Cambridge, has been elected the first chancellor. Dr. Curran is to be the first principal and vice-chancellor. The University of Strathclyde will naturally devote itself to the advancement of learning mainly through the fundamental and applied sciences. Unlike the other four universities in Scotland it will not have a Rector.

U.S. Air Force Office of Scientific Research : Dr. W. J. Price

Dr. William J. Price, former chief scientist of the Aerospace Research Laboratories, Wright–Patterson Air Force Base, Ohio, has been appointed executive director of the U.S. Air Force Office of Scientific Research, Washington. The Air Force Office of Scientific Research is responsible for the administration of grant and contract awards to industry, universities and other institutions whose proposals for research projects have been selected by the Air Force for support. Dr. Price was chief of the Modern Physics Research Branch at the Aerospace Research Laboratories prior to becoming chief scientist there in 1959. Since receiving his Doctorate in Physics from Rensselaer Polytechnic Institute in 1948, he has worked almost continuously for the Government. During 1949–54 he was associate professor of physics at the Air Force Institute of Technology (AFIT). In February 1954 he was appointed head of the Department of Physics there. He held this post until 1957, when he went to the Aerospace Research Laboratories as chief of the Modern Physics Research Branch.

The Finance of Education

The report of a conference on the finance of education, organized by the 1963 Campaign for Education, and

held on April 23, contains the text of the addresses of the two principal speakers, Sir William Alexander and Mr. J. Vaizey, together with a report of the discussion (*The Finance of Education*. Pp. ii+34. London: 1963. Campaign for Education, c/o Hamilton House, Mabledon Place, London, W.C.1, 1963. 2*s*. 6*d*.). Both speakers strongly urged a reduction in the number of education authorities from the present 146 to about 50, each with a population of about one million, but the discussions emphasized that there was no single answer to the problem of educational finance and that some of the proposals now being canvassed have disadvantages or dangers. Sir William Alexander pointed out that expenditure on education was increasing at the rate of about 9 per cent per annum and was already 4·9 per cent of the national product, while productivity was increasing by only about 2·5 per cent, and he did not think the present system could stand the strain of the next two decades. He thought a percentage grant system relating the contribution by the central Government directly to the expenditure that a local authority incurred on the education service was essential; however, he did not think that the financial problem could be considered by itself. Further, it was necessary to consider what was the highest proportion of the gross national product that Britain could invest in education without detriment to the national economy, and the total manpower she could invest in teaching without detriment to increased growth. Such fundamental questions required consideration by an independent commission. Mr. Vaizey suggested that these fundamental questions were more for consideration by the profession itself than by a Royal Commission, and he looked for the association of teachers with such developments just as the medical profession was associated with the National Health Service. While he welcomed the increased concern of the central Government with education and thought that the crucial question in the finance of education (in which higher education represented an increasing proportion) was the future of the rates as an educational tax, he stressed the importance of regional bodies. Prof. Bondi agreed as to the extent to which innovation and experiment came from the periphery, not from the centre.

Educational Statistics for Britain

The second volume of the educational statistics for 1962 has recently been published by the Ministry of Education (*Statistics of Education*, Part 2, 1962. Pp. 115. London: H.M.S.O., 1963. 20*s*. net). The topics dealt with include General Certificate of Education examinations, further education and teacher training (excluding, however, the universities), scholarships and awards, the school health service, school meals and milk, and educational building. As regards Advanced Level examinations for the General Certificate of Education, it is interesting to note the increasing number of candidates offering economics at that examination. During the past decade the number has increased from about 900 to 5,000. There are also considerable increases in the number of candidates passing science and mathematics; the number of girls passing these subjects has increased faster than the number of boys, but the disproportion still remains very great, except in the biological sciences. In the science group, mathematics passes have increased most in the past decade, and passes in the biological sciences least. The further education section contains figures on the colleges of advanced technology, containing 4,800 students on full-time, and a further 6,200 on sandwich courses. The statistics on students completing teacher training in 1961–62 are affected by the introduction of the three-year course in training colleges; of 7,700 qualifying in that year 3,400 were graduates, a figure which has not changed significantly during the past three years. The section on awards makes it possible to obtain some idea of the wastage rate of university students; of the local authority award holders 13·3 per cent of the men and 12 per cent of the women were classed as premature terminations, and a further 4·9 per cent of the men and 4·0 per cent of the women failed their final examination, thus indicating that just less than one in five of undergraduate award holders fails to complete his or her course satisfactorily.

Scientific Research in British Universities and Colleges, 1962–63

The 1963 edition of *Scientific Research in British Universities and Colleges* contains brief notes on scientific research in progress in British universities and colleges, and describes the nature of the projects in sufficient detail to indicate the scope of the research done by the various science departments and individual teams of investigators. The social sciences and industrial administration are not included except where work in these fields normally occurs within the science faculty or technology department. Information is supplied for 76 university colleges, institutes and medical schools, and 48 colleges of technology. Each university, college, etc., is divided up under subject headings giving staff, their qualifications and fields of research. A name index and a subject index are provided, also a list of addresses of the various universities and colleges, including telephone numbers. Immediate reference to any name or aspect of research is given by means of a duplex code system. The publication is sponsored by the Department of Scientific and Industrial Research under an arrangement with the British Council (1962–63. Pp. xxvi+670. London: H.M.S.O., 1963. 32*s*. 6*d*. net).

The Belgian University Foundation

The forty-second annual report of the Belgian University Foundation covering the year ended September 30, 1962, records twenty-four grants to postgraduate students during the year totalling 590,000 francs, subsidies to publications of some 2·8 million francs, of which 2·3 million francs were for periodical publications, and grants to scientific societies totalling 896,000 francs (Pp. 152. Brussels: Fondation Universitaire, 1963). It includes also the twenty-third annual report of its Bureau of Statistics, which gives the number of students in 1961–62 as 32,726, of whom 19·7 per cent were women. The largest number of students were in the University of Louvain, and 13·47 per cent of the total were in science, 25·16 per cent in medicine, pharmacy and related fields, 12·16 per cent in applied science, 13·17 per cent in commercial science, and 6·01 per cent in social, political or economic science. The report also deals with the prizes awarded by the Foundation, conferences and reunions held under its auspices, the centre for scientific societies and documentation services which it supports, including an index of periodicals, and lists twenty-six fellowships awarded for 1962–63 by the Belgian American Educational Foundation, as well as of bursaries. Notes are included on the activities of the various other institutions with which the Foundation has particular relations.

The Journal of the Association of Public Analysts

Analysts, consultants and chemists employed in the food and drugs industries will welcome the appearance of the quarterly *Journal of the Association of Public Analysts* (1, No. 1. Pp. 1–24. London: Association of Public Analysts, 1963, Annual subscription, 30*s*.). It is intended that this new journal will include analytical and technical contributions, correspondence, reports on legal matters and the Association's views on food standards. The first issue includes the following papers: "Detection of Insecticide Residues in Fruit and Vegetables", by P. S. Hall; "Determination of Chlorinated Pesticide Residues", by J. A. Potter; "Differentiation of Canned Processed and Canned Garden Peas", by A. Houlbrooke; "Analytical Data for Grapefruit Juice", by J. G. Sherratt and

R. Sinar; and "Determination of the Almond Content of Marzipan", by H. E. Monk. Minimum standards of composition are quoted for sausages, luncheon meat, flavoured milk, marzipan and candied peel. These represent the official views of the Association in relation to selected foods for which no legal standards exist. Under the heading "Items of Interest", D. R. A. Davies discusses some of the newer types of 'bacteria-free' milk such as 'Uperised' (Swiss) and 'Palarized' (Danish) which do not possess the 'boiled' taste and brownish colour of conventionally produced sterilized milk, and keep for at least a month. In their preparation, superheated steam is incorporated into the preheated milk, but automatic control should ensure that the composition remains unchanged. Two samples of 'Palarized' milk examined by Davies gave freezing-point depressions of 0·536° and 0·534° C, and he reported on them as "genuine despite the possible addition of water as steam". The *Journal* should become a very useful medium for the exchange of ideas, technical information, compositional data and analytical methods in the fields of food, drugs and water. Workers who are not directly involved in public analyst's work are cordially invited to send in contributions for future issues.

Australian Journal of Soil Research

RESEARCH workers in Australia in the numerous branches of soil science have earned an international reputation for work of the highest quality, and it is gratifying that the Commonwealth Scientific and Industrial Research Organization, in collaboration with the Australian Academy of Science, has arranged to produce a new periodical *Australian Journal of Soil Research* (1, No. 1 (February, 1963). Pp. 1–128. Melbourne: Commonwealth Scientific and Industrial Research Organization, 1963. 10*s*. per issue). It is intended to provide a means of communicating advances in the examination of soils and soil-plant relationships, and will be published only as the accumulation of suitable contributions dictates. The first issue contains 12 papers dealing with such topics as flow properties, water movement, aluminium compounds, polyphenolic constituents in leaves, weathering of dolerite and examinations of the Riverine Plain, New South Wales, and of æolian materials in Victoria.

Radiobiological Dosimetry

Radiobiological Dosimetry is one of a series of five reports issued by the International Commission on Radiological Units and Measurements which deal with various aspects of radiation measurements, and which have replaced the single and complete statement of the recommendations of the International Commission on Radiological Units and Measurements previously issued every three years (United States Department of Commerce: National Bureau of Standards. Handbook No. 88. Pp. vii+26. Washington, D.C.: Government Printing Office, 1963. 25 cents). The subject-matter has grown so much and, in some cases, has become so specialized that a single volume is no longer practicable. The report deals with radiobiological dosimetry and considers methods of improving the accuracy and intercomparability of dose measurements in radiobiology. Many conflicting results reported in the radiobiological literature no doubt stem from faulty dosimetry; in some papers essential data concerning the radiation source were either omitted altogether or described inadequately. There should be no more excuse for this if radiobiologists read the report on *Radiobiological Dosimetry*, since it sets out in clear terms the basic principles of dosimetry in radiobiological experiments, lists different classes of irradiation, and ends up with examples of the form in which exposure arrangements should be reported for publication. An appendix to the report contains a very useful description of various radiation quantities and units, including those, like kerma, which have been introduced only recently.

Antibiotic Dips for Preserving Fish Fillets

CHLORTETRACYCLINE (CTC) and oxytetracycline (OTC) have been used for some years in Canada for prolonging the storage-life of fish. A few instances have been reported, however, where dipping in antibiotic solutions has been found to be ineffective in commercial practice. The difficulties encountered are discussed in Bulletin No. 138 of the Fisheries Research Board of Canada, entitled "*Problems in the Use of Antibiotic Dips for the Preservation of Fresh Atlantic Groundfish Fillets*", by C. H. Castell and J. Dale (Pp. vi+70. Ottawa: Queen's Printer, 1963. 1.30 dollars). The Bulletin discusses the use of CTC dips and stresses that antibiotics cannot be expected to be effective if applied to fish that are already spoiling or to apparently good fillets with a relatively high *p*H. Most of the bacteria found on fish are sensitive to CTC, and increasing the concentration of the antibiotic up to (but not necessarily beyond) 25–30 p.p.m. causes a corresponding extension of the storage life. Different fish tissues absorb the CTC from solution at different rates and its activity is gradually lost during prolonged storage. Various substances cause a decrease in the effectiveness of the CTC, namely, strong oxidizing agents, strong alkalis and certain metallic salts, so it is recommended that such compounds should not be present in dipping solutions. It is probable that the greatest single cause for decreased effectiveness of CTC in preserving fillets is alkalinity derived from dissolved solids in water, alkaline detergents and the developed alkalinity in the muscle itself. Fillets dipped commercially for 10 sec in solutions containing 10 p.p.m. of CTC were found to contain 0·1–0·6 p.p.m. (cf. legal maximum 5 p.p.m.).

Palæontology in the U.S.S.R.

THREE further volumes of *Principles of Palæontology* (*Osnovy Paleontologii*) have been published (*Nature*, 197, 854; 1963): (1) Mollusca: Cephalopoda I, Nautiloidea, Endoceratoidea, Actinoceratoidea, Bactriodea; Ammonoidea (Agoniatitidea, Goniatitidea, Clymeniidea), edited by V. E. Ruzhenzev, written by seven authors (Pp. 438+82 plates, 417 text-figs. 1962. Price 4 *r*. 35 *k*.). (2) Porifera, Archaeocyathidea, Coelenterata, Vermes, edited by B. S. Sokolov, written by 16 authors (Pp. 485+57 plates, 421 text-figs. 1962. Price 5 *r*. 7 *k*.). (3) Arthropoda; Trachaean, Chelicerata, edited by B. B. Rodendorf, written by ten authors (Pp. 560+22 plates, 1,535 text-figs. Price 4 *r*. 92 *k*.). *Principles of Palæontology* is produced by the Palæontological Institute, Moscow Academy of Sciences (Lenin Prospect, 33, Moscow B-71) in collaboration with a number of other institutes.

Simon Memorial Prize

THE 1963 Simon Memorial Prize has been awarded by the committee of the Low Temperature Group of the Institute of Physics and the Physical Society to Prof. H. E. Hall, professor of physics in the University of Manchester, and Prof. W. F. Vinen, professor of physics in the University of Birmingham, for their work on liquid helium II. The Prize of £250 will be presented by the president, Sir Alan Wilson, at the Royal Institution, London, on October 28, when the prizewinners will give short addresses on their work. Visitors will be welcome; tickets are not required.

The Ramsay Memorial Fellowship

THE Ramsay Memorial Fellowships Trustees have made the following awards of new fellowships in chemistry for the year 1963–64. A General (British) fellowship to Mr. C. D. Akon at University College, London; a Canadian fellowship to Dr. W. F. Reynolds at University College, London; a Japanese fellowship to Dr. Shiori Ishino at the University of Reading; a Netherlands fellowship to Mr. F. B. van Duijneveldt at the University of Sheffield; a New Zealand fellowship to Miss Margaret Kershaw at the University of Reading; Spanish fellowships to Dr.

J. A. Leal Ojeda at the University of Hull and Mr. Vicente Gonzalez Gonzalez at the Grassland Research Institute, Hurley; United States fellowships to Dr. P. O. Offenhartz at the University of Cambridge, Dr. Violet Simmons at the University of Oxford, and Dr. E. A. Thiele at the University of Cambridge. The following fellowships for the same year have been renewed: Mr. D. T. Dixon (New Zealand fellowship), at the University of Reading; Dr. D. Vazquez Martinez (Spanish fellowship), at the University of Cambridge.

The Beilby Medal and Prize, 1964

Awards from the Sir George Beilby Memorial Fund are made by the Administrators of the Fund, representing the Royal Institute of Chemistry, the Society of Chemical Industry and the Institute of Metals. Sir George Beilby was president of each of these three bodies, and they jointly sponsored the appeal for subscriptions whereby the Fund was raised as a memorial to him after his death in 1925. The Beilby Medal and Prize, which consists of a gold medal and a substantial sum of money, is specified as being "For Advancement in Science and Practice". Such an award is now being offered annually, but more than one may be made on the same occasion if there are several candidates of sufficiently outstanding merit. Thus, two awards were made in 1963, each carrying a prize of 100 guineas. The awards are made to British scientists in recognition of independent original work of exceptional merit, carried out continuously over a period of years and involving the development and application of scientific principles in any field related to the special interests of Sir George Beilby, namely chemical engineering, fuel technology or metallurgy, in their modern interpretations. The awards are intended as an encouragement to men and women (preferably under the age of 40) who have done distinguished work of practical significance in any of these fields. Awards for 1964 are shortly to come up for consideration. Outstanding work of the nature indicated may be brought to the notice of the Administrators, either by persons who desire to recommend the candidate or by the candidate himself, not later than December 31, by letter addressed to the Convener of the Administrators, Sir George Beilby Memorial Fund, the Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

The Perkin Centenary Trust: Awards

The Perkin Centenary Fellowship for 1963 has been awarded to Mr. N. S. Rao (India), who has been a post-graduate student at the University of Birmingham for the past year. The Fellowship will be tenable from October 1 in the Department of Chemistry, Imperial College of Science and Technology, London, where Mr. Rao will work under the supervision of Dr. T. S. West. Perkin Centenary Scholarships have been awarded to Mr. H. A. J. Butterworth (Ashton-under-Lyne), tenable at the Royal College of Advanced Technology, Salford, and to Mr. C. R. Laycock (Bradford), tenable at the Liverpool College of Technology. Mr. Butterworth and Mr. Laycock are laboratory assistants with the Clayton Aniline Co., Ltd., and the Technical Service Department of the General Industries Division of Allied Colloids (Manufacturing) Co., Ltd., respectively. The Perkin Centenary Trust was established as a memorial to the discovery in 1856 of the first important synthetic dyestuff, 'mauveine', by William Henry Perkin. The centenary of this event was acknowledged by celebrations held in London in 1956 and widely supported by many organizations having an interest in chemistry, including the Royal Society, the Chemical Society, the Society of Chemical Industry, the Society of Dyers and Colourists, the Royal Institute of Chemistry, and the Association of British Chemical Manufacturers.

Courses on Management

The wide variety of courses described in a brochure published by the Urwick Management Centre is a remarkable tribute to the way in which management education is developing in Britain. The courses include short seminars for top management, longer courses for potential managing directors and for heads of organization and methods departments, as well as a mixture of seminars and courses on various aspects of marketing and selling and on production planning and control. Among the more specialized courses are a series specially designed for the construction industries and a course specially designed to show the application of network analysis techniques to industrial and commercial problems. Scientists should be particularly interested in the courses and seminars dealing with the management of research. The content of each course and seminar is described in the brochure as well as the way the course or seminar is arranged. Full details of the Centre, which has been in existence for fifteen years, and receives annually some 700 executives, may be obtained from the Director, Urwick Management Centre, Baylis House, Slough, Bucks.

Second International Congress of Medical Photography and Cinematography

It has been found impracticable to organize the second International Congress of Medical Photography and Cinematography in Great Britain in the foreseeable future, owing to the fact that, apart from a few notable exceptions, the support which was expected from many quarters has not been forthcoming. However, it is hoped that it may be possible to organize a limited number of small international symposia on specific problems associated with photography, cinematography and television in the service of medicine. Further information can be obtained from Mr. K. Moreman, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2.

Announcements

The Kamerlingh-Onnes Medal of the Netherlands Society for Cooling Technique has been awarded to Dr. Franklin Kidd and Mr. C. West for their work on the influence of temperature, moisture and composition of gases on the behaviour of fruit.

Dr. H. Lehmann has been appointed University biochemist to Addenbrooke's Hospital, Cambridge, as from November 1, and the Medical Research Council's Abnormal Hæmoglobin Research Unit, of which he is honorary director and which has been housed at St. Bartholomew's Hospital, London, will move with him to Cambridge.

The next award of the Meldola Medal, the gift of the Society of Maccabaeans, will be made early in 1964 to the chemist who, being a British subject and less than 30 years of age on December 31, 1963, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Institute of Chemistry before December 31, 1963. Further information can be obtained from the President, the Royal Institute of Chemistry, 30 Russell Square, London, W.C.1, the envelope being marked "Meldola Medal".

A course of six lectures on "Recent Advances in Virology" will be held at the South-east Essex Technical College, Dagenham, on successive Thursdays commencing October 31. Further information can be obtained from the principal, Dr. F. Heathcoat, South-east Essex Technical College, Longbridge Road, Dagenham.

The Thomson Lecture of the Society of Instrument Technology will be delivered by Prof. Dennis Gabor at the Royal Institution on October 24. The subject of Prof. Gabor's Lecture will be "Technology, Life and Leisure". Admission is by ticket only, which can be obtained from the Secretary, Society of Instrument Technology, 20 Peel Street, London, W.8.

THE U.K. ATOMIC ENERGY AUTHORITY

THE accounts of the Atomic Energy Authority for the year ended March 31, 1962*, were issued last April, together with the report of the Comptroller and Auditor-General, Sir Edmund Compton. They are now included in the ninth annual report of the Authority for the year 1962–63†, which adds a further appendix comparing the 1962–63 grants and the 1963–64 estimates, which stand at £108,124,000 and £114,897,000 gross receipts—the latter bringing the net expenditure to £67,876,000 and £60,204,000, respectively. Of the gross total, salaries, etc., account for £43,185,000 and £42,198,000, respectively; research, development and design contracts with industry, universities, etc., for £4,881,000 and £4,612,000; contributions towards the International Atomic Energy Projects Dragon and Halden for £2,329,000 and £2,195,000; and grants to the National Institute for Research in Nuclear Science for £7,205,000 and £8,445,000, respectively.

While the form of the balance sheet for 1961–62 is substantially changed from previous presentations, as a result of some change in security requirements, and the published information has been expanded, the operating account is still withheld from publication on security grounds. General research in the year ended March 31, 1962, is put at £40,557,186, weapons research, excluding military contracts, at £8,263,215, and grants to the National Institute for Research in Nuclear Science at £5,278,470 and to international projects at £1,809,115. Moreover, in the Consolidated Civil Trading Account, which shows a net profit of £24,080, contributions to research and development appear at £591,423, compared with £632,153 in the previous year. Considering the prospective future income of the Authority for the civil use of nuclear energy, Sir Edmund notes that in 1957 the Authority concluded that the possibilities of the magnox reactor system, derived from the Calder Hall reactor, would be largely exhausted in the first stage of the nuclear power programme. Further development of the advanced gas-cooled reactor would permit higher operating temperatures and fuel ratings with consequent greater efficiency. The construction of such a prototype was approved in December 1957, and, when completed in 1962, this had cost about £9·7 million, while some £10 million capital and current expenditure had been incurred in the development and production of beryllium for canning the fuel elements—a method of canning later found to be unsatisfactory and abandoned in favour of stainless steel. Further expenditure will be incurred in developing the system to full commercial use, and Sir Edmund adds that the Electricity Boards are not committed to the adoption of this system and that the Authority's own view is that the adoption of a particular system by the Generating Boards in the United Kingdom or overseas should await the demonstration of its feasibility, safety and economics.

Expenditure on the development of magnox fuel elements for civil use is charged partly to the Exchequer as non-trading expenditure and partly to trading, and an item of £14,047,382 for major development projects, charged to the Exchequer, includes some expenditure on research and development in support of the nuclear power programme. 'Genetic' development, in which the basic designs of the reactor and its fuel are established, and the feasibility, reliability and economics of the system are demonstrated, is regarded by the Authority as forming part of its statutory obligation to undertake civil research and development. After commercial exploitation begins, the cost of development is regarded as part of the cost of production and charged in the Trading Account. As from April 1, 1962, expenditure on further development of magnox fuel elements became regarded as appropriate to the commercial exploitation phase and sales prices scheduled for the following five years.

In the Authority's annual report the percentage deployment of effort between reactor projects in terms of qualified scientists and engineers, excluding basic research, is now given as follows: advanced gas-cooled reactor, 23; steam-generating heavy water reactor, 15; fast reactor, 36; civil marine-propulsion reactor, 13; and high-temperature gas-cooled reactor, 13. Generation of electricity from the magnox reactors at Calder Hall and Chapelcross increased by 13 per cent, and the reactors of the Central Electricity Generating Board at Bradwell and Berkeley have started delivering to the grid, as did the advanced gas-cooled reactor at Windscale in February, while by March 31 the Dounreay fast reactor had generated 2·5 million kWh, of which 10 per cent had been exported to the grid. Nor was progress confined to the reactor field. During the year, the world's first two cobalt-60 industrial irradiation plants, designed specifically for sterilization of medical products, began continuous operation, one at Slough and the other at Edinburgh. Both were built under licence from the Authority following large-scale experiments at the Wantage Research Laboratory. On the other hand, with the meeting of the Government's requirements for enriched uranium for defence, it was decided to reduce the output of the diffusion plant at Capenhurst to the minimum level necessary to keep the plant in product; a substantial reduction of manpower at the factory had to be effected by the end of 1962. About 50 per cent of the redundant employees have found alternative employment inside or outside the Authority.

Of the expenditure in 1962–63 on the Authority's programme of civil research and development of £48 million, some £35 million was current expenditure and £13 million capital facilities. Of 2,825 graduates or professional scientists or engineers employed on the programme at March 31, 200 were working for other bodies on repayment; 1,880 were engaged on the reactor development programme and the reactor systems research, 470 on general research, 190 on plasma physics and fusion research, and 85 on isotopes research. Some account of the research on reactor systems, which includes nuclear data for reactor calculations, experimental measurements in reactor physics and heat transfer in reactors, as well as instrumentation, fuel materials, canning and structural materials, and moderators and coolants, is given in a separate chapter in the report.

The ninth report also includes chapters on general research, on plasma physics and fusion research, on nuclear weapons and test direction, on commercial operations and raw materials, on health and safety, on information services, and on the Authority's international relations and on staff. Reductions in manpower raised considerable problems and recruitment was on a much lower scale, but the lower number of graduate scientific and engineering recruits was broadly sufficient for the reduced need; in 1963 the reduction will be mainly on the engineering side, but some scientific entrants will be required. Critical

* Atomic Energy Authority Act, 1954. Accounts 1961–62: Balance Sheet of The United Kingdom Atomic Energy Authority as at 31 March, 1962, and Accounts for the year ended 31 March, 1962; together with the Report of the Comptroller and Auditor-General thereon. (House of Commons Paper No. 187.) Pp. 14. (London: H.M.S.O., 1963.) 1*s.* 6*d.* net.

† United Kingdom Atomic Energy Authority. Ninth Annual Report for the period 1st April, 1962–31st March, 1963. Pp. i x + 87 + 7 plates. (London: H.M.S.O., 1963.) 6*s.* 6*d.* net. (See also p. 1121 of this issue of *Nature*.)

appraisal of training methods continued, and the variety and scope of internal training was wide, with special emphasis on the training of industrial supervisors. At the Harwell Reactor School, which was attended by 522 students, of whom 26 were from overseas and 262 were Authority staff, two new courses for Authority staff were an extension course in reactor engineering and a course on applied mathematics for engineers; a course for university engineering staff and one for science masters were also held. At the Calder Operations School, where 711 people (213 from overseas) have now attended, standard courses were held for physicists and engineers, and a new course for lecturers at universities and technical colleges.

Some research is included in the Authority's health and safety programme, for example, measurements of radioactivity in air and in rain-water, the study of radiological protection, including the development of instruments for precise measurements of radiation dose-rates down to very low levels and the determination of plutonium in urine and in the body. There was a marked increase in the Authority's commercial activities. Sales of radioisotopes rose from £1·4 million in 1961–62 to £1·473 million, of graphite from £2·843 million to £3 million, and of reactor fuel from £9·6 million to £22·5 million. This year the chapter on general research discusses the programme as a whole, which, on the basic side, aims to supply fundamental scientific knowledge for future developments of nuclear energy, and also to supply the scientific understanding needed to solve problems arising during the rapid development of the present programme. Much of the general applied research programme is concerned with the interactions of neutrons and other radiations with matter on both the nuclear and atomic scale.

In nuclear chemistry, work continued on the determination, by both radiochemical and mass spectrometric methods, of the proportions of fission product atoms formed with various mass numbers in fission by low-energy neutrons. In theoretical physics, special attention was directed to investigations of the magnetic and electronic properties of transition metals and their alloys, the properties of defects and defect clusters in metals, and the scattering of neutron beams by liquids and solids. In the physics of the solid-state, the individual atomic contributions to the magnetization of ferromagnetic alloys were identified by neutron diffraction techniques, and the weak magnetic properties of vanadium–chromium alloys, in conjunction with other physical measurements, have established the validity of the band models of electronic structure of these alloys. Other research on radiation damage is now concentrated on elucidating the underlying atomic mechanisms, and at the Atomic Weapons Research Establishment the electrical properties of semiconductors are being exploited to provide a sensitive method for analysing mechanisms of radiation damage. A new variable energy cyclotron is being built at Harwell for general studies on the physical and chemical effects of radiation. Research continues on the physical metallurgy of uranium, plutonium and other metals of technical interest as well as on the physical and chemical properties of the oxides, carbides, nitrides and sulphides of fissile and fertile elements. Besides chemical engineering research in the role of bubbles in gas and solid movement, an extensive programme of research is being undertaken in electronics.

The Culham Laboratory programme remains concerned with stability studies on a wide range of plasma containment systems, including both pinch configurations and magnetic traps. *Zeta* remains the main toroidal pinch experiment, and has been modified to increase both the initial rate of rise of current in the discharge and the time during which the current is held constant after the initial rise. Besides the study of several linear pinch systems, three methods of injecting particles into a magnetic mirror trap are being studied. Effort has been devoted to the production of computer programmes for magnetohydrodynamic and electromagnetic problems, and a long-term programme of technological studies to support the laboratory's scientific programme has been established.

BIOLOGY OF RADIOIODINE

THE Hanford symposium on "The Biology of Radioiodine" was held in Richland, Washington, during July 17–19, under the joint sponsorship of the U.S. Atomic Energy Commission and the Hanford Laboratories of the General Electric Co. (contract No. *AT*(45–1)–1350 between the Atomic Energy Commission and the General Electric Co.). About 150 visiting scientists from Belgium, Canada, France, India, Japan, United Arab Republic, United Kingdom, West Germany and the United States joined more than 70 scientists of Hanford Laboratories for two and a half days of formal presentations and a tour of the facilities of the Laboratories.

Participants were welcomed to the sessions of the first day by Dr. H. A. Kornberg, manager of the Biology Laboratory of Hanford Laboratories. He noted that this symposium on radioiodine was the second in an annual series, the first having been held in May 1962, on the "Biology of the Transuranium Elements", under the general chairmanship of Dr. R. C. Thompson. The third symposium, to be held in the spring of 1964, will be concerned with the "Biology of Radioactive Particles and Gases" and will be under the general chairmanship of Dr. W. J. Bair.

The symposium was devoted to evaluating the nature, effects and control of radioiodine in the biosphere under the following four general categories:

(1) "Physical Origin and Dispersion of Radioiodine", which covered sources of environmental radioiodine in nuclear industrial operations, accidents and weapons tests, and the nature and form of iodine dispersed and potentially available to the biota.

(2) "Biological Disposition of Radioiodine", which concerned entry of iodine-131 into and passage through biological systems and included uptake of different radioisotopes of iodine by means of various routes of entry in several species of animals, including those in an aquatic environment.

(3) "Biological Effects of Radioiodine", which emphasized comparative early and late effects of single and prolonged exposure in young and adults of various species and included carcinogenic effects on the thyroid of iodine and X-irradiation.

(4) "Prophylactic and Therapeutic Measures for Radioiodine", covering protective measures to reduce the thyroid uptake of radioiodine or its effects on the thyroid.

Principal fields within these general categories were reviewed by authorities and each review paper was followed by brief reports of unpublished work from many laboratories. Only a few of the fifty papers are here summarized.

The session chairmen were Drs. S. A. Lough and H. D. Bruner of the U.S. Atomic Energy Commission, Dr. A. H. Wolff of the U.S. Public Health Service, Dr. B. M. Dobyns of the Cleveland Metropolitan General Hospital, Dr. E. E. Pochin of the University College Hospital Medical School, London, Dr. Stuart Lindsay of the San Francisco Medical

Center at the University of California, and Dr. C. L. Comar of Cornell University: Dr. Shields Warren was the honorary general chairman.

In a succinct "Statement of the Problem", Dr. H. D. Bruner (Division of Biology and Medicine of the U.S. Atomic Energy Commission) began the scientific sessions by giving a brief history of radioiodine and pointing out the problem areas of concern. Historically, he related, the thyroid was considered a radio-resistant organ, and not many years ago it had been assigned a permissive yearly dose of 30 rems. Following reports of increased thyroid tumour incidence in children as a consequence of exposure to X-irradiation early in life, the Federal Radiation Council recommended a decrease in annual exposure, concluding that a thyroid radiation dose from iodine-131 of 0·5 rem/year would call for some control measures. As a result, Dr. Bruner pointed out, a dose of 0·5 rem has thus become a limiting case for normal peacetime operation.

J. Z. Holland (Division of Biology and Medicine, U.S. Atomic Energy Commission) comprehensively reviewed the origin and disposition of radioiodine. He compared the relative biological significance of the several radioisotopes of iodine, concluding that even in cases of heavy environmental contamination with the shorter-lived radioiodines, the delays in the food chain would tend to reduce their relative importance, with the result that longer-lived iodine-131 is of unique importance. The availability of parent tellurium (of radioiodine isotopes) could conceivably modify this conclusion; however, data presented at the symposium indicated that tellurium-132–iodine-132 would not represent a significant hazard to man following a contamination event involving pastures grazed by cows, etc.

In his review, Mr. Holland pointed out that one may expect extreme irregularity in the distribution of iodine-131 following a release incident. This results from the interaction of its relatively high abundance and short half-life with the buoyant rise imparted by the releasing event and the atmospheric eddies and rain clouds acting over periods of a few hours to days. Additional complications are introduced by the behaviour of iodine precursors during releases and by the chemical behaviour of iodine. Mr. Holland pointed out that iodine reacts with many materials, including trace substances in the atmosphere, and that it is readily oxidized and reduced through a wide range of valence states. The only conclusion which could be drawn at the present time regarding the partitioning of iodine-131 between vapour and particulates, soluble and insoluble forms and among elemental, reduced and oxidized states is that none is clearly dominant over any great range of conditions. Of interest, too, was Holland's observation that after deposition iodine-131 apparently sublimes and may be re-cycled by means of the atmosphere. However, it was noted by work in two laboratories that up to 90 per cent of the iodine in old fall-out may be in the particulate form, but that the iodine could be quite easily leached, the majority being in the reduced state. Releases from separations plant stacks at Hanford Laboratories were observed to be mainly in a gaseous state for at least 25 miles. Measurements performed at Harwell of stable iodine in the atmosphere indicated most of it was also in a gaseous state.

Dr. L. Machta (U.S. Weather Bureau) concluded the first session by explaining that three meteorological mechanisms can probably explain all incidents of higher-than-normal iodine-131-levels in the Public Health Service milk surveillance network since September 1961: (1) low-altitude transport of debris from atmospheric and vented underground tests in Nevada; (2) subsidence within an anticyclone; (3) rainout of debris moving in the troposphere or even in the lower stratosphere.

Prof. M. Eisenbud (New York University) pointed out that although there have been about twenty years of large-scale experience with iodine-131, we do not, as yet, have the correct yardstick with which to estimate the dose that would be received by individuals in an environment where the air or water is contaminated with iodine-131. He predicted that direct measurements of the radio-iodine content of milk would be for some time the indicator of choice when it is necessary to appraise the public health significance of environmental contamination by iodine-131.

Two reports dealt with the problem of deposition of iodine on to plants from airborne iodine-131. In the first, Drs. Barry and Chamberlain (Atomic Energy of Canada and U.K. Atomic Energy Authority, Harwell, respectively) stated that the absorption of iodine-132 on to leaves of the broad bean is controlled by the rate of diffusion of the gas through stomata. They found that in dry air the rate of deposition was one-tenth or less that in humid air. Dr. F. P. Hungate *et al.* (Hanford Laboratories) observed distinctly greater deposition of freshly generated iodine-131 inside leaves as compared with less pure and more particulate-laden off-gas from a melted fuel element. Translocation to other plant parts was slight.

Dr. C. P. Straub and J. H. Fooks summarized some co-operative field investigations involving environmental factors that influenced level of iodine-131 in milk. Of special interest were the results of an investigation performed in co-operation with the St. Louis Health Department. They observed that less iodine-131 was found in the milk of cows grazing well-fertilized pastures.

Under the general subject of "Comparative Uptake of Iodine by Different Species", more than twenty papers were presented. In the area utilizing animals as biological monitors, the work reported extended from using rabbits around the National Reactor Testing Station in Idaho to observations in thyroids of North American deer in many locations and thyroids of pigs in Michigan. The use of the latter species was suggested because their thyroids always showed higher iodine-131 concentrations than the human or fœtal thyroids sampled. Substantial uptake of radioiodine by means of the skin was reported. It was noted by workers at Hanford Laboratories that thyroid uptake of iodine-131 after the application of a $Na^{131}I$ solution to either a clipped area of skin or to a hairless area of sheep was about a third that observed following oral or parenteral routes of administration.

Of interest to medical diagnostic laboratories was the report that thyroid metabolic techniques are feasible with small doses of 1–10 nc. iodine-131 using a method described by Dr. M. A. Van Dilla and M. J. Fulwyler (Los Alamos Scientific Laboratory).

A series of papers from the Institute of Industrial Medicine, New York University, included means for estimating the distribution of thyroid doses due to iodine-131 in an exposed population, the dose of iodine-131 to human thyroids in New York City from nuclear tests in 1962 (up to 40 mrads) and on thyroid weights of a large number of adults (they suggest a mean weight of 16·7 g, which is less than at present considered for the standard man, that is, 20 g).

In a review of several investigations, Dr. G. Gaffney (U.S. Public Health Service) reported, under the general topic of "Radioiodine in Man", that at Brookhaven Drs. S. Cohn and E. A. Gusmano could not detect iodine-131 in thyroids of new-born whose mothers had thyroid burdens up to 230 pc. and milk values up to 100 pc./qt. Work at Northeastern Radiological Health Laboratory by Drs. F. Visalli and A. S. Goldin indicated that in children ingestion was the principal mode of entry of iodine-131, and thyroid uptake of iodine-131 could be suppressed but not blocked in children by giving 0·5 mg of iodine-127/day. Moreover, he and his co-workers had found the vehicle of intake of iodine-131 from fall-out was fresh foods, principally milk. However, uptake in man and animals'

thyroids was similar whether the iodine-131 was in a water solution or in milk, according to Dr. C. L. Comar and co-workers at Cornell.

Dr. R. J. Garner reviewed the available information on the comparative early and late effects of single and prolonged exposure to radioiodine in young and adults of various animal species. He emphasized the work on the animals of economic importance, which included chiefly the sheep, pig and cow, and took special note of the work performed at Hanford Laboratories since 1950 and work at the Compton Research Station on cows by Garner and associates. Garner's review served to point out the radioresistance of the thyroid gland of the domestic animal, with doses in excess of 5,000 rads being required before any sign of damage can be expected to appear.

H. Hollister of the Division of Biology and Medicine discussed the problems of estimating the hazards from radioiodine following a nuclear attack. In his paper, Mr. Hollister defined hypothetical nuclear attack situations and considered how information on the deposition and intake of radioiodine would be used to arrive at an estimate of the exposure dose to man and to the effect on grazing animals. He pointed out that interpretable information is needed at dose-levels quite in contrast to those of usual concern for weapons testing and reactor accidents.

Dr. I. Doniach (London Hospital Medical College) then presented a comprehensive review of "The Effects, Including Carcinogenesis, of Iodine-131 and X-rays on the Thyroid of Experimental Animals". Results of various investigators show a similarity in biological action of X-rays and iodine-131 on the thyroid. In the measurement of four different effects, 1,000 rads from X-rays has a similar quantitative effect to a calculated mean dose of about 10,000 rads from iodine-131. This comparative effect was somewhat less than the twenty-fold greater effectiveness of X-rays compared with iodine-131 reported by Dr. R. O. McClellan and associates at Hanford Laboratories on the early changes seen with these two forms of exposure in sheep. Dr. S. Lindsay, in this same session, concluded that thyrotropic stimulation after sub-total thyroidectomy is a promoting factor in the development of both benign and malignant thyroid neoplasms in the rat and that it may also initiate the development of some malignant neoplasms. In an extension of these findings in animals, Dr. E. L. Saenger *et al.* presented a very complete analytical review of the available data on the carcinogenic effects of iodine-131 compared with X-irradiation in man. These workers concluded that thyroid carcinoma will only rarely develop with X-ray doses of 2,000–6,000 r. after a latent period of 10–35 years, and that it is probable that X-ray doses of 100 r. or greater to children's thyroids will increase the incidence of carcinoma. Irradiation of the adult thyroid with iodine-131 cannot as yet be implicated as a carcinoma-causing mechanism; however, doses of iodine-131 to children seem to increase the susceptibility of their thyroids to neoplastic change. Dr. Saenger stated that the carcinogenic dose-level for a child from iodine-131 would probably not be below 600 rads, considering the differing dose rate between iodine-131 and X-rays, fractionation of dose and irregular distribution.

In the only paper in the session devoted to carcinogenic effects of iodine-131 compared with X-irradiation in man, Drs. Dolphin and Beach (U.K. Atomic Energy Establishment, Harwell) reported on a survey of the literature which they had performed. They concluded that the latent period from tumour development appeared to be independent of the thyroid dose in the dose range considered ($< 40{,}000$ rads), but did show a fairly distinct bimodal response. They estimated a total thyroid tumour incidence of $3{\cdot}5/10^6$ people/rad.

Dr. F. W. Lengemann, in his review of "Prophylactic and Therapeutic Measures for Radioiodine Contamination", pointed out that the best policy is to prevent the entrance of iodine-131 into the food chain since this is apparently the major route of contamination for those not occupationally exposed. Because milk appears to be the chief vector of iodine-131 in the United States and some other countries, he proposed four means of limiting the access to the food chain by means of milk: (1) eliminate milk from the diet; (2) using ion-exchange columns to remove radioiodine; (3) feeding milk-producing animals with stored feed; (4) the substitution of fresh milk by stored milk or milk products. An added consideration was the use of thyroid-blocking agents at a number of places in the feed–milk–man cycle. The foregoing possibilities were expanded into an interesting discussion of some of the advantages and disadvantages of each.

Dr. W. J. Bair *et al.* (Hanford Laboratories) stated in their brief paper that the release of stable iodine simultaneously with a release of iodine-131 to reduce the thyroid uptake of iodine-131 is feasible on a biological basis, but that physical and chemical phenomena accompanying the release of iodine vapours must be considered in a final evaluation of this procedure as a method of decreasing the hazard of reactor-released iodine-131.

Dr. Stumpf, reporting on work performed with Prof. E. H. Graul *et al.* at the University of Marburg, proposed, on the basis of their investigations on elimination of iodine-131 that: (1) a combined treatment with thyroid-stimulating hormone and potassium perchlorate be used to give optimal results at time intervals exceeding 24 h after administration of iodine-131; (2) gastric lavage (with injection of potassium perchlorate) be used within the first few hours following exposure to iodine-131; (3) ingestion of salivary secretions be avoided.

In the final paper of the symposium, E. C. Watson (Hanford Laboratories), reporting on a co-operative endeavour, noted that increasing the cow's dietary intake of stable iodine to 2 g/day reduced uptake of iodine-131 by the thyroid of milk drinkers by 50–80 per cent. Other workers from Hanford Laboratories noted that by increasing the stable iodine content of the diet of the cow from 5 mg to 2 g/day, levels of iodine-131 in milk decreased by 50 per cent and the cow's thyroid burden was reduced by more than 90 per cent.

One of the highlights of the entire symposium was a very spirited, timely and thought-provoking banquet speech in which Prof. E. E. Pochin addressed the question: "What is a Permissible Dose ?" "Perhaps it is more reasonable to look at what is a permissible risk", he said, and went on to cite some interesting comparative levels of risk in established industries to-day, based on fatal accidents per year or per million people employed. Prof. Pochin stated that, although we still have a great deal to learn about quantitation of risks in radiation exposure, we have even further to go in attempting to estimate quantitatively the benefits that may be associated with any procedure involving radiation exposure. "In the equation between hazard and benefit", he stated, "it is silly to be tidy-minded about the left-hand side of the equation if we have no figures on the right-hand side with which to compare it. I am sure that more quantitative thinking is needed on the benefits as well as on the risks". Prof. Pochin went on to say that the attempt to set levels of radiation protection on the quantitative basis is very necessary, but a very ambitious one. Many of the difficulties and also criticisms arise simply from the fact that no comparable attempt is being made to deal as quantitatively with many of the other hazards of life or work. This, in fact, may be one of the greatest values of radiation protection work—it may extend the use of hazard evaluation into other fields and there promote development studies of the justification for risks on a factual basis.

Collectively, the papers presented at this symposium represented the present state of our developing knowledge and should promote proper evaluation of the hazards of radioiodine.

L. K. Bustad

THE ONTARIO RESEARCH FOUNDATION

THE annual report of the Ontario Research Foundation for 1962* includes the reports of the director, Dr. A. D. Misener, and the director of research, A. E. R. Westman, the financial statement, lists of scientific and technical papers published during the year and of research awards and details of staff and advisory committees, etc. Contract research for industry increased from 34 contracts, valued at some 522,000 dollars, in 1961, to 48, valued at 660,000 dollars, in 1962, largely as a result of the activities of the project development group. Reference is made to the new research community developing around the Foundation's new location west of Toronto. The Canadian Gas Association has now taken over responsibility for operating the gas appliance testing facilities set up and operated by the Foundation since 1956. Unsponsored research in the Department of Biochemistry was concerned with selectivity in hydrogenation and the molecular structure of fatty acids, and in a search for new antibiotics from insects two compounds were isolated, one being identified as a small peptide or protein, and the other as a substituted quinone. A microbiological examination showed that in the biological treatment of wastes and sewage no flocculation occurs if the bacterial cells are broken up.

In the Department of Chemistry, a kinetic study was made of reactions of active nitrogen with simple alcohols, and the structure and thermal stability of molecular complexes of urea with long-chain hydrocarbon derivatives and the mechanism of retardation in the setting of plaster of Paris were investigated. Research on phosphate glasses was concerned mainly with constitution studies by means of paper chromatography, and studies on nucleation and crystallization in metaphosphate glasses continued. Metal physics research in the Division of Engineering and Metallurgy included the fundamental investigation of ultrasonic-wave attenuation in elastically strained ferrous materials in an effort to correlate dislocation and domain movements with metal breakdown or fatigue. The investigation was extended to pure metals, such as iron, molybdenum, tantalum and niobium, and an investigation of the ductile-brittle phenomenon in Armco iron at low temperatures by ultrasonic methods was started, as well as an investigation of the iron–carbon–uranium alloy system. In the Ore Dressing Division, a 'flash roaster' was further developed, particular attention being directed to burner design, and several applications of ultrasonic energy were tentatively explored.

The Department of Parasitology continued its studies of parasites occurring in the mammals, birds and fish of Ontario, and an investigation of tapeworms of foxes and dogs suggested that infection with the larval stage of *Taenia crassiceps*, due to eating mice, results in resistance to infection, which is probably due to the production of certain types of white blood cells. The determination of the life-history of 'throat-bots' in deer was concluded and the research has demonstrated the production of two generations of flies each year and the slow growth of bots in the throat in winter. Radioactive phosphorus was used to measure the size of blood meal, frequency of feeding, longevity and flight range of black flies feeding on ducks. In the Department of Physics, application investigations of the atmospheric electron probe continued, experimental studies in vacuum technology continued, and work was completed on a new type of high-voltage heat-to-electricity converter. The Department of Physiography continued to extend its physiographic survey into Northern Ontario with the mapping of surface deposits in Muskoka, Parry Sound, and the main glacial features were detected by a transverse along the Canadian National Railway between Fossmill and Petawawa and by aeroplane flights over Algonquin Park. Field study of the effect of weather on corn growth and development continued on a reduced scale, and investigations on the influence of Lakes Erie and Huron on the Ontario climate also continued, and microclimatic investigations of light intensity within the crops, soil moisture, etc., for alfalfa and bromegrass. In the Textiles Department, work on the molecular cross-linking of cellulosic fibres to improve certain properties of cotton and viscose rayon fabrics, and on the elastic behaviour of wool and hair, have been resumed.

* Ontario Research Foundation. Annual Report 1962. Pp. 66. (Toronto: Ontario Research Foundation, 1963).

THE NATIONAL CHEMICAL LABORATORY

THE report of the National Chemical Laboratory, 1962, includes the reports of the Steering Committee and of the Director*; that of the Director includes accounts of the work of the six research groups and lists of publications during the year. The planned transition of the work of the Extraction of Metals Group from a programme entirely devoted to work for the Atomic Energy Authority to one of basic research as part of the Laboratory's own work proceeded smoothly. The Steering Committee considers that increased effort in chemical thermodynamics, particularly of inorganic substances, is needed, and that the Corrosion of Metals Group should be expanded to permit study of the corrosion problems of some of the important new metals such as vanadium, niobium and titanium, and also corrosion problems at high temperatures. It does not consider that the Laboratory should at present undertake research on fuel cells, unless requested to do so, under contract, by some other organization. It considers, however, that co-operative arrangements, such as that under which an industrial firm has seconded a member of its own staff to work in the Laboratory for three years on thermodynamic measurements of phosphorus compounds, should be strongly encouraged in view of the complex apparatus involved. It has been agreed that the Properties of Pure Materials Research Sub-Committee shall cease to exist as a joint sub-committee of the Laboratory and the National Physical Laboratory, but the sub-committee has been renamed the Chemical Thermodynamics Research Sub-Committee and will advise the Steering Committee on such work at the National Chemical Laboratory.

The Director's report directs special attention to the promise of the application of high-temperature flame technology by the Extraction of Metals Group in mineral processing, for example, to fuse and quench individual beryl particles with the minimum of interaction with accompanying gangue material and the virtual elimination of container material problems. The research sponsored by the Atomic Energy Authority in the same Group on

* Department of Scientific and Industrial Research. Report of the National Chemical Laboratory 1962: Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1962. Pp. iv+91+4 plates. (London: H.M.S.O., 1963.) 6s. 6d. net; 1.30 dollars.

the extraction of uranium and the chemical processing of low-grade beryllium ores has been further curtailed and the effort devoted to fundamental problems in inorganic chemistry increased, while besides flame technology other new techniques like the rotary contractor for solvent extraction from pulps are being applied in a wider context. In the Inorganic Group, studies on the separation of the rare earths have now ceased, as have those on the ultra-purification of elements, and interest has now shifted to the solid-state chemistry of rare-earth compounds. The catalytic properties of europium are proving of interest, as are also the reflectance spectra of the rare earths, while the section set up to work on X-ray diffraction has been widely used and a new section created to deal with electrochemistry.

Considerable changes are taking place in the organic chemical work of the New Materials Group, where the work on boron–nitrogen polymers is being tapered off and comparable study of the less-known organo-aluminium–nitrogen compounds has led to the characterization of low-polymeric substances of a new type and unusual molecular structures. Progress is reported in the synthesis and study of organic semiconducting material and in the understanding of intermolecular electron-transfer processes. Work on the physical chemistry of ion-exchange has now permitted the relations between the selectivity of a resin, its degree of cross-linking and its exchange capacity to be framed in rather general terms, and these investigations, and those of the selectivity and transport process in ion-exchange membranes, are relevant both to technological applications of such materials and to an understanding of membrane phenomena, etc., in biological systems.

The Chemical Thermodynamics Programme has entered a highly productive phase, and during the coming year the responsibilities of the Group will be extended to include a more general concern with chemical physics. The Laboratory is equipped with both static and rotating bomb calorimeters, and besides measurements of the thermodynamic properties of organic oxygen compounds, measurements have been made on organic fluorine compounds to establish bond–energy relations and to provide highly accurate heats of combustion of substances which may be used as secondary standards in the combustion calorimetry of organo-fluorine compounds. Critical temperature measurements have been made on a further eighteen compounds, including twelve substituted phenols with critical temperatures between 420° and 460° C, and for the four butanols critical densities and pressures were also measured. An investigation of the thermodynamic properties of non-stoichiometric compounds has been started and a solid-state electrochemical cell constructed. A batch of *m*-ethylphenol with a purity of 99·91 moles per cent has been synthesized from acetophenone as well as highly purified samples of *p*-ethylphenol, diethyl ketone, 3-methyl-6-*t*.-butylphenol, methyl *n*-propyl ketone and 2 : 5-xylen-1-ol.

In the Corrosion of Metals Group, research on metallic corrosion remains a continuing theme and during the year has been concentrated on corrosion in flowing water; passivation or inhibition and the specific role of the anions in a corrosive solution; and anaerobic corrosion in soils and polluted waters. As well as fundamental studies, work on underground corrosion, essentially microbiological, has included a survey of the aggressiveness of soil at various sites in England and Wales and a continued long-term experiment on corrosion under conditions presented by the River Thames. Topics to which the Isotope Applications Unit has devoted much time to include the possible utilization of the Mössbauer effect for studying structural chemical problems, the labelling and pulverizing of coal, the distribution of activity along threads or wires and liquid scintillation counting. The Director's Research Unit has studied the interaction of oxygen with tungsten surfaces by electron microscopy and the accommodation of copper atoms evaporated into ultra-high vacuum on to a clean tungsten surface.

The staff in post at the end of the year included 54 scientific officers, 7 research fellows, 104 experimental officers, 29 scientific assistants, 2 works technical officers, 20 executive and clerical, and 44 industrial, the authorized complement being 286 compared with 293 at the end of 1961. No progress has been made in relieving the serious shortage of accommodation.

THE EMPLOYMENT OF CAMBRIDGE GRADUATES

THE results of a survey, *The Employment of Cambridge Graduates**, made by Mrs. Christine Craig to help the University of Cambridge Appointments Boards, has been published by the Cambridge University Press. It covers the present occupation of Cambridge graduates of 1952 and 1953, the levels of responsibility and the salaries they have attained, the satisfaction they derive from their occupations and how they have progressed to their present positions. The questionnaires were sent out during July and August 1961, and completed questionnaires were received from 2,630 men and 494 women, or 70 per cent of all the men and 80 per cent of all the women graduates on the College lists for the appropriate years. The results are presented in six chapters covering, respectively, the graduates' background, their current employment, earnings and satisfaction of those working in the United Kingdom, the past and the future, men working overseas, and the women graduates.

Both men and women come of predominantly bourgeois background, only 2 per cent of the men being children of semi-skilled or unskilled workers. Nearly two-thirds of men, but a lesser proportion of the women, were educated at independent schools, but while most women went direct to Cambridge from school, only 17 per cent of the men did so, 66 per cent being otherwise occupied for one to three years, usually in National Service. Of the men, 31 per cent graduated in science or mathematics, 11 per cent in engineering, 9 per cent achieving first-class honours, 59 per cent second-class honours and 6 per cent an ordinary degree. Most of the men were in the age-group 29–33 and all but 434 were working in the United Kingdom, 349 of those being British nationals. At the time of the survey, 35 per cent of the women were working full time and 16 per cent part time, and out of 207 married women, 50 were working full time and 78 per cent part time, a very high proportion being teachers in schools and universities.

Of all men working in the United Kingdom, about 24 per cent were employed in teaching and 24 per cent in industry, of whom about a quarter were employed in research, design and development work, a similar proportion in sales and marketing, less than a fifth in production management and a slightly smaller proportion in general management. Of the teachers, three-fifths were schoolmasters, of whom only a quarter were teaching science, mathematics or engineering subjects, whereas in the universities and technical colleges, men teaching the latter subjects outnumbered those teaching arts subjects in the proportion 57 : 43. Medicine and commerce each claimed about 8 per cent of the whole group; 9 per cent were public servants in the Civil Service, local authorities or the Atomic Energy Authority (4 per cent in the Scientific Civil Service, 3 per cent in professional or specialist classes,

* *The Employment of Cambridge Graduates*. By Christine Craig. Pp. xi+102. (Cambridge: At the University Press, 1963.) 10*s*.

2 per cent in administration). Nearly 60 per cent of engineering graduates and 41 per cent of scientists and mathematicians were in industry, about 20 per cent of the latter being in universities and technical colleges and 16 per cent teaching in schools. About two-fifths of the arts men in industry were in commercial departments and a quarter in general management or the central services of industrial groups.

More than a third of all men with first-class honours were in university posts, about one in seven being in industry and professional practice. Only three schoolmasters in 100 had first-class honours and nearly 10 per cent had ordinary degrees. Most Ph.D.s were in universities or had research appointments elsewhere. Taking the total earnings, the middle members of the group of men working in the United Kingdom were receiving £1,500–£1,600; for basic earnings, the medium was £1,425, but for a quarter total earnings were less than £1,250; for another quarter they exceeded £2,000, and for 12 per cent £2,500. A third of the men in industry were earning more than £2,000, but nearly a quarter less than £1,500. Three-quarters of the schoolmasters fell in the group earning between £1,000 and £1,500 and only 3 per cent were earning more than £2,000, while only 7 per cent of university and technical college teachers earned more than £2,000. Teaching was the only occupation in which the men who considered their salary a fair return were outnumbered by those who thought otherwise, but even so, 50 per cent were satisfied. About a third of those employed in universities, in medicine and in private practice were dissatisfied, and about a quarter in industry. About 30 per cent of men in the universities expressed profound satisfaction. Men who entered paid employment within six months of graduation were mostly paid £400–£600 a year, and for four years after graduation the average salaries of men who had taken postgraduate courses were slightly lower than those of men who had entered employment immediately after graduation. While only 3 per cent of the men who went into the Church (6 per cent of the total) or the medical profession afterwards changed their profession, 58 per cent of the others changed their employers at least once between starting their first job and the time of the survey, 16 per cent making radical changes of occupation. About half the men expected to be with the same employer or in the same business in five years time.

Of the 434 men working overseas, 60 were in the United States, 58 in Canada and 131 in various African territories, and it is probable, but not certain, that at least half of those in Canada and a substantial proportion of those in the United States were permanently resident overseas. Of the 19 per cent in Government service, most were in Africa; 20 per cent were concerned with education in schools, 7 per cent were employed in universities, mostly in the United States, 20 per cent in industry and 13 per cent in commerce. Of the scientists and mathematicians, 16 per cent, and of the engineers 13 per cent, were overseas, 25 engineers or scientists being in the United States and 20 in Canada; of the 29 Ph.D.s overseas, 17 were in the United States, almost all in university posts. A third of the men overseas were earning the equivalent of £3,000 or more.

RIOMETER OBSERVATIONS OF IONOSPHERIC ABSORPTION DUE TO THE SOLAR FLARES OF APRIL 15, 1963

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AND

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THE occurrence of a strong solar flare offers an excellent opportunity for determining the dependence of ionospheric absorption on the zenith angle of the incoming ionizing radiation. Early calculations for a simple Chapman layer[1] indicated a dependence of the absorption on the zenith angle θ of the form $(\cos\theta)^{3/2}$. Some recent experimental and theoretical results show that the 3/2 power is generally too high an exponent. Calculations by Nicolet[2] have shown that an important parameter in determining the dependence of absorption on zenith angle is the effective recombination coefficient α. The classical derivation assumes that both α and H, the scale height, are constant. However, this is not the case in any of the ionospheric regions. A recent work by Mitra and Jain[3] also considers the variability with altitude of α.

A riometer network, installed by the Air Force Cambridge Research Laboratories at various locations around the world, has yielded absorption data on the two Type II+ solar flares which occurred on April 15, 1963, beginning at 1123 U.T. and 1614 U.T., respectively. The first flare was accompanied by a severe noise storm which began about ten minutes after the conclusion of the first major period of absorption. All the riometers located on the sunlit side of the Earth observed these flares and absorption data obtained are given in this paper. The dependence of absorption on zenith angle is quite close to the value obtained by Appleton.

Riometer locations. Riometers which observed the solar flares of April 15 are listed in Table 1, along with the solar zenith angles at flare onset. All operating frequencies were 30 Mc/s with the exception of Bedford, Massachusetts, which also operated at 22 Mc/s, and Athens, Greece, where data was also obtained on 27·6 Mc/s and 58 Mc/s.

Table 1. SOLAR ZENITH ANGLES AT 1123 U.T., APRIL 15, 1963. COMPUTED FOR RIOMETER SITES

Site locations	Solar zenith angle
Accra, Ghana	10°
Addis Ababa, Ethiopia	29°
Athens, Greece	34°
Hermanus, Republic of South Africa	45°
New Delhi, India	63°
Bedford, Massachusetts	76°

Table 2 lists solar zenith angle information for the flare of 1614 U.T., April 15, 1953.

Table 2. SOLAR ZENITH ANGLE AT 1614 U.T., APRIL 15, 1963. COMPUTED FOR RIOMETER SITES

Site locations	Solar zenith angle
Bedford, Massachusetts	34°
Sacramento Peak, New Mexico	45°
Great Whale River, Quebec	47°
Accra, Ghana	62°
Athens, Greece	80°

Antennæ used were two-element, vertically directed Yagis operating over an artificial ground screen. Antenna beam-width was about 60° in the E-plane.

Absorption data. In some of the measurements, local interference was quite severe, and the accuracy of the measurements was somewhat degraded. However, at

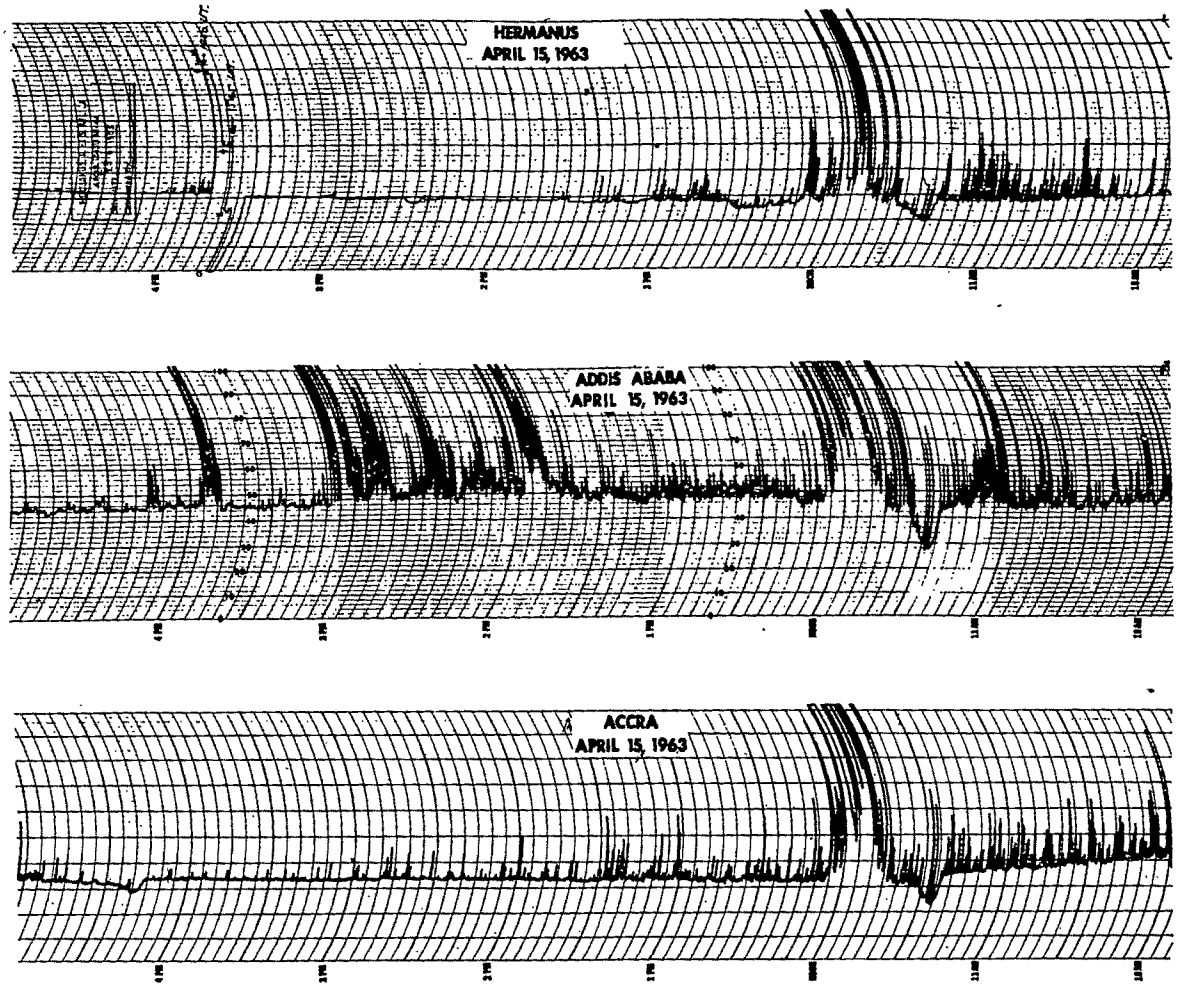


Fig. 1. Riometer effects on 30 Mc/s at African sites due to solar flare of April 15, 1963, at 1123 U.T.

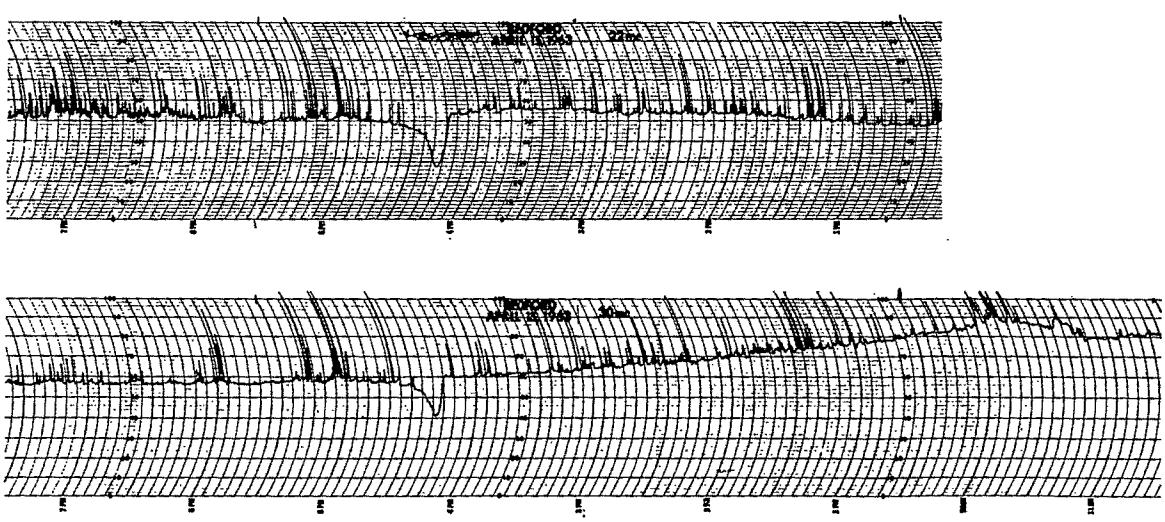


Fig. 2. Riometer effects on 22 Mc/s and 30 Mc/s at Bedford, Massachusetts, due to solar flare of April 15, 1963, at 1614 U.T.

those sites where relatively quiet conditions prevailed, measurements to $\pm 0{\cdot}1$ db are considered to be valid. In Fig. 1 is shown a photograph of the data obtained at three African sites. The occurrence of the first solar flare at 1123 U.T. is easily seen at all stations. The second solar flare is observed at Accra, which was the only station in sunlight at the time of the flare. 22 Mc/s and 30 Mc/s data obtained on the second flare at Bedford, Massachusetts, is shown in Fig. 2.

In Fig. 3 we have graphed the values of absorption due to the 1123 U.T. flare, as a function of solar zenith angle. On the same graph are plotted $\cos^n$ curves for $n = 1$ and $n = 3/2$. Within the limits of observational error, the 3/2 power curve is a better fit. The point at zenith angle of 10° (corresponding to Accra, Ghana) does not fall close to either curve. This anomaly may be due to the fact that the noise observed at Ghana was quite extreme and may have prevented the riometer signal from reaching

its minimum due to absorption. The data from the flare at 1614 U.T. is shown in Fig. 4 and once again, for comparison, the cosine function with the unity and three-halves exponents are shown. The quality of the data and the inaccuracy of the measurements at 80° zenith angle (due to a wide trace on the particular riometer) makes it somewhat more difficult to decide which curve the data is following. The 3/2 power curve appears to be a better fit, but this conclusion is not as clear as that reached from the earlier flare.

Frequency dependence. The operation of multi-frequency riometer installations at Athens, Greece and Bedford, Massachusetts, enables a study of the absorption dependence on frequency. For non-deviative propagation in the quasi-longitudinal mode, where $f \gg \nu$, the collision frequency, this absorption has often been shown to be dependent on the inverse square of the frequency. Fig. 5 shows this dependency and indicates that the absorption measured was occurring in the lower ionosphere.

Attenuation rate. Fig. 6 shows the elapsed time between onset of the flare and the time at which maximum absorption was recorded. These values show no clear dependence on zenith angle and indicate a time of about 3 min for the first flare and about 4 min for the second flare. The relation of the relaxation time of the absorption to the rate of recombination and electron attachment in the lower ionosphere is presently being studied.

Second absorption phase. Careful inspection of the data from Hermanus, Bedford, and Sacramento Peak, shows that a second absorption phase appeared. In the case of Hermanus, this second absorption reaches its maximum at about 1230 U.T. on the first flare. The second absorption phase at Bedford is somewhat complicated by the occurrence of local and solar noise; however, preliminary analysis indicates that the absorption maximum occurred at about 1740 U.T. Information relayed from Cape Hallet, Antarctica, indicates the occurrence of an absorption increase about one-half hour following each flare. Since this second absorption phase is not observed at the equatorial sites, it is possibly due to the arrival of solar charged particles[4].

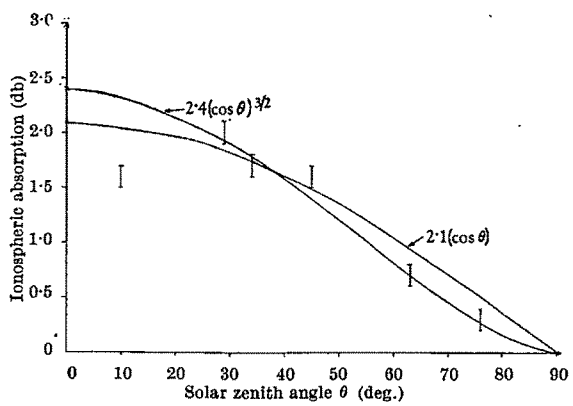


Fig. 3. 1123 U.T., April 15, 1963, solar flare absorption 30 Mc/s riometer

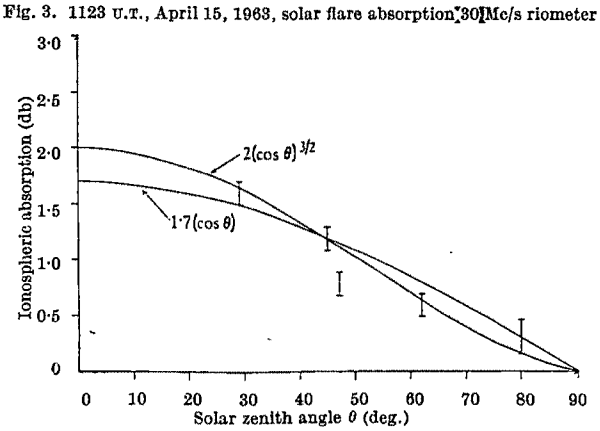


Fig. 4. 1614 U.T., April 15, 1963, solar flare absorption 30-Mc/s riometer

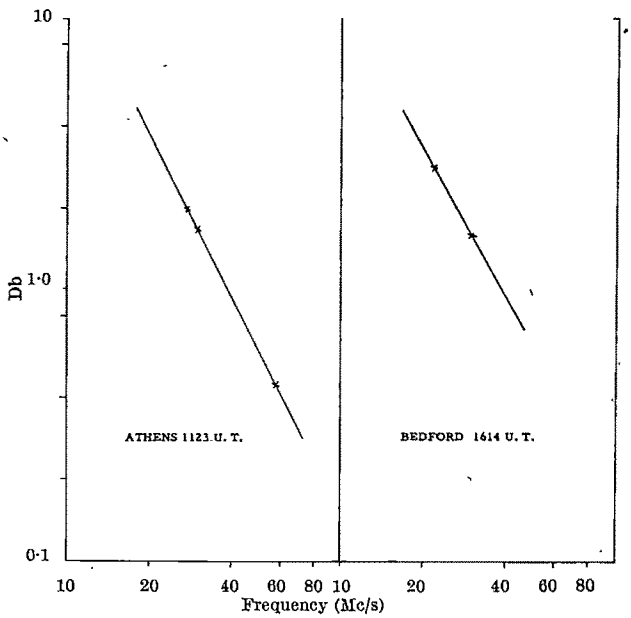


Fig. 5. Solar flares April 15, 1963, 1123 U.T. and 1614 U.T. Frequency dependence of absorption

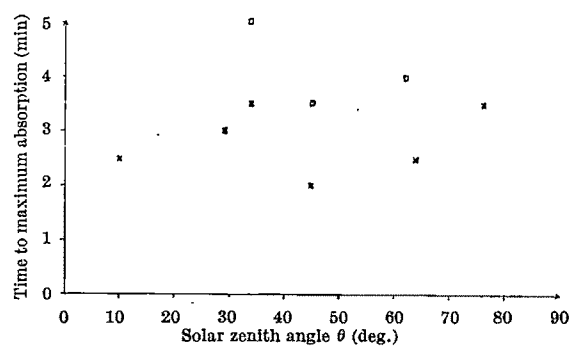


Fig. 6. 30 Mc/s riometer April 15, 1963. Time from flare onset to maximum absorption
×, Flare of 1123 U.T.; □, flare of 1414 U.T.

Conclusions. A network of riometers operating at 30 Mc/s has enabled a comprehensive study of the ionospheric absorption due to a solar flare. Riometers located at, or near, the geomagnetic equator have indicated effects which may be attributed solely to electromagnetic radiation. As one progresses farther from the equator it is possible to see the beginning of effects due to charged particles. A riometer located near the south geomagnetic pole indicated no electromagnetic effects and strong absorption effects due to incoming charged particles.

Since the electromagnetic and corpuscular effects can be separated, it is possible to determine the dependence on solar zenith angle of the flare-induced absorption at low and medium latitudes. This functional dependence is found to be close to the $\cos^{3/2}$ relationship derived from the simple Chapman layer. The dependence of this absorption on riometer frequency is found to be f^{-2}, which is the value expected for absorption in the quasi-longitudinal mode at medium latitude.

We thank the following scientific organizations for their co-operation: University of Ghana, Legon; University College, Addis Ababa; National Physical Laboratory, New Delhi; Magnetic Observatory, Hermanus; University of Athens; National Bureau of Standards, Boulder, Colorado.

[1] Appleton, E. V., *Proc. Roy. Soc.*, A, 162, 451 (1937).
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[4] Warwick, Constance S., *Sky and Telescope*, 24 (3) (1962).

A NEW CALCULATION FOR THE AGE OF THE EARTH FROM ABUNDANCES OF LEAD ISOTOPES

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THE only precise estimates that exist to-day for the age of the Earth are based on measurements of the isotopic composition of lead. Numerous calculations have been reported in the past[1], but only two[2,3] are of significance at the present time. In both, the assumption is implicit that the isotopic composition for the various lead samples can be related to an initial primeval lead by equations derived for a system which is closed with respect to uranium and lead. One calculation[2] assumes that the primeval lead isotope abundances are identical with those now observed in the troilite phase of iron meteorites, and evaluates the time required to change such lead into that found in one or more terrestrial samples of known age and isotopic composition. The second calculation[3] assumes that the leads now observed in stone meteorites represent the products of evolution from the primeval isotope ratios to their present values. Theory shows that such leads, in the absence of events fractionating lead and uranium during the lifetime of the meteorite, should have isotope ratios which lie on a straight line, and that the slope of this straight line can be used to determine the age of the meteorite system. Apart from the difficulty in proving the common assumption that equations for a closed system may be used in these calculations, the accuracy of the first calculation is limited by the uncertainty in assigning ages to the samples used, and of the second calculation by the uncertainty in equating the age of the Earth to the age of the meteorite system. The approximate equality of the values obtained by the two calculations provides some experimental justification for both procedures.

Recently considerable progress has been made in interpreting the isotopic composition of terrestrial lead samples[4]. The results suggest a third method for calculating the age of the Earth from lead isotope abundances which is basically distinct from the two mentioned above. In common with the others, the new method assumes that the samples used in the calculation can be interpreted by equations appropriate to evolution of the lead isotope abundances in a single closed system. It does not, however, require estimates to be made for any of the ages of the samples used, and it does not require the assumption that the age of the Earth is the same as the age of meteorites. It does assume that terrestrial leads have evolved in the Earth from lead similar in isotopic composition to that in the troilite phase of iron meteorites, and in fact the method provides an estimate for the time at which the Earth's lead had this isotopic composition. It also requires the assumption that the primary growth process for lead isotopes in the Earth occurred in a system or systems in which the uranium/lead ratio differed by a very small amount from the mean value, an assumption for which considerable evidence has been accumulating[5,6].

The calculation itself is very similar to one proposed several years ago by Allan, Farquhar and Russell[7]. If a closed system can be assumed for a particular lead sample, its age can be determined from both the $^{206}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ ratios according to the equations:

$$t = \frac{1}{\lambda}\log_e\left\{e^{\lambda t_0} - \frac{x - a_0}{\alpha V}\right\}$$
$$t' = \frac{1}{\lambda'}\log_e\left\{e^{\lambda' t_0} - \frac{y - b_0}{V}\right\} \qquad (1)$$

The symbols are defined in Table 1. In the right-hand side of equations (1) there are two unknown parameters, namely, V and t_0. (We have made the assumption that a unique value of V will describe the suite of samples closely enough.) It is formally possible to carry out a least-squares analysis of measured lead isotope ratios to determine values of both the foregoing parameters which give the most concordant t and t' values. Such a procedure is able to yield a reasonably precise value for t_0; we have followed two procedures and obtained two numerical estimates as detailed here.

Table 1. Definition of Symbols

Isotope ratio	Present ($t=0$)	Time t	Primeval ($t=t_0$)
$^{206}Pb/^{204}Pb$	a	x	a_0
$^{207}Pb/^{204}Pb$	b	y	b_0
$^{238}U/^{204}Pb$	αV	$\alpha V e^{\lambda t}$	$\alpha V e^{\lambda t_0}$
$^{235}U/^{204}Pb$	V	$V e^{\lambda' t}$	$V e^{\lambda' t_0}$

Physical constants: $\alpha = 137{\cdot}8$; $\lambda = 0{\cdot}1537 \times 10^{-9}$ yr.$^{-1}$; $\lambda' = 0{\cdot}9722 \times 10^{-9}$ yr.$^{-1}$.

First calculation. The first calculation requires that the mean, ordinary-lead growth-curve originates at the primeval abundances $(a_0, b_0) = (9{\cdot}56, 10{\cdot}42)$ given by Murthy and Patterson[8]. In order to select suitable samples which are unlikely to be anomalous we have used analyses for galenas from deposits selected by Stanton[9] as being representative of his 'conformable' or 'pyritic conformable' classification. Previous work[4,5] has indicated that this geological criterion, although not perfect, more successfully excludes anomalous leads than any other known.

Table 2. Summary of Results

Location	x	y	z	t_{app} (Gyr.)	$(^{238}U/^{204}Pb)_{app}$	Residual	t_0 (Gyr.)
1. Primeval Abundances, Murthy and Patterson, (1962)	9·56	10·42	29·71	4·53	9·21	475	4·53
2. Manitouwadge, Ontario (Mean of 3 samples)	13·403	14·565	33·545	3·03	9·62	600	4·48
3. Broken Hill, Australia (Mean of 6 samples)	16·117	15·539	36·087	1·61	8·92	575	4·56
4. Mount Isa, Australia (Mean of 5 samples)	16·210	15·593	36·219	1·46	8·50	1,530	4·61
5. Sullivan Mine, B.C. (Mean of 2 samples)	16·633	15·638	36·577	1·28	8·79	1,000	4·58
6. Captain's Flat, Australia (Mean of 7 samples)	18·178	15·766	38·564	0·47	9·26	508	4·52
7. Cobar, Australia (Mean of 10 samples)	18·205	15·771	38·543	0·46	9·29	543	4·52
8. Bathurst, New Brunswick (Mean of 3 samples)	18·291	15·781	38·526	0·42$_5$	9·34	606	4·52
9. Hall's Peak, Australia (Mean of 6 samples)	18·490	15·754	38·770	0·15	9·04	492	4·54
10. White Island, New Zealand (Mean of 2 analyses)	18·885	15·750	39·070	−0·25	8·85	640	4·56
Mean ± standard deviation of the mean					9·08 ± 0·11		4·54 ± 0·02

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We have used only isotopic analyses carried out at the University of British Columbia, since we believe these to be the most precise galena analyses available. The analyses are shown in Table 2. Samples from Rosebery, Tasmania (18·374, 15·746, 38·469), which should be included on the basis of the foregoing criteria, have been excluded from the calculation. They are known to be part of an anomalous lead suite which includes vein leads from Mount Farrell. Some of the other samples may be slightly anomalous, too. Fortunately, the calculation is extremely insensitive to this provided that the anomalous component is not more than about 10 per cent of the total lead. For example, the inclusion of Rosebery would increase t_0 by less than 0·25 per cent, and the rejection of any sample used in the calculation (except for a_0 and b_0) would alter the result by a similar small amount.

We have interpreted best concordance between t and t' as corresponding to the minimum value of the expression:

$$\text{residual} = \sum_{i=1}^{n} W_i(t_i - t'_i)^2 \qquad (2)$$

The weight function, W_i, is included after Bullard and Stanley[1] to ensure that the result obtained is independent of the exact nature of the definition of the residual and is the most probable value. It is defined by the relationships:

$$1/W_i = \left(\frac{\partial \Delta t_i}{\partial x}\right)^2 \delta x^2 + \left(\frac{\partial \Delta t_i}{\partial y}\right)^2 \delta y^2$$

and:

$$\Delta t_i = t_i - t_i'$$

where δx and δy are the estimated experimental uncertainties in the measured ratios. The present calculation assumes $\delta x = \delta y$; the numerical value is immaterial.

The expression for Δt_i is non-linear in both V and t_0, and we have expanded it as a Taylor series in δV, the deviation from an approximate value **V**. Then condition (2) becomes*:

$$\text{residual} = \sum_{i=1}^{n} \mathbf{W}_i \left(\Delta t_i + \frac{\partial \Delta t_i}{\partial V}\,\delta V + \ldots\right)^2 = \text{minimum}$$

* Bold-faced symbols indicate that the quantity has been evaluated with **V**. By $\frac{\partial \Delta t_i}{\partial V}$ we mean the value of the derivative obtained with **V**.

The best value of V corresponding to a given t_0 is obtained by applying repeatedly corrections given by:

$$\delta V = -\frac{\sum_{i=1}^{n} \left\{\mathbf{W}_i \cdot \Delta t_i \cdot \frac{\partial \Delta t_i}{\partial V}\right\}}{\sum_{i=1}^{n} \left\{\mathbf{W}_i \cdot \left(\frac{\partial \Delta t_i}{\partial V}\right)^2\right\}}$$

until the procedure converges to the best V value. In all cases we have tried, the process converges to a unique result, although as many as sixteen iterations may be required.

The least-squares solution for V was obtained for each of t_0 = 4·49, 4·52, 4·55, 4·58, 4·61, and the minimum residual evaluated for each. These are plotted on an arbitrary scale in Fig. 1. The well-defined minimum at 4·53 Gyr. is the best value for t_0 in a least-squares sense. The corresponding value for αV is 9·21 atoms/atom. The sharpness of the minimum is a measure of the precision of the estimate of t_0. A good approximation to the standard deviation of t_0 is obtained by dividing the half-width at double minimum residual by the square root of two less than the number of samples. The standard deviation so evaluated is 0·03 Gyr. (obtained by rounding up 0·025).

For the purpose of comparison, we have carried out a similar, though simpler analysis of meteoritic data, with results shown in Fig. 2. In this case the stone meteorites were assumed to be of zero age, and would therefore lie on the isochron of zero age, given by the equation:

$$\frac{y - b_0}{x - a_0} = \frac{e^{\lambda' t_0} - 1}{\alpha(e^{\lambda t_0} - 1)}$$

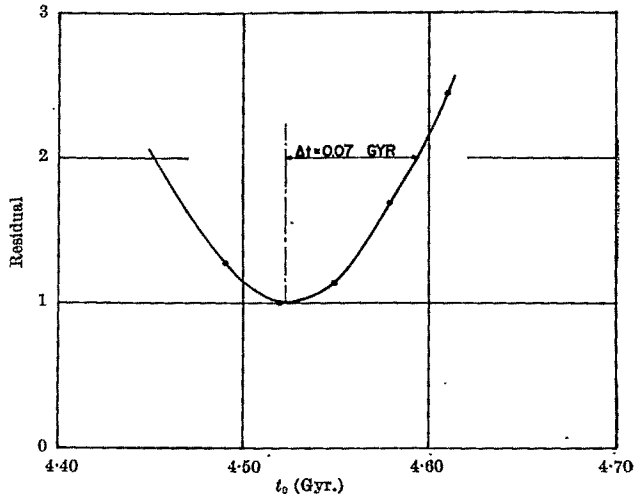


Fig. 1

The residual, equal to the sum of the perpendicular distances between the observed meteoritic ratios and the isochron of zero age, was calculated for different values of t_0. The two curves show the result of using analyses for only three stone meteorites by Patterson, and forty-eight of various types from several laboratories. The ages and standard deviations obtained from the figure are 4·56 ± 0·03 Gyr. and 4·56 ± 0·02 Gyr. This compares well with Murthy and Patterson[7], who give a slope of 0·59 ± 0·01 for the zero isochron, which corresponds to t_0 equal to 4·55 ± 0·03 Gyr.

Second calculation. A criticism of the first calculation is that it assumes an exact knowledge of the primeval lead abundances. Substituting the older Patterson values (9·50, 10·36) changes the result by a negligible amount, but the 1·5 per cent uncertainty of the ratios estimated by Murthy and Patterson could result in an error of forty million years in t_0.

The following procedure avoids placing excessive weight on the primeval abundances. In equations (1), with the assumption that an average V provides a reasonable representation of the samples, a_0, b_0 and t_0 need not be interpreted as primeval values, but can correspond to any point through which the growth-curve is forced to pass. Then it is formally possible to force the curve through any of the samples in Table 2, and calculate the apparent age of the sample and the apparent V-value. From these two numbers it is possible to use either of

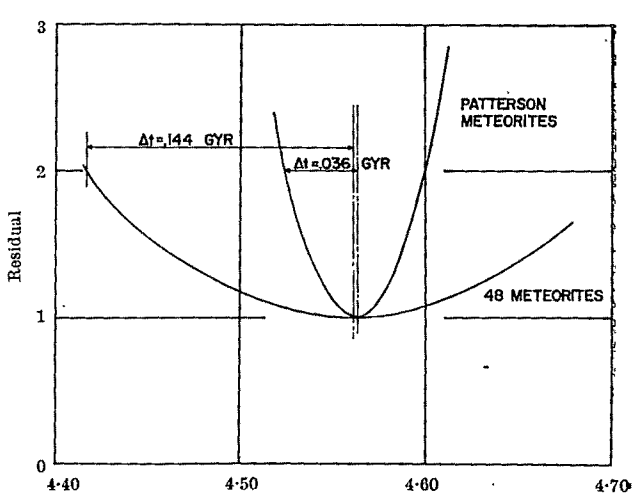


Fig. 2

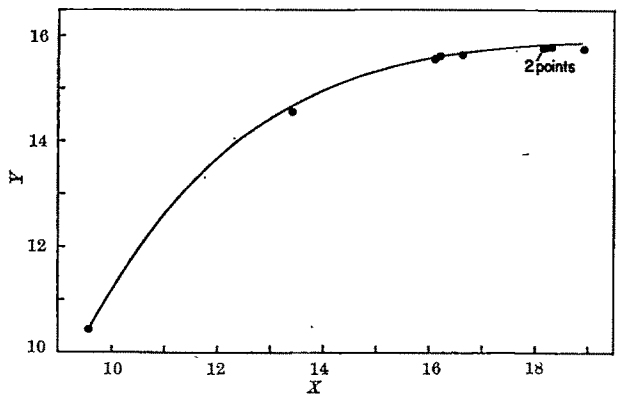


Fig. 3

equations (1) to calculate the time t_0 at which terrestrial-lead had the primeval ratios. Using the mathematical procedures described here, the growth-curve was forced through each point in turn, the two values of t_0 were calculated in each case and the results were averaged to give the ages shown in Table 2. The greatest difference between the two times, which was obtained when the curve was forced through the Mount Isa abundances, amounted to less than 0·3 per cent. This agreement supports the basic assumption of a unique growth curve. By averaging the t_0 values obtained, we arrive at a result which does not weight the primeval abundances so heavily. The result obtained is 4·54 ± 0·02 Gyr., where the uncertainty quoted is the standard deviation of the mean (rounded up from 0·014).

Conclusion. Either of the results obtained, 4·53 ± 0·03 Gyr. or 4·54 ± 0·02 Gyr., provides an estimate of the time at which terrestrial lead had the same isotopic composition as the troilite phase of iron meteorites. The procedure avoids both the necessity of assigning ages to terrestrial samples and the assumption that the age of the Earth is equivalent to the age of meteorites. Thus the agreement of the present result with the age of meteorites obtained from the slope of the meteoritic isochron (4·55 ± 0·03 Gyr.) provides valid independent evidence that the two events occurred at about the same time.

The essential assumption of the calculation, that the samples used can be represented by a single growth curve, is justified by Fig. 3, which shows the experimental points plotted on such a growth-curve calculated for $t_0 = 4{\cdot}53$ Gyr. and $V = 0{\cdot}0670$ ($aV = 9{\cdot}21$).

This work was supported by the National Research Council of Canada and the Petroleum Research Fund of the American Chemical Society. Calculations were carried out using the IBM 7090 computer operated by the Institute of Computer Science, University of Toronto.

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ADSORPTION OF KRYPTON AND XENON ON EVAPORATED METAL FILMS IN RELATION TO THE DETERMINATION OF SURFACE AREA AND THE NATURE OF ADSORBED CARBON MONOXIDE

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THERE is a growing tendency to use the adsorption of xenon for surface area measurements, especially for small areas, because the low value of P_0(Xe) enables useful values of P/P_0 to be reached with conventional apparatus without incurring the penalty of large dead volume corrections. However, there is some uncertainty concerning the effective cross-sectional area (σ) of xenon which should be used for this purpose. Thus, Singleton and Halsey[1], using nitrogen as the reference adsorbate with $\sigma(N_2) = 16{\cdot}2$ A^2, obtain values of σ(Xe) as follows: 20·2 A^2 on carbon black, 18·2 A^2 on silver iodide and 27·3 A^2 on anatase; the Brunauer–Emmett–Teller method was used, except for carbon black for which it was unsatisfactory and the point B was used instead. Later work would seem to favour a value σ(Xe) = about 24 A^2. Van Heerden and Zwietering[2], using carbonyl iron as the adsorbent, obtained σ(Xe) = 22·3 A^2 from a comparison between the adsorption of xenon, krypton and ethylene; no details of this study are provided. Pritchard[3] gives σ(Xe) = 24 A^2; this value was obtained by comparison of the monolayer volume, estimated by following the change in surface potential when xenon was adsorbed on copper, with the krypton Brunauer–Emmett–Teller area, using σ(Kr) = 19·5 A^2. Support for this value has been given recently by Cannon[4] who reports σ(Xe) = 25 A^2 as the result of comparison of Brunauer–Emmett–Teller monolayer volumes for xenon and krypton, again with σ(Kr) = 19·5 A^2, nickel powder and oxidized nickel powder being the adsorbents. However, the need for caution in assigning a value to σ(Xe) is indicated by the work of Ponec and Knor[5], who adsorbed xenon and krypton on evaporated films of nickel, and obtained σ(Xe) = 13·8 or 17·2 A^2, depending on the method of assessment of the monolayer volume, with σ(Kr) = 19·5 A^2 as the reference value; theirs is the only published result in which the effective area of xenon is smaller than that of krypton. Again, Anderson and Baker[6] have attempted to estimate σ(Xe) using evaporated films of nickel and tungsten, and they emphasize the importance of lattice parameters in determining the availability of sites for adsorption. They interpret the variety of σ(Xe) values reported by Singleton and Halsey[1] in these terms and quote average effective values σ(Xe) = 23·0 and 19·5 A^2 for polycrystalline tungsten and nickel films respectively, and σ(Kr) = 17·9 A^2 for nickel films.

This article describes an investigation into the value of the ratio σ(Xe)/σ(Kr) determined for evaporated films of titanium, zirconium, iron, cobalt, nickel, molybdenum, tungsten, tantalum and platinum at 77° K. The procedure followed in every case was to obtain successively the adsorption isotherm for xenon, krypton and then xenon again, on the same film with complete removal of adsorbate prior to each determination. The requirement that the first and the last xenon isotherms be superimposable, or very nearly so, leaves no doubt that all the isotherms refer to precisely the same surface. The films were

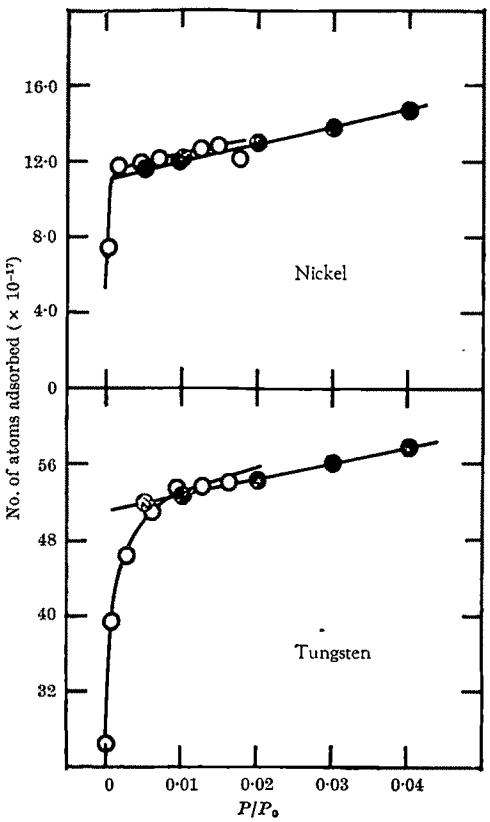


Fig. 1. The 77° K isotherms for the adsorption of krypton (○) and xenon (●) on evaporated films of nickel and tungsten

deposited in a vacuum of the order of 10^{-8} torr using a conventional adsorption apparatus adapted for bakeout by inclusion of water-cooled greased stopcocks[7]. The krypton used was supplied by British Oxygen Co., Ltd., in break-seal ampoules, the purity being 99–100 per cent, with xenon as the possible impurity. The xenon used was similarly supplied, but was found to contain a more volatile impurity which was removed by repeated distillation into a trap at 77° K with pumping. The value of $P_0(\mathrm{Xe}) = 2{\cdot}3 \times 10^{-3}$ torr at the temperature of a liquid nitrogen cold trap was obtained on several occasions and with different samples of the gas. Corrections for thermal transpiration were made by means of the equation of Bennett and Tompkins[8], which was found by Podgurski and Davis[9] to be very satisfactory for xenon. Our value for $P_0(\mathrm{Xe})$ is not seriously different from $P_0(\mathrm{Xe}) = 1{\cdot}7 \times 10^{-3}$ torr at 77° K derived from the very careful measurements of Podgurski and Davis and such discrepancy as there is corresponds to an uncertainty of only one degree in bath temperature. For the purpose of Brunauer–Emmett–Teller calculations, $P_0(\mathrm{Kr}) = 3$ torr was used; at the very low values of P/P_0 involved, the method is not sensitive to P_0 and the equation is virtually the Langmuir isotherm. The results are summarized in Table 1 and two typical isotherms are shown in Fig. 1. The Brunauer–Emmett–Teller equation provides a useful

Table 1. Comparison of Monolayer Values obtained for Krypton and Xenon on the same Evaporated Film by the Brunauer–Emmett–Teller (B.E.T.) and Point *B* Methods

Metal	Weight of film (mg)	Monolayer coverages (atoms × 10^{-17}) Point *B* Kr	Point *B* Xe	B.E.T. Kr	B.E.T. Xe
Co	64·8	12·4	13·9	14·1	16·2
Ni	65·3	11·6	11·0	12·5	13·2
Pt	35·1	8·2	8·5	10·2	10·7
Fe	83·8	74	67	84	75
Mo	77·0	—	192	241	226
Ta	45·8	43	44	53	53
W	32·4	53	51	56	56
Ti	15·8	—	20·8	23·2	22·3
Zr	35·3	—	16·9	21·2	19·8

criterion of monolayer coverage only when applied to the isotherm in the region of point *B*. Because the heats of adsorption for these systems are relatively great, the point *B* occurs at relative pressures of 0·01 or less. If Brunauer–Emmett–Teller plots are used for relative pressures much greater than this, unreliable estimates of the monolayer values are likely to be obtained, even though the plots may be reasonably straight; this criticism may be made of the data of Cannon[4]. Again, when very small areas are used, great care must be made in applying various corrections and difficulties may arise owing to this cause also[4].

Examination of Table 1 reveals a notable similarity in the numbers of krypton and xenon atoms adsorbed by the same film for a given criterion of monolayer coverage. Only in the case of iron is the number of krypton atoms in a monolayer significantly greater than of xenon. We conclude, consequently, that, in general, the effective areas of krypton and xenon atoms on these metals are virtually the same.

If it were not for the long tradition of assigning unique areas to physically adsorbed molecules, and some recent experimental work, this result would perhaps not be too surprising. The field emission microscope has amply demonstrated the importance of co-ordination number of a substrate site in the adsorption of the inert gases[10,11], and the relatively large energy of activation for surface diffusion observed for krypton and xenon on tungsten[11,12] is further evidence that these adatoms are located on surface sites. We have tried to assess the interatomic energy for all the various conceivable configurations of krypton and xenon atoms on these metals, using 6–9 and 6–12 Lennard–Jones type potential energy functions and the exp–6 function as limiting cases[13,14], with the object of excluding those configurations that seem to involve a prohibitively large repulsive energy. The conclusions reached from this analysis, which will be presented in detail elsewhere, are that apart from zirconium, which has the largest lattice spacings, there is no likelihood of adsorbing more krypton atoms than xenon atoms on these metals, and, even for zirconium, the possibility of doing so is not strong.

Additional support for the view that these atoms are held on surface sites can be found in the values of the ratio:

$$p = \frac{\text{No. of carbon monoxide molecules adsorbed}}{\text{No. of krypton atoms adsorbed by the clean surface}}$$

Since the clearest deductions can be made for titanium and zirconium, for which p (experiment) = 1·78 and 1·05 respectively, the discussion will here be confined to these two metals. It would not be expected, *a priori*, that titanium and zirconium would behave dissimilarly towards carbon monoxide and it is the present contention that, in fact, they do not. Their difference lies in the ability of zirconium to adsorb more krypton per given number of surface atoms than can titanium. The greater lattice spacings of zirconium make available sites which may be occupied by krypton atoms without excessive interatomic repulsion, while occupation of the corresponding sites on the surface of titanium would involve prohibitive overlap by neighbouring atoms. The argument can be extended to show that carbon monoxide is held on these two metals by bridge bonds, each surface metal atom being bounded to only one carbon atom. Repulsive interaction between neighbouring molecules is insufficient to prevent each surface metal atom carrying one carbon monoxide molecule, if bonding were linear carbon monoxide, or being bounded to two carbon atoms, if there were orbitals available for this purpose. The observed values of p are much smaller than the values expected for these two possibilities, but in good agreement with the requirement that each surface atom has available only one orbital for bonding with carbon monoxide in a bridge

configuration. These conclusions are unequivocal for titanium and zirconium; for the other metals the distinctions are not clear and argument by analogy is necessary.

One of us (F. H. H.) is in receipt of a maintenance grant from the Department of Scientific and Industrial Research.

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PARTIAL REVERSIBILITY OF THE $Ca^{\cdot\cdot}$ – ITP-CONTRACTION OF GLYCEROL-EXTRACTED MUSCLE

By Prof. W. KUHN, I. TOTH and A. RAMEL
Physical Chemistry Institute, University of Basle

It is known that chemical energy can be transformed directly into mechanical energy by means of macromolecular filaments dilating and contracting under the influence of chemical changes. This energy transformation was found in all cases to be associated with the phenomenon that mechanical stretching produces a chemical equilibrium shift leading to an increase in the embedding medium of the reactants producing the contraction and a decrease in the activity of the reactants producing the dilation of the filaments (teinochemical principle)[1-3].

If $n_{1,\mathrm{tot}}$ is the number of moles of a given teinochemically active substance present in the total system (consisting of the gel filament and the embedding liquid), a_1, the activity of the substance in the embedding liquid, L, the length of the filament, and $\mathfrak{K}$, the mechanical force acting on it, the expression characteristic for a reversible and quantitative energy transformation reads:

$$-\left(\frac{\partial n_{1,\mathrm{tot}}}{\partial L}\right)_{\mathfrak{K}=\mathrm{const}} = \left(\frac{\partial \mathfrak{K}}{\partial L}\right)_{n_{1,\mathrm{tot}}=\mathrm{const}} \cdot \frac{1}{RT\left(\frac{\partial \ln a_1}{\partial L}\right)_{n_{1,\mathrm{tot}}=\mathrm{const}}} \qquad (1)$$

Equations similar to equation (1) hold independently for each teinochemically active substance present in the system[4]. The validity of equation (1) has been tested with a number of artificial filaments and afterwards also with filaments prepared from a natural muscle (suitably prepared earthworm muscular system)[5].

Corresponding experiments will here be described for the $Ca^{\cdot\cdot}$–ITP-contraction of the glycerol-extracted rabbit psoas muscle. We confirm the statements published by several authors that the glycerinated psoas contracts with ATP (or ITP) under certain conditions only if a minimum concentration of $Ca^{\cdot\cdot}$-ions is present in the system[7-9]. In our experiments the teinochemical behaviour of $Ca^{\cdot\cdot}$ in this system was tested with the use of equation (1).

Fibre bundles of glycerol-extracted rabbit psoas muscle, prepared according to the procedure described by Szent-Györgyi[6], were first soaked in de-ionized water to remove glycerol and afterwards in magnesium chloride solutions to extract exchangeable calcium.

All materials used were of analytical grade, purchased from E. Merck AG, Darmstadt.

ITP (trisodium salt, purum) was obtained from Fluka AG, Switzerland. Cations were removed before use with 'Dowex 50X8'. Concentrations were adjusted spectrophotometrically and the *p*H by neutralization with magnesium hydroxide.

Concentrations of calcium were determined spectrophotometrically with a Unicam 900 flame spectrophotometer.

Inorganic phosphate with a Unicam ultra-violet spectrophotometer 700, according to a modified procedure of Fiske–Subbarow, Unicam method sheet No. 500/6.

The length of the fibre bundles was measured cathetometrically.

For apparative details concerning the mechanical stretching of the bundles see ref. 3.

A psoas muscle sample of about 0·05 g, having a length (L) of 2·24 cm, was suspended at 5° C in 10 ml. of 0·01 M magnesium chloride. After 8 h the solution was replaced by a fresh one of equal composition, and extraction was continued under equal conditions for a further 8 h. About 0·2 μmole of $Ca^{\cdot\cdot}$ could be removed in the first step, and further 0·02 μmole approximately in the second.

The fibre bundle was then transferred into a cylindrical cell containing 2·5 ml. 0·01 M ITP, 0·01 M magnesium chloride, *p*H 7·4.

The unloaded fibre bundle contracted under these conditions from 2·2 cm to a length of 2·0 cm. This contraction is most likely caused by traces of $Ca^{\cdot\cdot}$ still present in the bundle.

Addition of calcium chloride in quantities of $3{\cdot}5 \times 10^{-8}$ to 7×10^{-8} moles in each step to the embedding solution produced an additional contraction, represented in Fig. 1. From the quantities indicated in the figure we obtain for the left-hand side of equation (1):

$$\left(\frac{\partial n_{1,\mathrm{tot}}}{\partial L}\right)_{\mathfrak{K}=0} = 1{\cdot}1 \times 10^{-6} \text{ mole cm}^{-1} \qquad (2)$$

The $Ca^{\cdot\cdot}$ concentration in the embedding liquid was at this stage of the experiment $4{\cdot}3 \times 10^{-5}$ mole/l., and the length of the unloaded muscle was 1·88 cm. The concentration of inorganic phosphate in the embedding medium increased by about 2×10^{-4} mole/l., indicating a corresponding decomposition of the ITP contained in the system.

The muscle was then stretched mechanically by a force $\Delta\mathfrak{K} = 1{,}800$ dynes, producing an increase in length of $\Delta L = 0{\cdot}24$ cm, corresponding to a value:

$$\left(\frac{\partial \mathfrak{K}}{\partial L}\right)_{n_{1,\mathrm{tot}}=\mathrm{const}} = 7{\cdot}5 \times 10^{3} \text{ dynes·cm}^{-1} \qquad (3a)$$

After establishment of the new equilibrium the $Ca^{\cdot\cdot}$ concentration was found to be $4{\cdot}7 \times 10^{-5}$ mole/l. The relative increase of the $Ca^{\cdot\cdot}$-concentration in the embedding liquid, associated with the mechanical stretching (3*a*), was thus:

$$\frac{1}{a_1}\frac{\partial a_1}{\partial L} = \left(\frac{\partial \ln a_1}{\partial L}\right)_{n_{1,\mathrm{tot}}=\mathrm{const}} = 0{\cdot}37 \text{ cm}^{-1} \qquad (3b)$$

With the numerical values (3*a*) and (3*b*) and with $RT = 2{\cdot}4 \times 10^{10}$ ergs/mole we obtain for the right-hand side of equation (1):

$$\left(\frac{\partial \mathfrak{R}}{\partial L}\right)_{n_{1,tot}} \Big/ RT\left(\frac{\partial \ln a_1}{\partial L}\right)_{n_{1,tot}} = 0{\cdot}85 \times 10^{-6}\ \text{mole.cm}^{-1} \quad (3c)$$

in rather good agreement with equation (2).

The mechanical force which had produced the stretching and the liberation of $Ca^{\cdot\cdot}$ from the muscle was finally reduced to zero; the muscle contracted correspondingly whereby the $Ca^{\cdot\cdot}$-concentration in the embedding medium decreased from $4{\cdot}7 \times 10^{-5}$ to $4{\cdot}44 \times 10^{-5}$ mole/l. It is noticed that the uptake of $Ca^{\cdot\cdot}$ when reducing the load is only about 70 per cent of the release of $Ca^{\cdot\cdot}$ when increasing the load. Analogous results were obtained with other muscle samples.

The values for $\left(\frac{\partial n_{1,tot}}{\partial L}\right)_{\mathfrak{R}}$ and for $\left(\frac{\partial \ln a_1}{\partial L}\right)_{n_{1,tot}}$ depend considerably on the actual calcium content of the muscle and also on the ITP concentration of the embedding liquid. Under favourable conditions (low calcium content of the muscle), a $Ca^{\cdot\cdot}$ concentration increase in the embedding liquid as high as by a factor of 3 could be observed to be associated with a 20 per cent mechanical stretching of the muscle. Stretching in presence of ITP and $Mg^{\cdot\cdot}$ can be applied as an alternative means instead of washing with magnesium chloride solutions to withdraw $Ca^{\cdot\cdot}$ from the muscle.

The $Ca^{\cdot\cdot}$ uptake and the $Ca^{\cdot\cdot}$ release associated with the contraction and the mechanical dilation of the glycerol-extracted psoas muscle proves that $Ca^{\cdot\cdot}$ under the conditions of our experiment is a teinochemically active substance which is bound chemically in the fibre when it contracts. Hence, $Ca^{\cdot\cdot}$ is not only a catalyst for other processes involved in the contraction.

If the process of contraction under these conditions includes formation of chemical bonds—fixing $Ca^{\cdot\cdot}$ inside the fibre—the free energy corresponding to the formation of these bonds undoubtedly becomes a part of the total chemical free energy which is displayed and the equivalent of which appears as mechanical energy produced in the contraction. The free energy corresponding to the reaction ITP → IDP + inorganic phosphate (which also occurs) will therefore only be a part of the total chemical free energy associated with the contraction. Equality of the mechanical energy produced in one contraction and the free energy of ITP (or ATP) splitting, which has been suggested by several authors, is therefore not to be expected. It can only be true approximatively, that is, if the free energy of the $Ca^{\cdot\cdot}$ binding is neglected.

Within the rough approximation in which the validity of equation (1) has been ascertained, the glycerol-extracted muscle in the presence of ITP must be considered to be a system able to transform into mechanical energy reversibly and quantitatively the free energy of dilution of $Ca^{\cdot\cdot}$ solutions or the free energy of chemical reactions by which $Ca^{\cdot\cdot}$-ions are produced or eliminated from the solution.

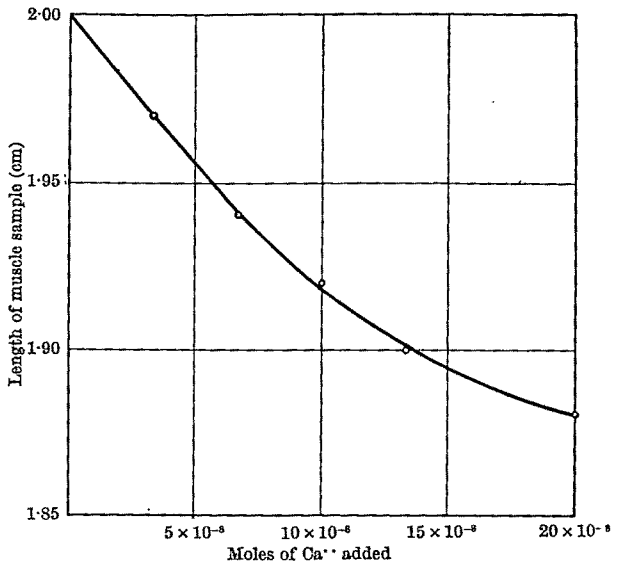


Fig. 1. Change of length of an unloaded washed muscle sample in 2·5 ml. of embedding liquid containing 10^{-3} moles of ITP and 10^{-2} moles of $Mg^{\cdot\cdot}$ per litre on addition of calcium chloride (abscissa)

We have stated that the reversibility observed is only partial in that the $Ca^{\cdot\cdot}$ release and uptake was only about 70 per cent reversible; it is partial, furthermore, in that the reversibility of the ITP splitting, which is also associated with the contraction, has not been ascertained yet. It is possible that the incomplete reversibility hitherto observed results from the presence in the system of enzymes catalysing irreversible reactions superimposed to the 'ideally' reversible $Ca^{\cdot\cdot}$–ITP-contraction.

The 70 per cent reversibility of $Ca^{\cdot\cdot}$ uptake and release associated with a mechanical change in length of the glycerol-extracted psoas muscle, satisfying approximately the teinochemical equation (1), indicates that the teinochemical laws established in the case of artificial macromolecular systems are also valid in principle for the glycerol-extracted muscle and we believe that this represents a first demonstration of an at least partial teinochemical reversibility of the elementary process of muscle contraction.

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EFFECTS OF AIR, OXYGEN AND 20 PER CENT CARBON DIOXIDE ON THE RABBIT DURING SURFACE COOLING DOWN TO CARDIAC ARREST

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I HAVE shown[1] that in the rat the inhalation of 20 per cent carbon dioxide in oxygen, as opposed to 25 per cent air in oxygen, significantly increases the cooling rate while the animal is in a deep freeze at 0° C; an effect dependent on the prior removal of the fur.

The work recorded here has been undertaken to ascertain whether 20 per cent carbon dioxide accelerates cooling in the rabbit and, if so, whether this acceleration is affected by pentobarbitone anæsthesia; the effects of both 20 per cent carbon dioxide and hypothermia on the electroencephalograph, the electrocardiograph and the arterial pressure have also been assessed.

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Table 1

	Air with pentobarbitone	20% CO_2 in air	20% CO_2 in air with pentobarbitone	Oxygen with pentobarbitone	20% CO_2 in oxygen	20% CO_2 in oxygen with pentobarbitone	Oxygen	Air
Time to unconsciousness (min)	155	180	105	160	225	115	1,000	1,100
Temperature when unconscious (° C)	19·6	21·8	21·8	19·2	21·4	22·2	16·0	17·0
Time to cardiac arrest (min)	220	260	175	220	315	195		
Temperature of cardiac arrest (° C)	12·0	11·8	8·4	10·0	7·2	7·0		

Body fur (40 g ± 10 g) was removed with scissors under pentobarbitone anæsthesia more than 48 h before each experiment. Forty-eight male rabbits of various strains (mean weight 2·8 kg ± 400 g) were used. The rabbit was placed in a cylindrical tin 25 × 15 cm (obtained from the Pharmacy Department) to one end of which was fitted a 'Perspex' chamber 12·5 cm × 12·5 cm × 12·5 cm, to which was attached a brass tube to receive gas tubing from the gas cylinders. The other end of the tin comprised a detachable lid, protected on the inside by a baffle, from which the effluent gases passed through another brass tube.

The rabbits were then subjected to chilling in the aforementioned tin at −20° C (± 4° C) with various inspiratory gas mixtures—air, oxygen, or 20 per cent carbon dioxide in either gas—with or without pentobarbitone anæsthesia, according to prior random selection. The inspiratory gas flow rate was 2 l./min. Pentobarbitone anæsthesia, when appropriate, was effected by an intravenous solution of pentobarbitone 50 mg/kg (± 15 mg/kg).

On reaching a temperature of about 20° C rectal the rabbit is deeply unconscious, and tracheotomy is quickly performed without mechanical restraint, ventilation afterwards being maintained by manual operation of an anæsthetic bag until cardiac arrest.

After tracheotomy the rabbit was placed on the floor of the deep freeze until cardiac arrest; electroencephalographic, electrocardiographic and temperature recordings being started in those rabbits initially unanæsthetized, and being continued in those already under pentobarbitone anæsthesia. The initially unanæsthetized rabbits breathing pure air and those breathing pure oxygen were left in the tin until unconsciousness supervened, their duration in the tin and final rectal temperature being noted. The interval between unconsciousness and death was rapid (about 10 min) in those animals given neither 20 per cent carbon dioxide nor pentobarbitone, though time was sufficient for some electrocardiographs to be recorded and these were all found to be grossly disrupted. Two rabbits in each of these groups showed no change in rectal temperature or conscious level after more than 20 h.

For the recording of arterial pressure, four unclipped anæsthetized rabbits were used; the rabbit was placed directly on the floor of the deep freeze at 0° C and the actual height of the column of blood in a polythene tube, inserted into the abdominal aorta through the femoral artery, was measured. The tube was lubricated with 1 : 10,000 units heparin in normal saline. The four gas mixtures were supplied, one to each rabbit.

The temperature depression seen with 20 per cent carbon dioxide is greater than three-fold that seen with either pure air or oxygen (Table 1); the temperature depression with 20 per cent carbon dioxide is nearly doubled by prior pentobarbitone anæsthesia. The cooling rate with 20 per cent carbon dioxide and pentobarbitone anæsthesia is significantly faster, at the 0·1 per cent level (Table 2), than with pentobarbitone anæsthesia alone, but not proportionately so (Table 1)—this must be due to relative underventilation with increasing carbon dioxide accumulation during anæsthesia[2]. This contention is supported by the results obtained in a prior series of randomized experiments on 42 rabbits where chilling to 20° C rectal was effected with air, oxygen, and both 10 and 20 per cent carbon dioxide in both gases, during pentobarbitone anæsthesia with prior tracheotomy and simultaneous re-breathing (achieved by closing the expiratory value of the anæsthetic bag): in these 42 experiments the cooling rates were uniform.

The temperatures of cardiac arrest are uniform as are those of the onset of unconsciousness and the differences in cooling rate between the various groups are seen in the duration of the interval to unconsciousness (Table 1). While the differences in duration to cardiac arrest between those animals breathing oxygen as opposed to air (both with and without 20 per cent carbon dioxide and/or pentobarbitone anæsthesia) favour oxygen at the 5 per cent level of significance, the difference between those animals breathing air or oxygen with 20 per cent carbon dioxide, as opposed to those animals breathing air or oxygen under pentobarbitone anæsthesia, with or without carbon dioxide, favour 20 per cent carbon dioxide as opposed to pentobarbitone anæsthesia in terms of survival time at the 0·1 per cent level of significance (Table 2). These differences in survival times are presumably explained by the increased tissue oxygen uptake produced by high

Table 2

	No. of rabbits	Time to cardiac arrest (min)	Significance (analysis of variance) (%)
Oxygen/pentobarbitone 20% CO_2 in oxygen 20% CO_2 in oxygen/pentobarbitone	18	246·1	5·0
Air/pentobarbitone 20% CO_2 in air 20% Co_2 in air/pentobarbitone	18	217·8	
20% CO_2 in oxygen 20% CO_2 in air	12	287·1	
Oxygen/pentobarbitone Air/pentobarbitone 20% CO_2 in oxygen/pentobarbitone 20% CO_2 in air/pentobarbitone	24	204·4	0·1
	No. of rabbits	**Cooling rate °C/min**	**Significance (%)**
Oxygen/pentobarbitone 20% CO_2 in oxygen/pentobarbitone	12	0·141	0·1
Air/pentobarbitone 20% CO_2 in air/pentobarbitone	12	0·131	
Oxygen/pentobarbitone Air/pentobarbitone	12	0·121	0·1
20% CO_2 in oxygen/pentobarbitone 20% CO_2 in air/pentobarbitone	12	0·150	

Significant linear cooling trends at 0·1 per cent level, with no evidence of curvature

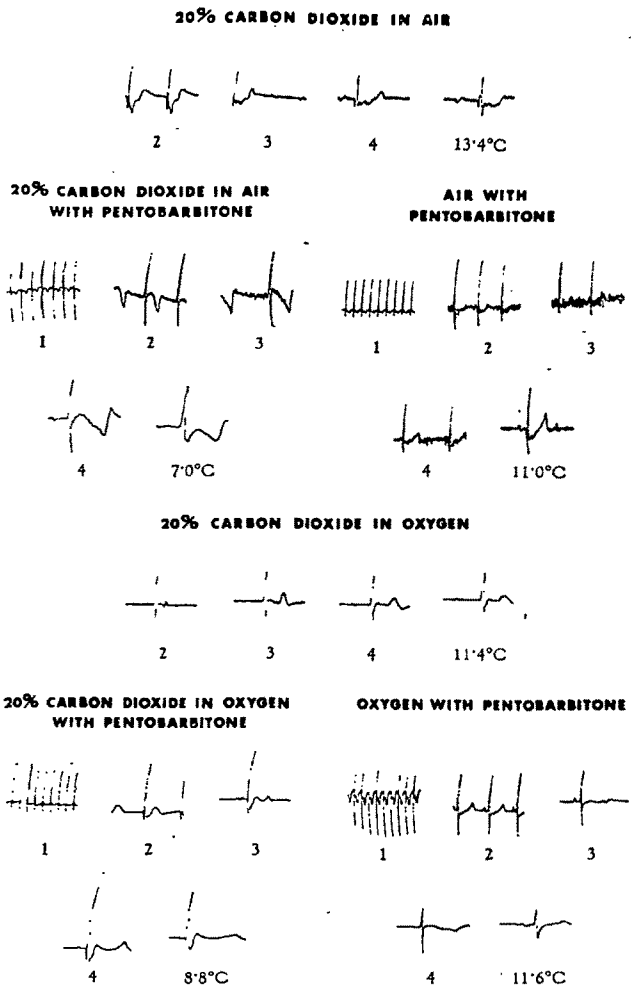


Fig. 1. The E.C.G. during hypothermia. 1, 38°–35° C; 2, 23°–20° C; 3, 18°–15° C; 4, 16°–12° C; 5, lowest actual temperature recorded. Each E.C.G. 'unit' occupies 2 sec of time-marker

levels of alveolar carbon dioxide concentration by the Bohr effect[3]. The lack of significance of the differences in the periods from unconsciousness to cardiac arrest is most reasonably ascribed to circulatory stasis after the onset of unconsciousness (Fig. 2) and minimal tissue oxygen uptake thereafter[4,5].

The electroencephalograph ceases at about 20° C when complete unconsciousness occurs, and spontaneous respirations usually cease at about 16° C—regardless of the presence or absence of 20 per cent carbon dioxide and/or pentobarbitone anæsthesia; the conjunctival reflex is also regularly present until about 16° C, when significant changes in the electrocardiograph start to occur and the blood pressure has fallen to below a quarter of its initial level (Fig. 2).

With adequate ventilation (note one animal 'died' at 24·8° C), the electrocardiograph is normal, except for an increasing bradycardia with gradual lengthening of the *PR* interval below about 25° C, until about 16° C, after which the *P* wave tends to diminish, the *ST* segment to become prolonged and either elevated or depressed, and the complex finally disrupts, to disappear at about 10° C (Fig. 1). The rhythm is regular while the configuration of the complex is preserved and an irregular rhythm presages impending dissolution of the complex. These changes in the electrocardiograph are dependent on temperature and not on the composition of the inspiratory gas mixture.

The role of an increased carbon dioxide tension of the blood in relation to cardiac arrhythmias during hypothermia is controversial, though there is plenty of evidence that hypercapnia is not in itself alone a cause of arrhythmias at normal temperatures[6–9] and recently evidence has been accruing to suggest that a raised carbon dioxide level, or a lowered blood *p*H, may actually delay the premature onset of arrhythmia during hypothermia[10] or facilitate the spontaneous reversion of arrhythmia occurring during hypothermia[11,12].

Earlier reports incriminating hypercapnia as a causative factor in the production of cardiac arrhythmia[13–15] may be criticized on the techniques used—excessive anæsthesia, the use of curare, and unduly rapid changes in inspiratory gas concentrations.

The observations of E.E.G., E.C.G., reflex activity and the maintenance of arterial pressure reported in this paper correspond with the reports of Smith[16], in the rabbit; Brendel *et al*[17] and Spohn *et al.*[18], in the dog; and Simpson[19] and Bryce-Smith *et al.*[20], in the monkey, during surface cooling by more conventional methods.

As already mentioned, a prior series of randomized experiments was performed on forty-two rabbits where chilling was stopped at 20° C rectal, at which temperature they were removed from the deep freeze. Of the first 19, in which spontaneous re-warming at room temperature was practised (18°–21° C), eight survived; and of the succeeding 23, in which re-warming was effected at 38° C ambient in an incubator, 17 survived. Survivors were killed after a fortnight, no disability having been observed. Thus, above cardiac arrest, survival was dependent on the rate of re-warming and not on the composition of the inspiratory gas mixture. Survival after chilling to 20° C rectal while inhaling 20 per cent carbon dioxide in air has been reported in the rabbit[21].

In the present series no survivals were obtained, though respiration was recommenced in two animals following cardiac arrest. The problem of survival after cardiac arrest in large mammals following surface cooling awaits solution[22], but success with small mammals[22] indicates that it is not insoluble.

The cooling rates reported here are significantly linear (at the 0·1 per cent level) above 20° C, to which temperature the reflexes, respiration and the blood pressure are maintained. The absence of any correlation of rates of body cooling with body weight, voluntary movement, shivering or anæsthetic dosage suggests that, in the absence of normal insulation (fur), the most important factor in

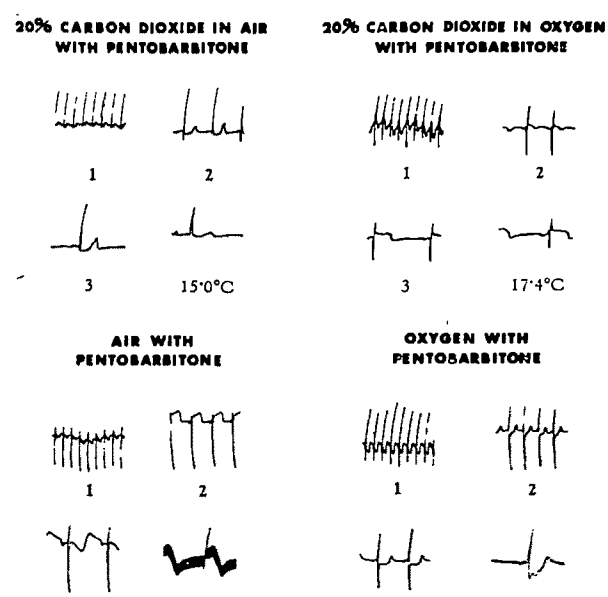


Fig. 2. Blood pressure and E.C.G. during hypothermia. 1, 38·8°–37·8° C; 2, 28·4°–24·4° C; 3, 20·6°–18·8° C; 4, lowest actual temperature recorded. Each E.C.G. 'unit' occupies 2 sec of time-marker. Arterial pressure; 1, 150–110 cm; 2, 120–100 cm; 3, 80–40 cm; 4, 40–20 cm

the depression of body temperature during surface cooling is the regulation of peripheral blood flow[23,24].

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VARIATIONS IN X-RAY RESPONSE DURING THE DIVISION CYCLE OF PARTIALLY SYNCHRONIZED CHINESE HAMSTER CELLS IN CULTURE

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INVESTIGATIONS of the response to X-radiation at different stages of the division-cycle of mammalian cells grown *in vitro* can provide important evidence as to the nature of radiation damage. Terasima and Tolmach[1,2], in studies on various facets of the response to X-radiation of partially synchronized HeLa cells in culture, found two periods of resistance during the first cycle. The first resistance phase was associated with the pre-DNA synthetic period (G_1) and the second with the latter stages of the DNA synthetic period (S) or the early stages of the pre-mitotic period (G_2). In later work, these authors[3] and also Erikson and Szybalski[4] have used 5-fluorodeoxyuridine (FUDR) as an inhibitor of thymidylic acid synthesis to achieve synchrony in cultures. Both studies show that X-ray resistance increases as soon as DNA synthesis begins. All these studies were confined to the first division-cycle after synchronization.

We have examined, throughout two division cycles, the survival of partially synchronized Chinese hamster cells after a single dose of 750 r. of 250-kVp. X-radiation. The method of Terasima and Tolmach for separating dividing HeLa cells from cultures was found to be quite unsuccessful with our *V*-79 clone of Chinese hamster lung cells grown in *HUT*-15 medium[5]. The most successful of many methods tried was the following. Log phase cultures, grown overnight on plastic Petri dishes in a humid atmosphere of 2 per cent carbon dioxide and air at 37° C, were cooled to 4° C in the refrigerator for 1 h and then re-incubated for 2–6 h at 37° C. The medium was removed and 4 ml. of 0·03 per cent trypsin were added without rinsing. The plates were then rocked gently for 5 min on a wrist-action shaker. Trypsin was removed from the individual plates and pooled. This suspension had a high proportion of dividing cells, as estimated from stained sections; up to 75 per cent of the cells were in mitosis in some experiments. In experiments for which substantial numbers of cells were needed, about 45 per cent of the cells pooled from 30–40 plates were found to be in mitosis initially. The status of the remaining 55 per cent was uncertain, but, from the data to be shown here, it seems probable that many of these must have just divided and were therefore in quite close synchrony with those in mitosis.

The cooling of these cells provides only a slight pulse of mitosis in the base culture and no marked synchrony[6] was achieved. Cooling, however, was found essential to provide a better differential attachment between rounded up (dividing) cells and flattened out (non-dividing) cells.

Immediately after separation, the partially synchronized cell-population was inoculated into four groups of plates. The first and second group received about 15,000 cells per plate and were used for growth studies and tritiated thymidine labelling studies, respectively. The third set received approximately 1,000 cells per plate for exposure to X-radiation and the fourth group received 150–200 cells for plating efficiency estimation. All these plates were incubated within 30 min of adding trypsin. Time zero on the graphs, all which pertain to the same experiment, corresponds to the time at which incubation began.

In the growth study, plates were removed from the incubator at intervals, trypsinized for 15 min and the suspension counted at fixed settings on a Coulter counter. The growth curve is shown in Fig. 1*A*. The curve is essentially flat for the first 13·5 h, while in the next 5 h practically all the cells divide and the curve is flat again for approximately 8 h more before the second division begins. The generation time is about 13 h. The index of synchrony proposed by Blumenthal and Zaler[7] is:

$$F = \frac{N_2}{N_1} - 2^{t/g}$$

where N_1 is the number of cells at time t_1; N_2 is the number of cells at time t_2; t is the time interval t_2–t_1; and g is the generation time. This index is 0 for log growth, +1 for doubling in zero time and −1 for periods of no cell doubling. For synchrony, positive values should be found at division followed by negative values in the next part of the cycle. For the data of Fig. 1*A*, F has a value close to −1·0 for the first 13·5 h, followed by $F = +0{\cdot}58$ while the cells are dividing (t_1 = 13·5 h, N_1 = 16,500 cells; t_2 = 18·5 h, N_2 = 31,000 cells). Thereafter, F is clearly negative again until the next division begins. The highest value found for F during the division period, in any of our experiments, was + 0·81. The actual number of cells found in the first cycle is consistent with our other data. For this experiment 45 per cent of the cells were

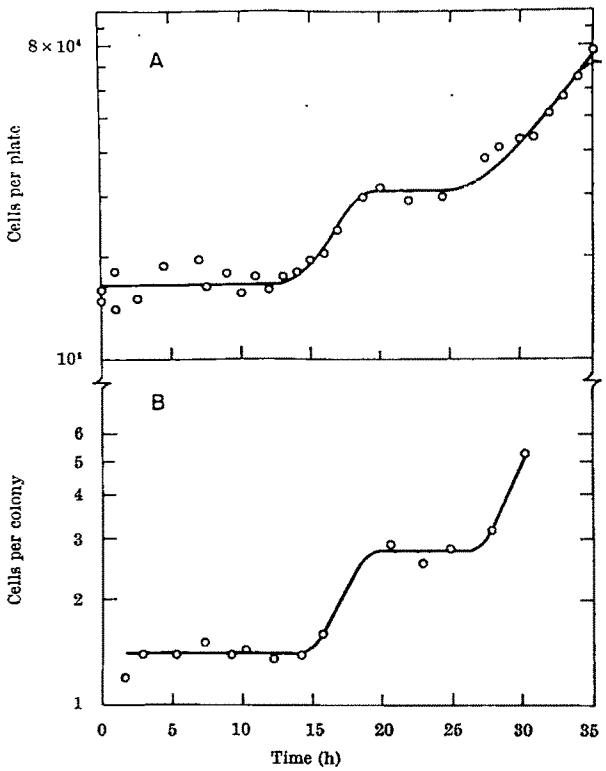


Fig. 1. (*A*) Growth of cells per plate measured by counting trypsinized cells on Coulter counter (Exp. *S*-3); (*B*) cells per colony as a function of time, measured from stained labelled plates (Exp. *S*-3)

in mitosis and would give two cells each after division. A total of (45 per cent × 2) + 55 per cent = 145 per cent of the initial number plated would be expected if all cells survived. A plating efficiency of 63 per cent (found from the fourth series of plates) would reduce this to 91 per cent if the non-survivors fail to attach and the plating efficiency is the same for both mitotic and non-mitotic cells. The actual value found was about 100 per cent.

For the labelling studies, 1 μc. of H^3-thymidine (3 c./mM) was added to the medium of the plates. The plates were incubated for ½ h at 37° C. All subsequent operations were carried out without removing the cells from the plates. The medium was poured off and the cells washed with buffered saline and fixed in acetic alcohol. Later, liquid nuclear emulsion was added in a thin layer. After about 4 weeks of exposure at 4° C to tritium β-particles the plates were developed at 18° C and stained with hæmatoxylin and eosin. These plates were assayed for cells per colony and percentage of labelled cells.

A plot of cells per colony obtained from the stained plates is shown in Fig. 1*B*. The ratio is constant during the first cycle at about 1·4. Since the 45 per cent mitotic cells provide a two-cell clone very soon after attachment, the expected average value of cells/clone is 1·45 if no more cells divide. It rises at division to twice this, ~ 2·8, and then remains constant in the second cycle until the next division begins. For this curve F = + 0·68 during division, which is somewhat higher than for Fig. 1*A*. As an indication of synchrony, this method, using directly stained plates, may be preferable to counting dilute cell suspensions on the Coulter counter.

The percentage of cells labelled at various times by the pulse of tritiated thymidine was assayed by examining at least 100 cells on each plate. An arbitrary limit of more than 12 grains per nucleus, found from grain counts on unlabelled plates, was taken as the criterion of a labelled cell. It was not often necessary to apply this criterion. The result, as a function of time, is shown in Fig. 2*A*. The percentage of labelled cells rises to 100 after about 9 h and then falls to zero at ~ 15 h, when mitosis is high. The rise and fall is repeated, possibly a little earlier, in the next cycle. The average S period, measured from 50 per cent on the ascending part of the curve to 50 per cent on the descending part of the curve of Fig. 2*A*, is about 6·5 h or one-half the cycle, in agreement with investigations on another line of Chinese hamster cells by Hsu, Dewey and Humphrey[8].

For radiation studies, duplicate plates from the third series were exposed at intervals to 750 r. (710 rads) of 250-kVp. X-radiation (half value layer = 0·9 mm copper, 100 rads/min). After 12 days of incubation the plates were stained with methylene blue and colonies counted. The normal surviving fraction of an asynchronous Chinese hamster population at this dose is about 0·12 and this is well beyond the shoulder region of the survival curves[5]. The number of cells surviving exposure at different times, expressed as a fraction of the controls, is shown in Fig. 2*B*. Survival ranges from about 0·1 initially to 0·31 at 9–10 h, only to fall again to about 0·1 at 14 h as division begins. This variation is repeated at a higher level, because of cell doubling, with approximately the same time relationships, in the next cycle. The peak of survival correlates closely with the peak of pulse-labelled cells but may follow it up to 1 h. The cells thus appear most resistant when synthesizing DNA. In a repeat experiment with the same percentage of mitosis in the stained section, very similar results were obtained, with maximum labelling preceding maximum resistance by about 1 h. Results of poorer resolution, but nevertheless exhibiting the same time relationships and correlation between DNA synthesis and survival, have been obtained in several experiments in which lower initial fractions of mitotic cells were available.

Other work has shown a close association of resistance to X-radiation and the onset of DNA synthesis. Studies with inhibitors of DNA synthesis, such as FUDR, in Detroit 98 cells[4] and HeLa cells[3] indicate an increase in resistance when the block is removed and DNA synthesis is resumed. In our case, maximum X-ray resistance

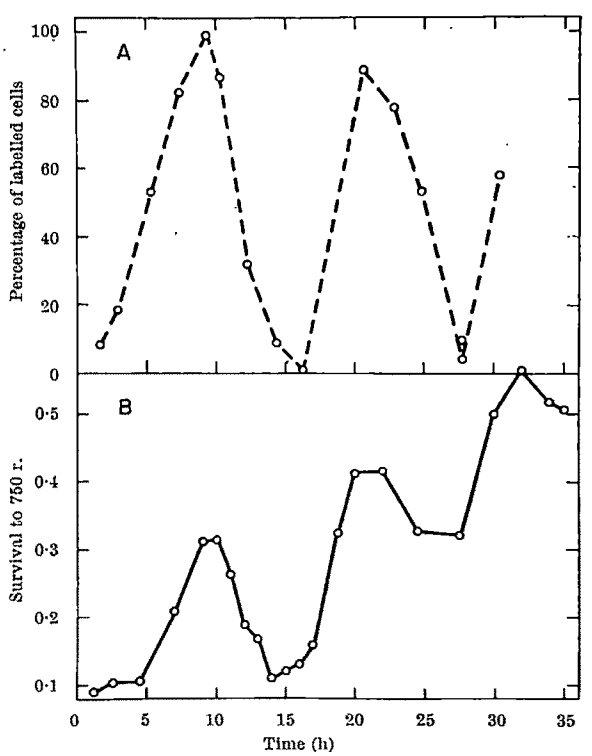


Fig. 2. (*A*) Percentage of cells labelled with tritiated thymidine as a function of time (Exp. *S*-3)0 (*B*) radiation survival to a single dose of 750 r. (710 rads) as a function of time (Exp. *S*-3)

appears to coincide almost exactly with the period of maximum labelling. An appreciable lag between these maxima, however, was reported for HeLa cells[2]. Such a lag may arise from growth differences between irradiated plates and labelled plates if the cell concentration is different. We do not know whether these effects were present in the experiments reported for HeLa cells, but our investigations on asynchronous Chinese hamster cells indicate that at concentrations of 1,000 and 15,000 cells per plate, growth differences, if any, are small. The sharp fall in resistance in the G_2 period (Fig. 2*B*) is consistent with the data of Hsu, Dewey and Humphrey[8]. They found, by ^{3}H-thymidine labelling of asynchronous Chinese hamster cells, 3–4 times less γ-ray induced chromosome damage for cells in *S* phase than for cells in G_2 phase. This would agree with our observations if chromosome damage is correlated with cell death. Different results were found by Dewey and Humphrey[9] in other cell lines. Finally, we observe only one peak of resistance in the cycle, during DNA synthesis, and none during the G_1 phase, as in the case of HeLa cells[2]. The studies with HeLa were limited to the first cycle following inoculation, in which there may be attachment problems. On the other hand, these hamster cells have such a short G_1 period that differences during this period for the two cell lines may not be unreasonable.

The survival to a single dose (Fig. 2*B*) does not determine the lethal response uniquely. However, the data for 750 r. given in Fig. 2*B* are consistent with the survival found for the asynchronous hamster population at this dose. Taking the area under the curve for a linear plot of the data of Fig. 2*B*, the average survival over the first 15 h, for cells with a multiplicity of 1·45 (cells per clone), is found to be 0·177. For single cells in an asynchronous population, the survival fraction at 750 r. should be $\frac{0{\cdot}177}{1{\cdot}45} = 0{\cdot}12$, which is the value actually found experimentally[5]. Full survival curves at different times will be necessary to compare the complete radiation responses of the various stages of the division cycle and correlate these with results for the asynchronous hamster population.

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CHOLINESTERASE-CONTAINING SYSTEMS OF THE BRAIN OF THE RAT

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THE thiocholine technique for the histochemical localization of cholinesterases, as originally applied to the rat brain by Koelle[1], showed a bewilderingly wide distribution of enzyme. Further investigations by ourselves have indicated that the pattern of cholinesterase activity varies in different areas[2,3]. In the forebrain these areas can be classified as follows: (*a*) those where the enzyme is intracytoplasmic, and causes the cell bodies of cholinesterase-containing neurones to stand out clearly from the surrounding grey matter, as in the subthalamus, globus pallidus, lateral preoptic area, diagonal band and medial septal nucleus; (*b*) those where the enzyme is intra-axonal, with the consequence that the cholinesterase-containing fibres can be readily distinguished from adjacent white matter, as in the dorsal fornix, fimbria, fasciculus retroflexus and ventral supraoptic decussation; (*c*) those where the staining is so dense that the precise location of enzyme is difficult to determine, as in the caudate-putamen, nucleus accumbens, pars ventralis of the lateral amygdaloid nucleus, and nucleus of the lateral olfactory tract.

Areas with generalized staining rather less dense than the foregoing include the midline and intralaminar thalamic nuclei, the ventral nucleus of the lateral geniculate body and the pretectal nuclei. In addition, one can recognize: (*d*) areas where the enzyme is in specific laminæ, as in the cingulate gyrus, which contains cholinesterase mainly in layers i and iii, the hippocampus where the enzyme is located on either side of the pyramidal layer and in the stratum lacunosum, the dentate gyrus with enzyme on either side of the granular layer, and the olfactory bulb, which contains enzyme in the glomerular and mitral cell layers; and (*e*) areas, lightly stained by the thiocholine method, where the distribution of cholinesterase appears to be diffuse, as in most regions of the neocortex. Certain areas of the brain show no evidence of cholinesterase activity: for example, the white matter of the internal capsule and of the cerebral peduncle, and the commissural fibres of the corpus callosum.

In order to clarify the picture in neurological terms, we adopted the device of placing lesions in selected areas of the brain and allowing the animal to survive for four days or more[4]. In this way we were able to show that interruption of cholinesterase-containing axons causes enzyme to pile up for a short distance on the cell body side of the cut and to disappear on the opposite side from the whole length of the severed fibres, including their terminals. The method, therefore, enabled us to determine the polarity and distribution of cholinesterase-containing tracts, which was often in doubt in normal material. We have since refined our technique by using radiofrequency current to produce thermal lesions accurately placed by means of a stereotaxic instrument[5]. In this way we have been able to interrupt cholinesterase-containing fibres with minimal damage to surrounding structures, and so build up a complete map of all such pathways in the forebrain (Fig. 1).

We have found that cholinesterase-containing fibres ascend from the brain stem into the forebrain by two main routes, which we propose to call the 'dorsal' and 'ventral tegmental pathways'. The dorsal tegmental pathway extends rostrally from the region of the nucleus cuneiformis of the mesencephalic tegmentum and projects on the pretectal area, the geniculate bodies, the mammillary region and the thalamus. In animals which have been subjected to a unilateral midbrain lesion involving this pathway and later killed, there is a loss of staining on the operated side which is particularly marked in certain nuclei to which the dorsal tegmental pathway contributes a dense terminal neuropil, namely, the pretectal nuclei (especially the nucleus pretectalis profundus), the parafascicular-centromedian nucleus, the intralaminar thalamic nuclei and the anterior thalamic nuclei.. The dorsal tegmental projection does not appear to reach cortical levels.

The ventral tegmental pathway extends rostrally through the substantia nigra and the region of the ventral tegmental nucleus to enter the subthalamic H_2 field and

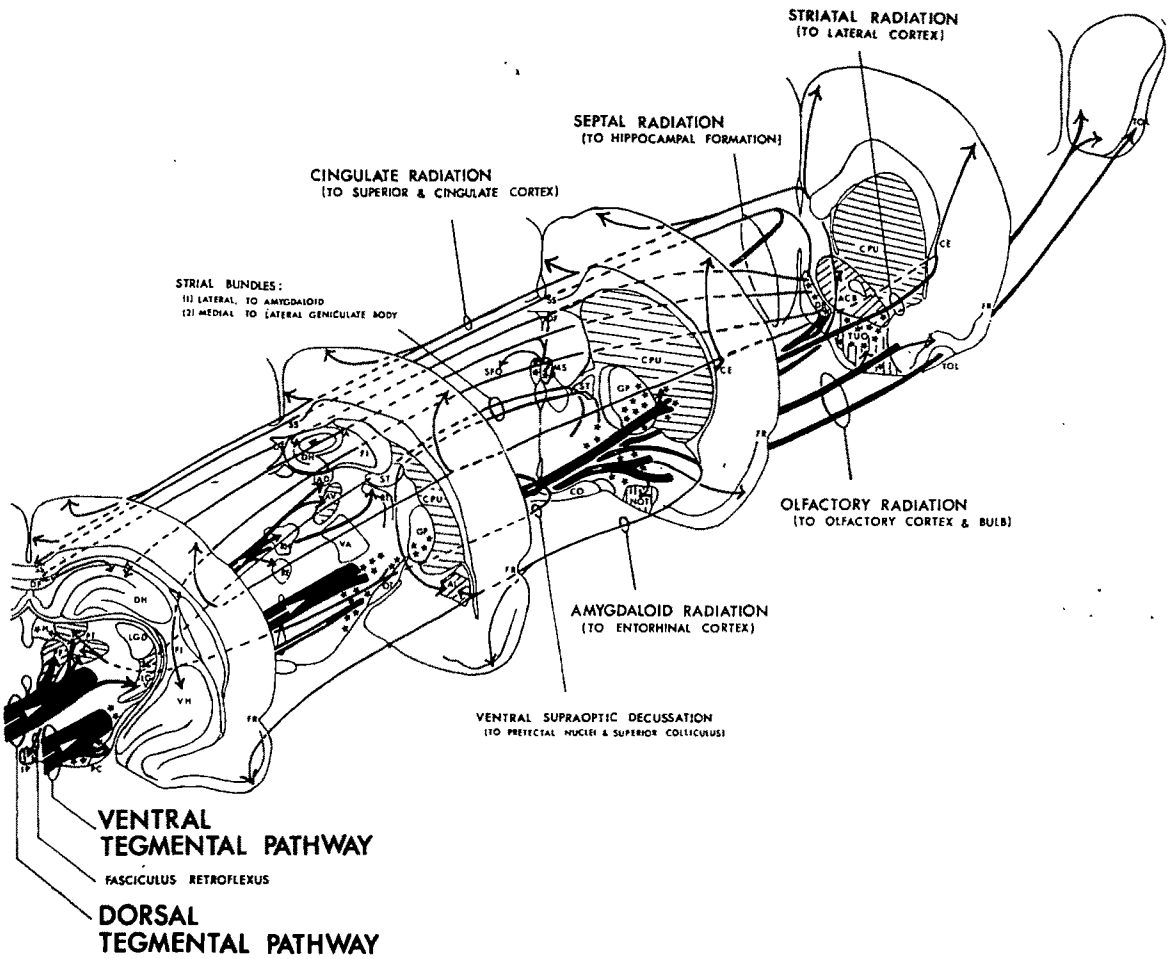


Fig. 1. Exploded diagram of rat forebrain, showing cholinesterase-containing pathways. Asterisks indicate distribution of AcChE-containing cells. Areas of dense neuropil are hatched. Natural spacing of sections increased 5 times. Abbreviations: *A*, alveus; *ACB*, nucleus accumbens; *AD*, anterodorsal thalamic nucleus; *AL*, lateral amygdaloid nucleus pars ventralis; *AV*, anteroventral thalamic nucleus; *CE*, external capsule; *CO*, optic chiasma; *CPU*, caudate-putamen; *DB*, diagonal band; *DF*, dorsal fornix; *DH*, dorsal hippocampus; *FI*, fimbria; *FR*, rhinal fissure; *GP*, globus pallidus; *H*, habenular nuclei; *IP*, interpeducular nucleus; *LGD*, dorsal nucleus of lateral geniculate body; *LGV*, ventral nucleus of lateral geniculate body; *MS*, medial septal nucleus; *NOT*, nucleus of lateral olfactory tract; *OT*, optic tract; *PC*, cerebral peduncle; *PF*, parafascicular-centromedian nucleus; *PT*, pretectal nuclei; *RE*, reticular thalamic nucleus; *SFO*, subfornical organ

the lateral hypothalamic area of the diencephalon. Some fibres which pierce the descending tracts of the basis pedunculi helps to form the so-called 'comb bundle'. In normal material, bundles belonging to the ventral tegmental pathway can be traced through the diencephalon as far as the globus pallidus: they correspond to the nigrostrial fibres of previous accounts[6]. In animals which have been subjected to a unilateral lesion of the ventral tegmental pathway at the level of the midbrain-diencephalic junction, these bundles lose their staining on the operated side, and there is an appreciable ipsilateral pallor of the normally densely stained neuropil of the caudate-putamen.

All cortical areas receive a projection of cholinesterase-containing fibres, which accounts for the loss of staining seen when these areas are denervated. We have found it convenient to group the corticopetal projections into five radiations, which we designate 'striatal', 'olfactory', 'amygdaloid', 'cingulate' and 'septal'. Fibres of the striatal radiation run in the external capsule (Fig. 2) and are distributed to the middle part of the lateral hemispheral cortex, extending as far down as the rhinal fissure. They reach the external capsule by traversing the caudate-putamen in company with cholinesterase-free bundles connecting the cortex with the internal capsule. Many apparently arise from cholinesterase-containing cells scattered throughout the globus pallidus and to a lesser extent in the caudate-putamen; others may have a more caudal origin from the ventral tegmental pathway. Lesions of the globus pallidus cause loss of fibre staining in the external capsule. The olfactory radiation can be traced from the lateral preoptic area and the olfactory tubercle, which include many cholinesterase-containing neurones, into the olfactory stalk and lateral olfactory tract, and from there to the prepyriform cortex and to the glomerular and mitral cell layers of the olfactory bulb. The normal staining of these layers is diminished by lesions involving the olfactory radiation. The amygdaloid radiation is continuous with the striatal radiation dorsally and the olfactory radiation anteriorly; its fibres pass from the lateral preoptic area through the amygdaloid, and are distributed via the lower part of the external capsule to postpyriform or entorhinal cortex. The cingulate radiation arches upwards in the bundle of Zuckerkandl in front of the genu of the corpus callosum, and travels back in the cingulum to supply superolateral and cingulate cortex. Finally, cholinesterase-containing cells of the diagonal band and medial septal nuclei give rise to fibres of the septal radiation, which is distributed to the hippocampus and dentate gyrus via the supracallosal striæ, dorsal fornix, alveus and fimbria (Fig. 3). Lesions of the septal radiation cause disappearance of the banded staining normally seen in the hippocampal formation (Fig. 4). This observation contradicts previous suggestions that afferent fibres in the fornix terminate only in the presubiculum[7] or in the dentate gyrus[8].

A portion of the septal radiation projects subcortically to two of the regions of the brain where the blood-brain



Fig. 2. Enzymatic pile-up following a lesion of the external capsule in fibres traversing the caudate-putamen. (×c. 124)

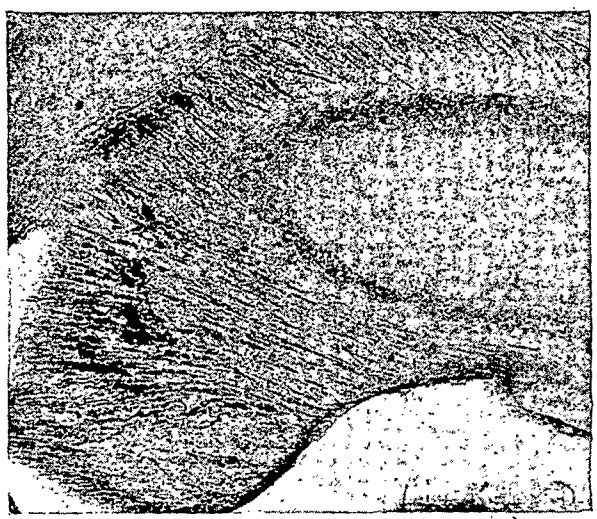


Fig. 3. Enzymatic pile-up in fibres of the alveus and fimbria. (×c. 26)

barrier is deficient, namely, the subfornical organ, otherwise known as the intercolumnar tubercle, and the supraoptic crest. The cholinesterase-containing fibres which innervate these organs arise from cells situated in the midline region of the septum, in the substance of the hippocampal commissure, and in the dorsal fornix.

We have also investigated experimentally a number of other subcortical pathways which carry cholinesterase-containing fibres. Those travelling with the 'fasciculus retroflexus' project on the interpeduncular nucleus. Fibres in the substance of the stria terminalis and at its lateral border, which we term the 'lateral strial bundle', project caudally, that is, against the main stream of the stria, and constitute a roundabout route from the lateral preoptic area to the amygdaloid. There is another group of fibres, on the medial side of the stria terminalis but not really part of it, which we have called the 'medial strial bundle': this projects on to the lateral geniculate body, especially on its more deeply staining ventral nucleus. The 'ventral supraoptic decussation' projects on to the pretectal area and superior colliculus. Interruption of the dorsal tegmental pathway produces loss of staining of the ascending fibres of the decussation, that is, those travelling forwards to the region of the optic chiasma. The medial strial bundle and the ventral supraoptic decussation can both be interpreted as aberrant loops of the dorsal tegmental pathway: the former an uncrossed bundle, and the latter a crossed one.

It is tempting to regard the cortical and sub-cortical pathways described here as part of a unified system, in which they represent the forebrain continuation of the brain stem reticular formation. This also consists, mainly if not entirely, of neurones rich in cholinesterase. The dorsal and ventral tegmental pathways correspond closely to the thalamic and extrathalamic portions respectively of the ascending reticular activating system described in cat by Starzl, Taylor and Magoun[9]. The problem remains as to whether these pathways, in view of their high concentration of cholinesterase, can be properly described as cholinergic. de Robertis *et al.*[10,11] have shown in homogenates of rat brain that acetylcholinesterase, acetylcholine and cholinacetylase are concentrated in subfractions containing nerve endings and synaptic vesicles. The association thus obtained between the three substances known to characterize cholinergic neurones in the peripheral nervous system would appear to justify the assumption that, in the central nervous system too, acetylcholinesterase is produced by cholinergic neurones and released at their endings.

For this reason we have thought it desirable to investigate the contribution to total staining made by true cholinesterase (AcChE) and pseudocholinesterase (ChE) in our material. Sections of normal brain were incubated in substrate solutions containing acetyl, butyryl and propionyl thiocholine, with and without the selective AcChE inhibitor (62*C*47) and ChE inhibitor (ethopropazine) recommended for rat brain by Bayliss and Todrick[12]. Comparison of the intensity of staining obtained with acetyl and propionyl substrates in the presence of ChE inhibitor indicated that the AcChE of rat brain hydrolyses propionyl thiocholine almost as effectively as acetyl thiocholine. On the other hand, there is no visible evidence of hydrolysis of butyryl thiocholine by AcChE even after long incubation: this is borne out by the fact that addition of AcChE inhibitor to Bu substrate did not affect the staining. Bayliss and Todrick[12] estimated that, when AcChE and ChE are both present, the contribution of AcChE to the total hydrolysis of butyrylcholine is 9 per cent. An indication of the substrate affinity of ChE is given by the intensity of staining of cerebral blood vessels (although we have found that certain blood vessels, especially those in the midline thalamic and cingulate regions, contain some AcChE). On this basis it

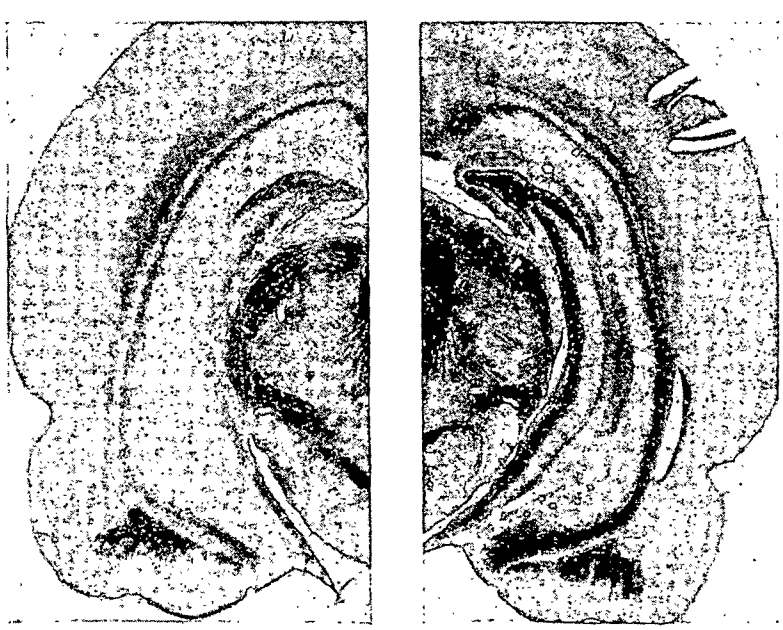


Fig. 4. Loss of staining in the hippocampus and dentate gyrus of the operated side 7 days after a fimbrial lesion. (×c. 9)



Fig. 5. Loss of staining around the motoneurones of the oculomotor nucleus 20 days after a lesion of the ventral tegmental nucleus. The nucleus of Edinger–Westphal is unaffected. (×c. 83)

may be said that ChE in rat brain hydrolyses propionyl thiocholine rather more effectively and acetyl thiocholine less effectively than butyryl thiocholine.

We have so far had no opportunity to study the hydrolysis of benzoyl thiocholine. Such an investigation would be of interest since the ability to hydrolyse benzoylcholine was used by Burgen and Chipman[13] as a criterion for the distribution of what they term ChE II in dog brain. Their findings do not tally with the localization of ChE in rat brain, as determined by us with the use of inhibitors: for example, we detected no ChE in the caudate. The discrepancy in the distribution of these enzymes may be due to lack of substrate specificity, since it has been shown that when AcChE and ChE are both present 30 per cent of the total hydrolysis of benzoylcholine is caused by AcChE[12]. Certainly our histochemical findings are consistent with the results obtained by Bayliss and Todrick using biochemical methods.

We conclude that two types of cholinesterase-containing neurones occur in rat brain: those which contain AcChE only, and those which contain ChE with or without some AcChE also present. Our findings in this respect run counter to those of previous workers, who have mostly held that central nervous ChE was located almost entirely in blood vessels and glia[14]. In the forebrain, the exclusively AcChE-containing neurones are those associated with the ventral tegmental pathway. They include the projections to the subfornical organ and supraoptic crest, and the radiations to the hippocampal formation, neocortex, olfactory cortex and the olfactory bulb described here, and also the dense neuropil of certain subcortical nuclei which appear to be associated with these radiations, that is, that of the caudate-putamen, the nucleus accumbens, the pars ventralis of the lateral nucleus of the amygdaloid, and the nucleus of the lateral olfactory tract. The ChE-containing neurones include those of the dorsal tegmental pathway, which project to the thalamus and contribute a dense neuropil to its midline and intralaminar nuclei, and also to the anteroventral thalamic nucleus. Other ChE-containing neurones of the forebrain are as follows: (1) the interstitial nucleus of the ventral hippocampal commissure—the neurones which constitute this nucleus can be seen in our material to project to the medial cortex of the frontal lobe; (2) the islets of Calleja, which project on to the superficial layer of the olfactory tubercle; (3) the anterior thalamic nuclei, whose projection on to layers i and iii of the cingulate gyrus is superimposed on the exclusively AcChE-containing projection of the cingulate radiation; (4) some cells of the lateral thalamic nucleus; (5) the nucleus reuniens of the thalamus, which possibly projects to the anterior cingulate cortex[15]; (6) the interstitial nucleus of the ventral supraoptic decussation, which contributes to the projection of that bundle on to the pretectal and tectal regions; (7) a few cells of the medial habenular nucleus, projecting on to the nucleus interpeduncularis.

In the brain stem the areas of grey matter which possess intracellular cholinesterase include the motor nuclei of cranial nerves, some cells of the sensory trigeminal nucleus, the lateral and superior vestibular nuclei, the red nucleus, the tegmental nuclei, nuclei of the reticular formation and cells of the stratum griseum profundum of the superior colliculus. As in the forebrain, it is possible to distinguish sites containing AcChE only and those in which the enzyme is partly ChE. Thus, the cells of the branchiomotor nuclei of cranial nerves V and VII and of the nucleus ambiguus contain AcChE only. The somatic motor nuclei of cranial nerves III, IV, VI and XII also contain AcChE only, with the exception of a group of cells forming the ventral portion of the hypoglossal nucleus in its caudal half. The AcChE of the somatic and branchiomotor nuclei is located not only within cell bodies but also in surrounding neuropil. We have been able to eliminate staining of pericellular neuropil in the oculomotor nucleus of one side by placing a lesion in the ipsilateral ventral tegmental nucleus (Fig. 5). This finding suggests that the neuropil is formed by presynaptic terminals of cholinesterase-containing neurones projecting on to the motor cells.

Of the visceral motor nuclei, the nucleus of Edinger-Westphal and the dorsal motor nucleus of the vagus possess a high concentration of intracellular ChE. The so-called interstitial nucleus of the ventral tegmental decussation also contains intracellular ChE, and appearances in normal material suggest that it may project on to the Edinger-Westphal nucleus and on to the deep pretectal nucleus. Unlike those of the visceral motor nuclei of III and X, the cells identified by us as belonging to the salivatory nucleus[2,16] contain only AcChE. The same is true of the preolivary nucleus and retro-olivary cells believed to be the source of the olivocochlear bundle of Rasmussen[17]. The nucleus coeruleus, sometimes described as the small-celled component of the mesencephalic trigeminal nucleus, also contains only AcChE and appears to send fibres to the cerebellum. ChE is distributed in other brain stem nuclei as follows: (1) the nucleus cuneiformis of the midbrain tegmentum, which appears to be, in part at least, the origin of the dorsal tegmental pathway; (2) the dorsal and deep tegmental nuclei (these nuclei appear to project to various centres which also contain ChE, for example, the nucleus cuneiformis, part of the interpeduncular nucleus, the superior and lateral vestibular nuclei via the dorsal longitudinal fasciculus, and the nucleus reticularis tegmenti pontis which relays to the cerebellum); (3) the nucleus reticularis pontis oralis et caudalis and the nucleus reticularis lateralis; (4) cells of the stratum griseum profundum of the optic tectum which appear to project on the superficial layer of the tectum. Our interpretation of the course taken by ChE-containing neurones in the brain stem, other than those of the dorsal tegmental pathway projecting on the thalamus, must be regarded as provisional only, since so far we have not placed experimental lesions caudal to the midbrain tegmentum.

The functional significance of intraneuronal ChE is at present obscure. In the absence of any evidence to the contrary, it seems reasonable to assume that neurones which contain AcChE only in high concentration, such as those forming the corticopetal radiations described in this article, are cholinergic, that is, operate by the release of acetylcholine. The likelihood that the cerebral cortex does come under cholinergic influences is enhanced by the finding that cortical cells are excited by acetylcholine[18]. Most of the ChE-containing neurones in the rat brain—the anterodorsal nucleus of the thalamus is an exception—also contain a high concentration of AcChE, and may

likewise be cholinergic. Thus the thalamus, which we have shown to contain ChE within the cell bodies of neurones belonging to the anterior group of nuclei and in nerve terminals derived from the dorsal tegmental pathway, has been found in dog to be rich in acetylcholine[19] and in cholinacetylase[20]. It has been suggested that ChE may act at sites of constant ACh production to prevent the concentration of ACh from passing beyond a certain level[21]: since ChE does not exhibit the phenomenon of substrate inhibition with respect to acetylcholine, it continues to act at concentrations of the latter which would inhibit AcChE. Another possibility is that the *in vivo* action of ChE is on higher choline or related esters. Koelle[22] has argued that the ChE which is present in large quantities in addition to AcChE in Auerbach's plexus may be concerned with the hydrolysis of such esters acting as local hormones or transmitting agents in the gut. These esters might act directly on the receptor or might have a potentiating effect, just as butyrylcholine will not only cause contraction of frog's rectus but will also sensitize the muscle to the action of acetylcholine[23]. It is not certain, however, that higher cholinesters occur in normal brain[24], other than γ-aminobutyrylcholine, which may function as an inhibitory transmitter[25].

Other sites of acetylcholine activity where ChE is present in addition to AcChE include the motor end plates of rat diaphragm[26]. We have studied the cholinesterase content of motor end plates in the tongue musculature of rat, and our observations suggest that the proportion of ChE is greatest in those of the intrinsic muscles. It is noteworthy that these muscles are believed to be innervated from the ventral part of the hypoglossal nucleus[27], which we have found to contain intraneuronal ChE. The other location for ChE in the medullary portion of the floor of the fourth ventricle is in the cells which form the dorsal motor nucleus of the vagus. These neurones are generally held to be cholinergic, although the evidence, based mainly on the acetylcholine content of the cervical vagal trunk[19], is not conclusive since some at least of the cholinergic fibres in the cervical vagus are probably derived from the sympathetic system[28].

Some importance may be attached to the distributional pattern of intraneuronal ChE in the central nervous system. It is noticeable, for example, that a number of the sites in which this enzyme is concentrated, such as the interstitial nucleus of the hippocampal commissure, the dorsal and deep tegmental nuclei, and the anterior thalamic nuclei, are on projection pathways related to the hippocampal fornix system. Other nuclei containing intracellular ChE, for example, the nucleus reticularis tegmenti pontis[29] and the nucleus reticularis lateralis[30], are known to project on to the cerebellum. Green and Morin[31] found electrophysiological evidence, which they were not then able to explain, for a functional relationship between fornix pathways and the cerebellar cortex. The projection of ChE-containing neurones on to the midline and intralaminar thalamic nuclei must, of course, be considered in relation to the diffuse thalamic projection system of Morison and Dempsey[32] which originates from these nuclei. It is significant, therefore, that high-frequency repetitive stimulation of the midline and intralaminar nuclei produces electrocortical arousal[33], and that a similar effect can be achieved by intra-arterial injection of ChE inhibitor[34]. Certain of the midline and intralaminar nuclei have been shown to project to the corpus striatum[35], and the final link with the cortex responsible for bringing about the arousal reaction may be achieved through the corticopetal projections of the AChE-containing, presumed cholinergic cells which we have found in this region.

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RESEMBLANCES BETWEEN PASSIVE ANAPHYLACTIC SENSITIZATION AND TRANSMISSION OF PASSIVE IMMUNITY

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RECENT advances in knowledge of passive anaphylactic sensitization and of transmission of passive immunity from mother to young have disclosed certain similarities in these two processes which it would seem worthwhile to consider, since it is possible that too little attention has been paid by those engaged in the one field to the results obtained in the other.

Hartley[1] was the first to suspect a similarity between the transmission of passive immunity from mother to fœtus and passive anaphylactic sensitization in guinea pigs and

to suggest that both processes depended on the acceptability of the antibody molecules to the cells. He knew that guinea pigs could be readily sensitized by antitoxins prepared in guinea pigs, rabbits or man, but not by those prepared in horses, oxen, sheep, goats or pigs. He showed that whereas guinea pig antitoxin was transmitted readily from mother to fœtus, horse antitoxin was scarcely transmitted at all. Further, he showed[2] that diphtheria antitoxin prepared in a guinea pig, when pepsin refined, lost the capacity both for passive sensitization and for passive transmission, although retaining its antibody activity.

During the past ten years much additional evidence has been obtained which tends to justify Hartley's assumption. To these advances, increasing knowledge of the structure of the γ-globulin molecule has contributed notably. It is unfortunate, however, that whereas most of the work on anaphylaxis has been on the guinea pig, most of that on the transmission of passive immunity has been on the rabbit, rat and mouse. Parallel studies on both these phenomena in a single species are needed for precise comparison and they should be rewarding.

Anaphylactic sensitization of guinea pigs *in vivo* and of guinea pig tissues *in vitro* is a selective process in so far as γ-globulin is the only effective agent, and the γ-globulins of some species are more effective than those of others. Humphrey and Mota[3] found, for example, that guinea pig and rabbit antibodies are the most effective, monkey, dog and human antibodies somewhat less effective, and rat, horse, goat and fowl antibodies ineffective. Antibodies from oxen and sheep are ineffective also[1], as are human auto-immune antibodies[3] and those to pollen[4].

Immunity is transmitted before birth in the rabbit and guinea pig and mainly after birth in the rat and the mouse; the γ-globulin being transmitted preferentially to the other serum proteins in all these animals. Antitoxins prepared in various species are transmitted preferentially in the order rabbit > human > guinea pig > dog > horse > ox in the rabbit. Rabbit antitoxins are transmitted in quantities about 100 times those of bovine antitoxins[5]. Rabbit, rat, guinea pig and other rodent antibodies are transmitted readily from the gut to the circulation in the suckling rat, but hyperimmune agglutinins to *Salmonella pullorum* from these species are transmitted several times more readily than agglutinins to *Brucella abortus* prepared in the same species[6]. Antibodies prepared in ox, sheep and fowl are not transmitted[7,8]. Some antibodies are transmitted preferentially in the order mouse > rat > guinea pig > rabbit > ox in the mouse[9,10]. However, when hyperimmune rabbit anti-*Brucella* serum containing a high titre of complete agglutinins is fed to suckling rats and mice a considerable quantity of incomplete agglutinins, with little or no complete agglutinins, appears in the circulation. Thus there is a notable similarity between passive anaphylactic sensitization and passive transmission of immunity in that both processes are selective between immune globulins derived from different species as well as in some cases between different antibodies derived from the same species.

Hartley[2] was the first to observe that diphtheria antitoxin prepared in guinea pigs, when pepsin refined, lost both the properties of passive sensitization of guinea pig smooth muscle tissue *in vivo* and *in vitro*, and of passive transmission from the maternal to the fœtal circulations in this species, although retaining unimpaired its antibody activity. The rate of transmission across the rabbit fœtal membranes of homologous diphtheria antitoxin is much reduced by pepsin refining[11]. Pepsin refining reduces the size of the antitoxin molecule by about one-third without impairing its antibody activity but with loss of most of its antigenicity. This suggested that the part of the molecule destroyed by pepsin was that responsible for anaphylactic sensitization and for the rate of transmission across the fœtal membranes. This part of the molecule also appears to be that which carries most of the antigenic determinants which distinguish the γ-globulin of one species from that of another. The discovery at this time[12] that the parts of rabbit γ-globulin molecules lost by pepsin digestion could be recovered after digestion with crystalline papain and separated chromatographically in a crystallizable state opened up the way for testing this hypothesis. This technique yielded also two fractions with antibody activity, each of which consisted of molecular fragments of about one-half the size of the product of pepsin digestion. Each fraction had incomplete or monovalent antibody activity as distinct from the complete or divalent activity of pepsinized antitoxin. These fractions were called I and II and the antigenic fraction was called III. It has been shown since[13] that a molecule of rabbit γ_1-globulin is composed of two identical pieces of fraction I together with one piece of fraction III, and that the molecule of rabbit γ_2-globulin is similarly constructed of two pieces of fraction II and one of fraction III. It has been shown[14,15] that neither fraction I nor fraction II of rabbit antibody is able to sensitize the skin of the guinea pig either for direct or reversed passive cutaneous anaphylaxis, whereas fraction III is an effective sensitizing agent in reversed passive cutaneous anaphylaxis. It was concluded that whereas guinea pig cells could readily fix fraction III, they could not fix fractions I or II of rabbit γ-globulin. Fraction III of rabbit γ-globulin, isotopically labelled with iodine-131, is transmitted across the membranes to the fœtal circulation of the rabbit eleven times as readily as fraction I and six times as readily as fraction II[16]. Fraction III was transmitted at nearly the rate (0·7) of whole rabbit γ-globulin similarly labelled. This suggested that the region of the γ-globulin molecule which contains the 'recognition unit' for transmission by homologous cells is also that (fraction III) which contains most of the antigenic groups for heterologous cells. The close correspondence of the conclusions that have been drawn from the use of these molecular fragments in passive anaphylactic sensitization and in the transmission of passive immunity is obvious.

Non-immune serum can interfere with the passive sensitization of guinea pig tissues by antibodies[17]. This interference is due to the γ-globulin fraction of the serum, not to the other serum proteins[18]. It can be demonstrated *in vivo* in passive general anaphylaxis in both guinea pigs and mice[19], and in passive cutaneous anaphylaxis in guinea pigs[20]. It can be demonstrated *in vitro* in guinea pig tissues by the Schultz–Dale reaction of smooth muscle[3,21] by histamine release from chopped lung[22] and by mast cell degranulation[3]. The γ-globulins of different species vary in their capacity to inhibit passive sensitization in guinea pigs; for example, the effectiveness of γ-globulins to interfere with the sensitization by rabbit antibodies of guinea pig smooth muscle tissue decreases in the order rabbit > man > dog > guinea pig > rat > horse > ox > pig > fowl > goat[23]. For complete inhibition of sensitization the non-specific γ-globulin must be present in amounts considerably in excess of the antibody, thus for inhibition of passive cutaneous sensitization in the guinea pig by rabbit antibody a ratio of antibody/non-specific γ-globulin of 1 : 100 is required[21]. Fraction III of the papain hydrolysed rabbit γ-globulin is even more effective than the whole γ-globulin in inhibiting the sensitization of guinea pig tissues by rabbit antibody, whereas fraction I is entirely without effect[15].

Certain heterologous sera interfere with the transmission of antibodies from the gut to the circulation of suckling mice and rats when administered orally at the same time[24]. The transmission of both homologous and heterologous antibodies can be interfered with in this way, but only the sera of certain species interfere. Rat, mouse, hamster and sheep sera do not interfere with the transmission of antibodies in either the suckling mouse or suckling rat. Rabbit, guinea pig, human and bovine

sera are effective in producing interference in both rats and mice; all do so about equally in the rat, whereas in the mouse the order is rabbit > guinea pig or human > bovine serum. The γ-globulin fraction is at least as effective as whole serum and can account for the whole of the interference, whereas the albumin fraction has no effect. However, to inhibit transmission of immunity, the γ-globulin, whether in serum or separated, must be in considerable excess relative to the antibody globulin. Fraction III of papain hydrolysed rabbit γ-globulin is several times as effective as whole rabbit γ-globulin in interfering with the transmission of antibodies in mice, whereas fractions I and II are ineffective[25]. Interference has not been observed in the transmission of antibodies across the fœtal membranes in rabbits and no information is available concerning other species.

Thus, the phenomenon of interference has been observed both with passive anaphylactic sensitization in the guinea pig and with passive transmission of immunity in the rat and the mouse. The sera of certain species interfere, whereas those of others do not, both in anaphylaxis and in transmission. In both processes the whole effect is due to the γ-globulin fraction of the serum, and fraction III of papain hydrolysed rabbit γ-globulin is considerably more effective than the whole γ-globulin, whereas fraction I is without effect. The interfering γ-globulin must be in substantial excess over the immune γ-globulin for interference to approach completion in either process. Both in anaphylaxis and in transmission interference has been attributed to competition for specific receptors on, or in, the cells.

The resemblances mentioned strongly support the assumption of an essentially similar mechanism of cellular attachment in both sensitization and transmission. There are, however, important differences between the two processes. Perhaps the most obvious is that, whereas it is the mast cells which are involved in sensitization, it is the endoderm cells of either the fœtal yolk-sac or of the fœtal or neonatal gut which mediate transmission in rabbits and rodents, although the syncitiotrophoblast may be concerned in primates[26]. Secondly, whereas sensitization only involves the assumption of attachment to receptors on the external surfaces of the cells, transmission requires transport across the cells from one pole to the other and, hence, the assumption of internal receptors. All the available evidence, both physiological[27] and electronmicrographic[28,29], indicates that in the transmission of immunity the uptake of the γ-globulin by the cells is pinocytotic and, consequently, not selective. This suggests that the receptors are probably in the walls of the pinocytotic vesicles, although they may be carried from the external surface into the vesicles when they are formed. The excess protein in solution, absorbed pinocytotically and not transmitted, is certainly degraded so that absorption and transmission are not commensurate[27].

Finally, whereas sensitization does not necessarily require a mechanism of release of the γ-globulin from the receptors, once attached, such a mechanism is implicit in the transmission of immunity.

Some of these differences between the two processes may be more apparent than real and careful comparison should result in further insight into both. It is tempting to speculate whether the observation[3] that the absorption of antibody by tissues in passive sensitization *in vivo* or *in vitro* is not commensurate with the capacity to sensitize might not be explained by pinocytosis, as in transmission. that the reversal of established sensitization by non-immune γ-globulin[30] might not depend on a mechanism of release from the receptors and subsequent competition between the free antibody and non-immune γ-globulin for the unoccupied receptors, and that the increase with lapse of time in the difficulty of reversing passive sensitization[22] might be due to the movement of the occupied receptors into the more inaccessible internal regions of the cells. Such rash speculations are warrantable at present only in so far as they may serve to indicate the lines along which further research might prove fruitful.

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VIRUS ISOLATION IN THE ZONAL ULTRACENTRIFUGE

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ZONAL centrifugation has been widely used for virus purification using either rate or equilibrium methods[1]. A limiting factor has been the small capacity of the swinging-bucket rotors generally used. Using a recently developed ultracentrifuge[2,3], I have purified large quantities of virus particles with both methods. This work is described in the present article and also a new technique which combines continuous-flow concentration with equilibrium centrifugation in cæsium chloride, followed by rate-zonal centrifugation in a sucrose gradient. The integrated method is applicable to large-scale virus isolation for research purposes or for vaccine production.

The general principles of the zonal ultracentrifuge have been previously presented[2,3]. The central problems in its development were the elimination of swinging buckets or centrifuge tubes of any sort, provision for sedimentation to occur in sector-shaped compartments, development of rapid methods for establishing and recovering large

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

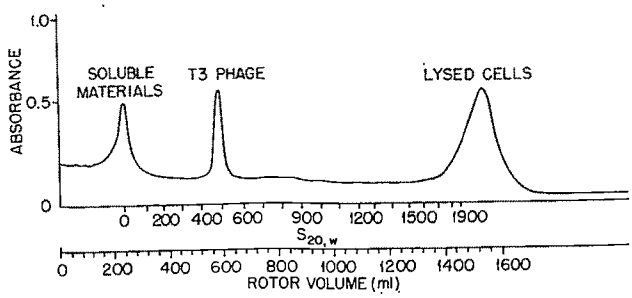


Fig. 1. Concentrated *T*3 phage lysate centrifuged 1 h at 20,000 r.p.m. in *B*-II zonal ultracentrifuge using a sucrose gradient described in text

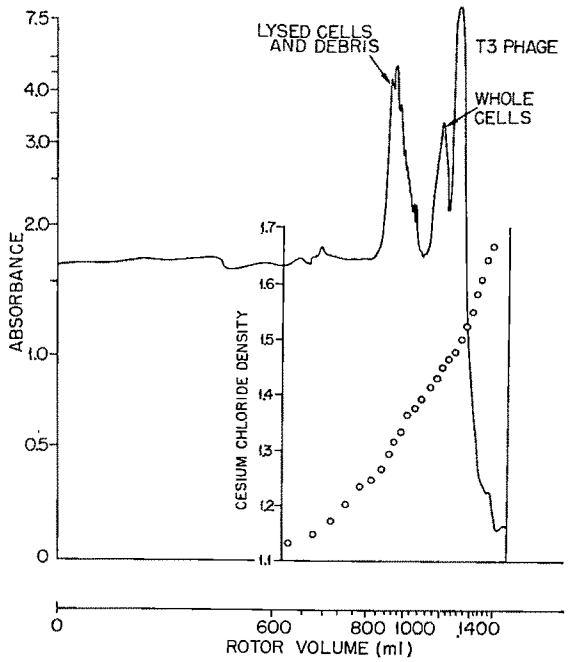


Fig. 2. Banding of *T*3 phage lysate in cæsium chloride after concentration by continuous-flow centrifugation

density gradients during rotation, and perfection of means for introducing the sample zone as a very thin layer at the top or (in instances where the particles of interest float up in the density gradient) at the lower, or denser, end of the gradient. For high-speed operation, vacuum chamber enclosure and high-speed seals for pumping fluid into and out of the spinning rotor are required. Fluid line connexions are to the rotor edge and to the centre core.

Centrifuge Operation

The rotor is loaded and unloaded while spinning. At an initial speed of 3,000 r.p.m. a sucrose density gradient starting with 10 per cent sucrose is pumped to the rotor edge. Centrifugal force stabilizes the fluid against the rotor wall and prevents mixing in the density gradient. As a stream of sucrose of continuously increasing density is pumped in peripherally, the light end of the gradient is displaced toward the centre core. When the complete gradient (1,200 ml. extending, in most viral work, linearly with radius from 10 to 30 per cent sucrose) is in the rotor, additional 55 per cent sucrose is pumped to the rotor edge to fill completely the 1,625 ml. internal rotor volume, and also to begin displacing a portion of the light end of the gradient out through the central core. At this time the direction of fluid-flow is reversed, and the sample is pumped in through the core, where it is displaced out free of the core by an overlay of additional fluid having a density less than that of the sample layer. Data on the thickness of the sample layer in the rotor has been presented previously[3]. When filling is complete, the rotor is brought up to operating speed to effect the desired separations, and then decelerated to 5,000 r.p.m. for unloading. The gradient is recovered by pumping 55 per cent sucrose to the rotor edge, thereby displacing the gradient toward the rotor core, where it is funnelled into the core exit line.

The gradient is thus recovered as a stream, with the light, overlying fluid recovered first, followed by the sample zone, and then the zones of successively more rapidly sedimenting particles. For most purposes the stream is monitored by recording absorbancy in the ultra-violet range using a quartz cell with a light path of 0·2 cm (ref. 4). All work was done in zonal rotor *B*-II, the configuration and dimensions of which have been previously reported[3].

Virus Purification

In initial studies[3], formaldehyde-treated types I, II and III poliovirus were purified from tissue culture supernatant concentrates. Most of the mass in the starting zone proved to be soluble material which did not sediment under the conditions used. A single virus peak was observed in each instance, which, when reconcentrated and observed in the analytical ultracentrifuge, gave only one peak having the expected sedimentation coefficient. With crude extracts containing brome mosaic virus, a single virus peak was also observed, while a very skewed peak was seen with crude extracts containing tobacco-mosaic virus.

For more extensive investigations, *T*3 *coli* phage from cells grown on a synthetic medium were used. As a test material, 20 l. of phage-infected *Escherichia coli* culture was pumped through the rotor at 20,000 r.p.m. and a flow rate of approximately 100 ml. per min. The sedimented material was scraped off the rotor wall and resuspended in buffer at *p*H 7·5. A small amount of this lysate concentrate was placed in 20 ml. of buffer and used as the sample layer in the run shown in Fig. 1. The sample contained some soluble material, phage particles, and lysed cells. An 'ovent-marking' pen along the lower edge of the chart registered each 40-ml. fraction as it was collected. The sedimentation coefficients were calculated using a modification of the equation suggested by Martin and Ames[5]. The sedimentation coefficient for the virus peak agrees very well with the value of 476 *S* reported by Swaby[6]. When examined by phase contrast microscopy,

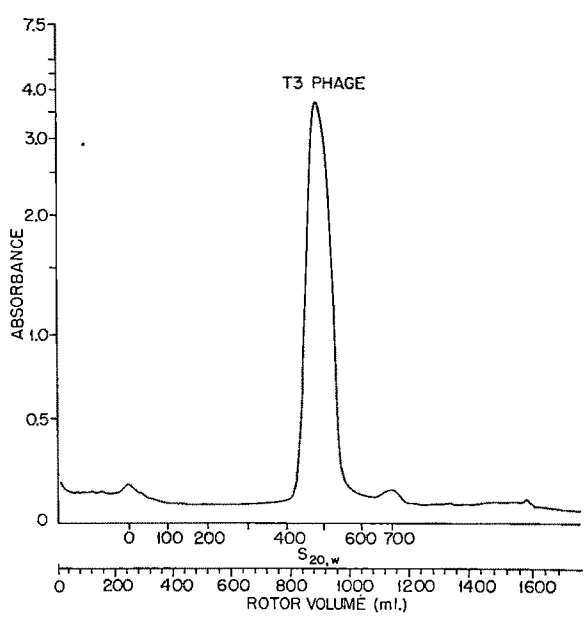


Fig. 3. Rate zonal sedimentation of *T*3 phage from phage peak shown in Fig. 2 in sucrose gradient centrifuged 2 h at 20,000 r.p.m.

the peak on the far right was found to contain lysed cell fragments. This run indicates the resolution which may be obtained with the present zonal ultracentrifuge prototype. Similar experiments were done with *T2* phage, where the separation was followed by infectivity assays.

Rotor *B*-II is not ideally suited to continuous flow work since flow through the rotor in the absence of a density gradient is not distributed evenly over the inner surface of the rotor. The following exploratory experiment was done, however, to see whether a combination of continuous flow and equilibrium-zonal centrifugation was feasible.

Fifteen litres of a low titre *T3* phage lysate was pumped through the rotor approximately 2 l./h with the rotor spinning at 20,000 r.p.m. at 5° C. The rotor was then decelerated to 5,000 r.p.m. and 100 ml. each of 1 : 3, 1 : 1 and 3 : 1 dilutions of a stock cæsium chloride solution (130 g in 70 ml. water) pumped to the rotor edge in that order. This was followed by 250 ml. of the stock cæsium chloride solution. These solutions form very thin layers at the rotor edge and rapidly diffuse to form a continuous density gradient.

After 2 h at 20,000 r.p.m. and 3 h at 5,000 r.p.m., the gradient was recovered by pumping saturated cæsium chloride to the rotor edge. The ultra-violet absorbancy of the effluent rotor stream was recorded and is shown in Fig. 2. Samples from the three peaks were examined by phase-contrast microscopy. The first peak contained lysed bacterial cells and debris, and the second only whole cells. The third peak, which banded at a density of 1·5, which is the density of *T3* phage[6], was optically empty. The ragged recording of the first peak is due to the presence of large clumps of material in the effluent stream.

The material in the third peak was diluted to decrease the density of the solvent and the virus was recovered by centrifuging at 150,000*g*. The pellet was suspended in 0·1 ionic strength Miller–Golder[7] buffer at *p*H 7·5 and rerun in the zonal ultra-centrifuge with the same gradient used for the separation shown in Fig. 1, but with a centrifuge time of 2 h at 20,000 r.p.m. The results, shown in Fig. 3, demonstrate that excellent purification of virus particles may be obtained using the combined continuous flow-density gradient method.

Rotors are now being designed which will allow a cæsium chloride gradient to be stabilized at the rotor wall while the dilute virus suspension is flowing through the rotor. The virus particles would then sediment into the density gradient rather than to the rotor wall and afterwards back into the cæsium chloride gradient. Better continuous-flow operation is also obtained by introducing the solution to be concentrated as a gradient of very gradually increasing density, or by slowly lowering the temperature of the rotor and the feed solution. In both instances, the solution is oriented against the rotor wall as it flows in, allowing efficient sedimentation to occur.

These experiments demonstrate the feasibility of zonal rotors for the large-scale purification of virus particles by rate-zonal and isopycnic zonal centrifugation, and in addition show that the latter may be combined with continuous-flow centrifugation for the concentration of virus from large volumes of solution.

Adventitious viruses have recently been discovered in vaccines previously considered safe for human use[8,9]. Since only those contaminating viruses for which specific tests are available can be detected, it is considered important to develop methods of large-scale virus purification which would decrease by several orders of magnitude the concentration of particles and viruses differing in sedimentation rate or density from the virus desired.

Where the viral nucleic acid may be removed by chemical treatment[10] or where a fraction of the naturally formed particles are devoid of nucleic acid, the possibility of isolating such hollow-headed or 'top-component' particles for the preparation of non-infective vaccines can now be considered. This approach is especially attractive where chemical inactivation of the virus results in extensive losses in antigenicity.

In most tissues, relatively few particles occur that have the sedimentation characteristics of virus particles. (Exceptions exist, as, for example, the hoof-and-mouth disease virus which has nearly the same sedimentation coefficient as a major class of ribosomes.) In the zonal ultracentrifuge, tissue breis yield a spectrum of separated particles. Fractions suspected of containing virus particles may be concentrated to small pellets for examination in the electron microscope or for infectivity assays. If the sedimentation coefficient of the virus is known, its position in the gradient may be easily calculated.

Poliovirus was provided by Dr. Jonas Salk, TMV by Dr. C. A. Knight, TMV and BMV-infected plant tissues by Dr. Myron Brakke, *T2* phage by Dr. Elliot Volkin, and *T3* phage by Dr. J. B. Bateman. I thank Dr. R. J. Huebner for advice and encouragement. This work was performed under the Joint National Institutes of Health–Atomic Energy Commission Zonal Centrifuge Program.

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PURIFICATION OF RADIOACTIVE FOOT-AND-MOUTH DISEASE VIRUS

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BEFORE the detailed investigation of the chemical composition and structure of a virus can be attempted it is necessary that it should be available in a pure state. Furthermore, the availability of radioactively labelled pure virus greatly facilitates biochemical studies of the early stages of virus multiplication. Preliminary investigations on the purification of small quantities of radioactively labelled foot-and-mouth disease (FMD) virus for these purposes are described in this article.

Monolayers of baby hamster kidney cells (*BHK* 21, Clone 13, ref. 1) grown in Roux flasks and containing about 10^8 cells were infected with 10^9 plaque-forming units of FMD virus of type *O* (strain 1) which had been passaged 5 times in cultured *BHK* 21 cells. After 15-min incubation at 37° the cell sheets were washed carefully three times with a medium deficient in phosphate (Hanks's saline with 0·001 M *tris* replacing the phosphate) and then incubated at 37° in 40 ml. of the same medium containing 50 μc. carrier-free, phosphorus-32-labelled phosphate. Because of the lability of FMD virus in environments at less than *p*H 7, care was taken to keep the *p*H of the medium greater than this value by periodic addition of

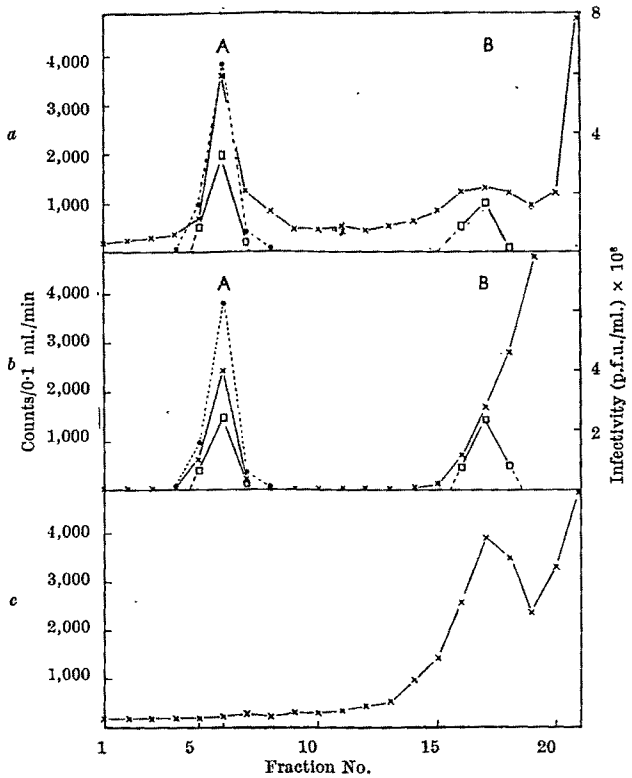


Fig. 1. Sucrose density gradient centrifugation (*S.W.* 39, 15–45 per cent sucrose, 35,000 r.p.m., 90 min) of phosphorus-32-labelled virus pellets mixed with phosphate (*a*), or deoxycholate (*b*), and of normal uninfected phosphorus-32-labelled cell extract (*c*). ×—×, Phosphorus-32; ●—●, plaque-forming units; □—□, complement-fixing activity

dilute sodium hydroxide. After 12-h incubation the medium was separated from the cells and cell debris by centrifugation at 2,000 r.p.m., cooled to 2° and mixed with an equal volume of cold saturated ammonium sulphate at *p*H 7·6 in 0·04 M phosphate buffer. All the infectivity and complement-fixing activity of FMD virus suspensions were precipitated by this procedure[2]. The precipitate was collected by centrifugation at 2,000 r.p.m. and re-suspended in 0·04 M phosphate, *p*H 7·6. Insoluble material was removed by centrifugation at 10,000 r.p.m. for 15 min in the *L* 40 head of the Spinco ultracentrifuge and the virus then deposited from the supernatant by centrifugation at 35,000 r.p.m. for 60 min. The pellet was dispersed in 0·04 M phosphate by vigorous pipetting followed by gentle agitation overnight at 2°. The recovery of virus infectivity and complement-fixing activity in these procedures is shown in Table 1.

Table 1. Concentration of Foot-and-Mouth Disease Virus (Type *O*) by Ammonium Sulphate Precipitation and Ultracentrifugation

	Volume (ml.)	Virus titre in mice (log ID_{50}/ml.)*	Complement-fixing activity (ml. 1/30 *C'* fixed by 1 ml. fraction)	Total Complement fixing activity
Initial virus	1,000	6·8	2·5	2,500
Ammonium sulphate precipitate	20	8·2	116·4	2,328
10,000 r.p.m. supernatant	20	8·5	116·4	2,328
35,000 r.p.m. supernatant	20	6·1	50·5	1,010
Pellet in phosphate	1	9·5	1,356	1,356
Pellet in 0·5 per cent deoxycholate	1	9·5	1,356	1,356

* Intraperitoneal inoculation of 0·03 ml. into 7-day-old mice.

Preliminary investigations with unlabelled virus showed that pellets prepared in this way contained cellular material because they fixed complement with cell antiserum prepared in rabbits. Partial fractionation of the virus and cellular protein could be obtained by chromatography on DEAE-cellulose. Sucrose density gradient centrifugation (*S.W.* 39, 15–45 per cent sucrose, 35,000 r.p.m., 90 min) of phosphorus-32-labelled virus pellets gave two main radioactive peaks, but there was a considerable background of radioactivity which was presumably due to cellular constituents (Fig. 1*a*). In experiments with uninfected cells, Dr. D. N. Planterose in this laboratory has found that the addition of sodium deoxycholate considerably reduces the rate of sedimentation of normal cell microsomes in sucrose gradients. Consequently, the effect of 0·5 per cent sodium deoxycholate on the behaviour of the virus in sucrose gradients was examined. The results in Fig. 1*b* show that the background radioactivity was almost completely eliminated. Furthermore, the amount of radioactivity found in peak *A* from an untreated pellet was reduced almost two-fold if the pellet was mixed with deoxycholate before centrifugation. As 0·5 per cent deoxycholate has no effect on the infectivity of FMD virus, the radioactive material which sedimented more slowly after deoxycholate treatment is presumably a phosphorus-containing cellular constituent. The amounts of complement-fixing activity and ultra-violet-absorbing substances (at 260 mμ) in peak *A* were also much lower, and the amounts in the later fractions correspondingly higher, when the pellet was treated with deoxycholate before centrifugation.

Uninfected cells which had been incubated in phosphorus-32-labelled medium for 12 h, lysed and the lysate concentrated in exactly the same way as virus suspensions gave the sucrose density gradient centrifugation pattern shown in Fig. 1*c*. It can be seen that there was no radioactive peak at the position corresponding to infective virus in Figs. 1*a* and 1*b*. In view of this observation, it was surprising that cellular material should be associated with peak *A* obtained from virus pellets centrifuged before treatment with deoxycholate. The loss from peak *A* of complement-fixing activity without loss of infectivity when the virus pellet was mixed with deoxycholate prior to centrifugation indicated that some of the 7 mμ viral protein sub-unit was present in peak *A* from untreated pellets. Previous experiments had shown that some of the 7-mμ sub-unit present in untreated virus collections was deposited with the 25-mμ infective component when spun for 1 h at 35,000 r.p.m. in the *L* 40 head, suggesting that the two components were combined. However, the 7-mμ component did not combine and deposit with purified 25-mμ component. These results indicate that there is present in untreated virus suspensions and pellets a complex of the 7-mμ component and cellular material, which has the same sedimentation characteristics in sucrose density gradients as the 25-mμ infective component but is disrupted by deoxycholate into smaller components which then sediment much more slowly in the sucrose gradient.

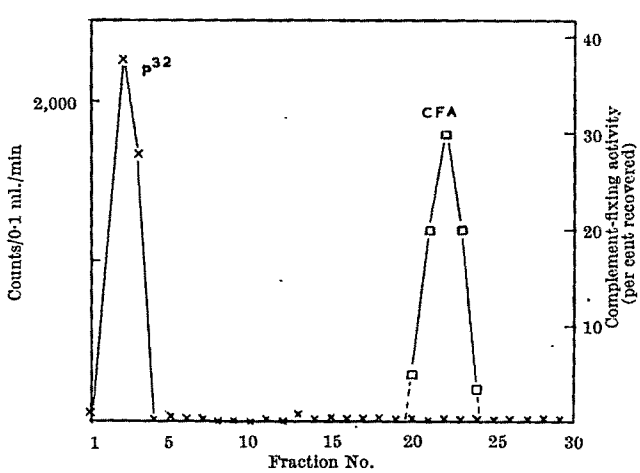


Fig. 2. Chromatography of peak *A* on 'Amberlite *IRC*-50' in 0·02 M phosphate, *p*H 6·8. Elution was carried out with increasing concentrations of phosphate. Fraction 21 was *c.* 0·2 M

The ratio of plaque-forming units and complement-fixing activity to radioactive counts in the individual fractions making up peak *A* in the sucrose gradients of deoxycholate-treated pellets (Fig. 1*b*) was remarkably constant, indicating that the material in the peak was homogeneous. A number of experiments revealed, however, that only about 5 per cent of the infectivity added to the gradient was being recovered in the fractions comprising peak *A*. These losses, which could also be demonstrated by complement-fixation tests, have been shown to be due to adsorption of virus on to the glass tubes in which the fractions were collected because recoveries of infectivity greater than 50 per cent were obtained when 10 per cent normal guinea pig serum was added to the tubes before collection of the fractions. Tubes coated with silicone also allowed good recoveries of virus.

The following experiments have been carried out to test whether peak *A* obtained by sucrose density centrifugation of deoxycholate-treated pellets is free from cellular constituents.

(1) Lowering of FMD virus suspensions to *p*H 6·8 in 0·02 M phosphate breaks down the virus into its infective RNA and 7-mμ protein sub-units[3,4]. These can then be separated by chromatography on 'Amberlite *IRC*-50', the RNA passing unadsorbed through the column, whereas the 7-mμ component is adsorbed and can afterwards be eluted with higher concentrations of phosphate[5]. Dilution of peak *A* in 0·02 M phosphate, *p*H 6·8, followed by chromatography on 'Amberlite *IRC*-50', gave the result in Fig. 2, which shows that all the radioactivity passed through the column without adsorption. The result also shows that the protein sub-unit does not contain any phosphorus. Similarly, sucrose density gradient centrifugation (*S.W.* 39, 15–45 per cent sucrose, 35,000 r.p.m., 90 min) of peak *A* after adjustment to *p*H 6·8 left the complement-fixing activity and radioactivity near the top of the tube. In contrast, this mild acid treatment did not alter the sedimentation characteristics of the radioactive constituents present in the pellets prepared from uninfected cells.

(2) Peak *A* was twice extracted with cold phenol in order to obtain the RNA. More than 80 per cent of the radioactivity was left in the aqueous RNA-containing layer. This preparation of RNA was then mixed with cellular RNA prepared by phenol extraction of unlabelled *BHK* cells and precipitated with 2 volumes of cold ethanol. After standing overnight at 0°, the precipitate was collected by centrifugation, dissolved in phosphate-buffered saline and either spun in a sucrose density gradient (*S.W.* 39, 5–20 per cent sucrose, 35,000 r.p.m., 4 h) or chromatographed on calcium phosphate[6]. In both procedures the peak of radioactivity did not coincide with any of the RNA peaks of the unlabelled cells.

(3) It is possible that unlabelled substances (for example, protein) could be present in peak *A*. However, adjustment of peak *A* to *p*H 6·8 before centrifugation in a sucrose gradient (*S.W.* 39, 15–45 per cent sucrose, 35,000 r.p.m., 90 min) did not leave any ultra-violet-absorbing substances in the position normally occupied by peak *A*. If there is a protein contaminant in peak *A* it must be broken down into smaller units at *p*H 6·8.

(4) Mixtures of the radioactive virus in peak *A* with either normal guinea pig serum or hyperimmune guinea pig serum against types *O* or *C* of the virus were centrifuged at 12,500 r.p.m. for 15 min in 2-ml. tubes in the *L* 40 head of the Spinco ultracentrifuge and the distribution of radioactivity in each tube was then measured. Some radioactivity was deposited even with normal serum or the heterotypic serum type *C*, but only the type-specific serum against type *O* caused complete precipitation of the radioactivity. Precipitation with the normal and heterotypic sera is likely to be caused by aggregation of the purified virus particles. The protein sub-unit (peak *B*, Fig. 1*b*) collected from one experiment in which radioactive virus was centrifuged was also examined in the same way. The radioactivity present in this peak was not deposited at 12,500 r.p.m. after mixing with the type-specific antiserum, thus affording more evidence that the virus sub-unit does not contain nucleoprotein.

(5) A suspension of unlabelled virus was mixed with an extract of phosphorus-32-labelled uninfected cells and concentrated with ammonium sulphate as described here. The pellet obtained by centrifuging for 1 h at 35,000 r.p.m. was then mixed with deoxycholate and centrifuged in a sucrose gradient (*S.W.* 39, 15–45 per cent sucrose, 35,000 r.p.m., 90 min). The virus peak which was obtained did not contain any radioactivity.

These experiments provide substantial evidence that the virus obtained in peak *A* of deoxycholate-treated pellets is free from substances found in uninfected cells. The possibility remains, however, that the virus peak contains non-viral substances which are found only in virus-infected cells. The methods described here are being applied to the purification of larger quantities of the virus and also to other immunological types of the virus grown in different cell systems.

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HISTONES AND GENE FUNCTION

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SINCE the proteins principally associated with the DNA in the chromosomes of the somatic cells of mammalian and other complex organisms are histones, and since similar proteins have not been found associated with the DNA of bacteria[1], it is an attractive hypothesis that they are concerned with cell differentiation. Stedman and Stedman[2], indeed, suggested in 1951 that histones were concerned with the control of gene expression by inhibiting the functioning of genes which are not required to function in particular types of cells. Since different genes may be expected to be active in different cell types we should expect them to have different complements of histones. Although cases of tissue specificity have been reported[3], histones from a number of mammalian tissues of several species show a remarkable similarity in their starch electrophoresis patterns[4]. On the other hand, it has been found that histones from a plant source (wheat germ) are markedly different[5].

Progress has been made in the fractionation of histones by chromatography and by various extraction and precipitation procedures[6] and a number of fractions with markedly different amino-acid analyses have been obtained, for example, the fractions termed *F*1 (lysine rich); *F2a*, *F2b* (intermediate); *F*3 (arginine rich)[7]. None of these, when examined by starch-gel electrophoresis, is free from subsidiary bands, but the fact that similar fractions prepared by different methods have closely constant compositions suggests that we are not dealing with a large number of components, having compositions which merge gradually into each other, but a limited

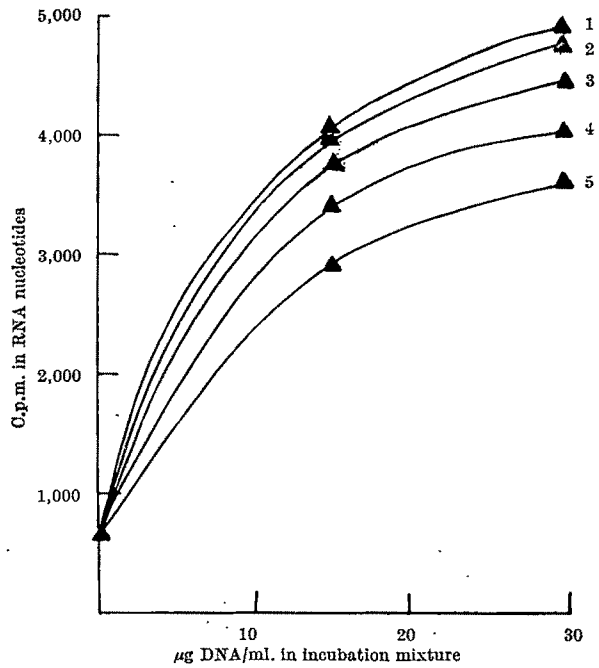


Fig. 1. RNA synthesis promoted by various DNA-histone complexes. The incubation mixture contained in 0·5 ml., 48 μmoles of *tris*-hydrochloric acid of *p*H 7·4; 4 μmoles of magnesium chloride; 2 μmoles ATP; 0·17 μmoles GTP; 0·17 μmoles CTP; 0·4 μcuries of [^{3}H]-UTP (125 μμmoles); enzyme preparation containing 92 μg of protein. Incubation for 3–15 min, 1 mg. carrier RNA added and acid insoluble alkali digest counted by liquid-scintillation
Histone DNA ratios (w/w): curve 1, 0; curve 2, 0·25; curve 3, 0·5; curve 4, 0·75; curve 5, 1·0

number of characteristically different proteins. If this were so we would then have to conclude that the different histones at most distinguish different classes of genes rather than individual genes. The possibility that each fraction contains a number of proteins of the same overall character, but with different amino-acid sequences, remains. This can only be settled by sequence studies which are in progress but insufficiently complete[8].

The discovery of the RNA polymerase, which uses DNA as a template[9], makes a quantitative measure of at least one aspect of gene function possible. In experiments by Huang and Bonner[10] it was found that with the native nucleohistone from pea seedlings and a polymerase from the same source, little synthesis occurred although the DNA itself was active, indicating inhibition of the process by the histone present. In reconstituted nucleohistones, prepared by adding histone to DNA in strong salt solutions, in which the complex is dissociated, and dialysing away the salt, marked inhibition of RNA synthesis occurs as compared with that in the presence of DNA, but the effects reported are variable[11].

We have attempted to obtain reproducible results in experiments of this kind, using DNA from calf thymus and histone fractions from the same source. Complexes of native calf thymus DNA and histone were formed by mixing DNA (250 μg/ml. in the final solution) and variable amounts of histone in 1 M sodium chloride and gradually dialysing away the salt against water and a suitable buffer over a period of 6 h, so that the final complex was in a medium of 0·05 M *tris* buffer at *p*H 7·4, as performed by Huang and Bonner[10]. For the most part, the complexes formed were gelatinous suspensions and were added to the incubation mixture as such. We found it impossible with these materials to obtain soluble complexes of the type described by Huang and Bonner[10]. Reducing the final concentration of the buffer to 0·01 M *tris* did not bring the complex into solution. The RNA polymerase used was purified from *B. megaterium* as described by Barr and Butler[12] and had an absolute requirement for DNA; RNA was inactive as primer.

Table 1 indicates the ability of the DNA to promote synthesis and the complete inability of the native nucleohistone in this respect. Boiling the nucleohistone solution for 5 min which denatures the DNA and may be expected to disturb any specific histone–DNA interaction, and possibly solubilize part of the histone, restores the ability to some extent.

Table 1. Priming Ability of Native Nucleohistone and DNA from Calf Thymus

Each incubation mixture contained 30 μg DNA and 101 μg of the enzyme protein

Primer used	C.p.m. in RNA
Calf thymus DNA	1,840
Calf thymus nucleoprotein	150
Boiled calf thymus nucleoprotein	530
Control (no primer)	120

Fig. 1 shows the ability of various complexes of DNA and histone to promote RNA synthesis. The 1 : 1 complex was 75 per cent as active as DNA, but some of this loss of activity may be due to the presence of traces of ribonucleases in the histone. Fig. 2 demonstrates the ability of complexes of DNA with different calf thymus histone fractions to promote RNA synthesis. The effects of the fractions are much larger and could not possibly be attributed to any ribonuclease contamination of the histones.

Complexes of calf thymus DNA and poly-*l*-lysine were also prepared and investigated in the same way. Table 2 shows that all complexes containing a ratio of poly-*l*-lysine to DNA of 0·5 or more (w/w) were completely inactive as primers.

It is evident from these results that the histone fractions produce much greater effects when present in excess of the natural amount than would be expected if they caused specific inhibitions of RNA synthesis on portions of the DNA molecules. *F2a*, *F2b* and *F3* comprise approximately 35, 25 and 20 per cent, respectively, of the

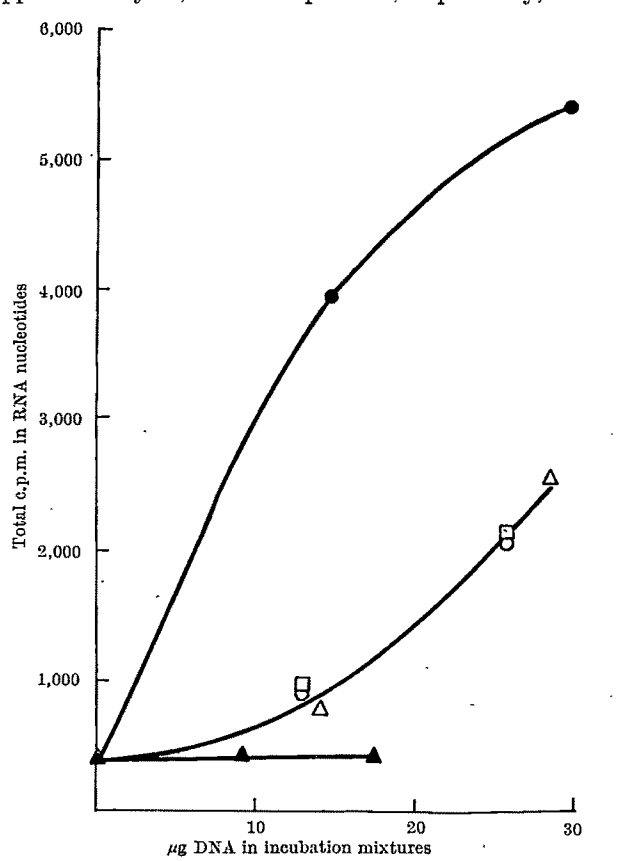


Fig. 2. RNA synthesis with complexes (1 : 1 w/w) of DNA and histone fractions. Conditions as Fig. 1
●, DNA; ▲, *F*1–DNA complex; □, *F2a*–DNA complex; ○, *F2b*–DNA complex; △, *F*3–DNA complex. The enzyme preparation contained 101 μg protein in each case

Table 2. Polylysine–DNA Complexes as Primers for RNA Synthesis

Wt. ratio Polylysine : DNA	Counts incorp./mols RNA	Inhibition (per cent)
0	3,790	
0·25	2,990	24
0·5	950	84
1·0	590	94
1·3	400	100
Control (no primer)	400	

All mixtures contain 40 μg DNA present in the various complexes. Incubation was carried out with 80 μg of enzyme-protein.

whole histone, but each causes more than 80 per cent inhibition of RNA synthesis if the complex containing 15 μg of DNA is used and 55 per cent inhibition with 30 μg DNA, while *F*1, which is about 20 per cent of the whole histone, causes total inhibition of RNA synthesis at all concentrations used. It follows that *F*1 inhibits RNA synthesis in all RNA molecules and not merely those with which it had a special relationship in the native nucleohistone. Moreover, the artificial peptide poly-*l*-lysine is an extremely effective inhibitor.

It clearly follows that the inhibition of RNA synthesis in reconstituted nucleohistone is unspecific. However, there is no evidence in our experiments that reconstitution of the original native material can be achieved by the methods used. The results obtained could be due to inability to prepare reconstituted nucleohistones in a suitable form. Nevertheless, reconstitutions from the whole histone are surprisingly active, and this suggests that the physical state of the material is not of prime importance. Whether there is any correspondence between the sequence of bases in DNA and the sequence of amino-acids in histone must remain an open question until further fractionations and sequence investigations have been carried out. But it may be observed that even if such a correspondence were shown to exist, some other mechanism would have to be postulated to ensure the presence and removal of particular histone molecules at the appropriate times. This is easier to envisage with a limited number of histone types as there could be proteolytic enzymes or endopeptidases which act specifically on one type, especially as it has been shown[13] that different histones have characteristic *N*-terminal groups.

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POLYURIDYLIC ACID AND AMINOACYL TRANSFER TO MAMMALIAN RIBOSOMES

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STIMULATION of amino-acid incorporation by synthetic polynucleotides in cell-free systems from *E. coli*[1–6] and mammalian tissues[7–12] has been reported. Previous investigations in this laboratory[12] demonstrated that aminoacyl transfer from *s*RNA to rat liver ribosomes was stimulated by the addition of several synthetic polynucleotides. This stimulation was greater when ribosomes were pre-incubated with crude soluble enzyme preparations that catalysed aminoacyl transfer. It was suggested that, on pre-incubation, a component of ribosomes, possibly messenger RNA, was removed by a factor present in crude soluble enzyme preparations; the stimulation on addition of polynucleotides to pre-incubated ribosomes suggested that endogenous messenger RNA was replaced by synthetic polynucleotides. Evidence presented here suggests that: (*a*) loss of endogenous messenger RNA activity and its replacement by polynucleotides are time-dependent processes; (*b*) the activity responsible for the loss of endogenous messenger RNA activity is different from the enzymes which catalyse aminoacyl transfer; (*c*) synthetic polynucleotide-enhanced incorporation represents the formation of amino-acid homopolymers.

Incorporation of amino-acids from aminoacyl *s*RNA into rat liver ribosomal protein is catalysed by the soluble portion of cells and requires guanosine triphosphate and glutathione. Most of the transferring activity in the non-particulate fraction of rat liver homogenates remains soluble after precipitation of the 'amino-acid-activating enzymes' fraction at *p*H 5 (*p*H 5 supernatant). Two protein fractions (aminoacyl transferases (or polymerases) I and II) have been resolved and partially purified in this laboratory, which are inactive individually but when combined catalyse aminoacyl transfer in presence of GTP and glutathione[13–16].

Incubation of ribosomes with polyuridylic acid (poly U) and *p*H 5 supernatant led to a two- to four-fold stimulation of phenylalanine incorporation from ^{14}C-phenylalanyl *s*RNA (Fig. 1, zero time values). Pre-incubation of ribosomes at 37° with *p*H 5 supernatant, GTP and glutathione, prior to addition of the labelled substrate ^{14}C-phenylalanyl *s*RNA, led to a decrease in the ability to incorporate amino-acid; most of the decrease in ribosomal activity occurred within the first few minutes of pre-incubation (Fig. 1). Addition of poly U to pre-incubated ribosomes led to a marked stimulation of aminoacyl transfer, particularly after the first few minutes of pre-incubation. These results are consistent with the time-dependent breakdown or release of endogenous ribosomal components which are then replaced by polynucleotides.

In contrast to experiments with *p*H 5 supernatant, addition of poly U to incubations with purified transferases I and II did not lead to a marked increase in phenylalanyl transfer. Further, ribosomes pre-incubated with transferases I and II did not exhibit a loss of activity as in experiments with *p*H 5 supernatant[12]. Experiments with ribosomes pre-incubated with *p*H 5 supernatant (Fig. 2*A*) indicated that incorporation was essentially completed after 4 min of incubation with ^{14}C phenylalanyl *s*RNA. Addition of poly U markedly stimulated phenylalanyl transfer as compared with the results with polycytidylic

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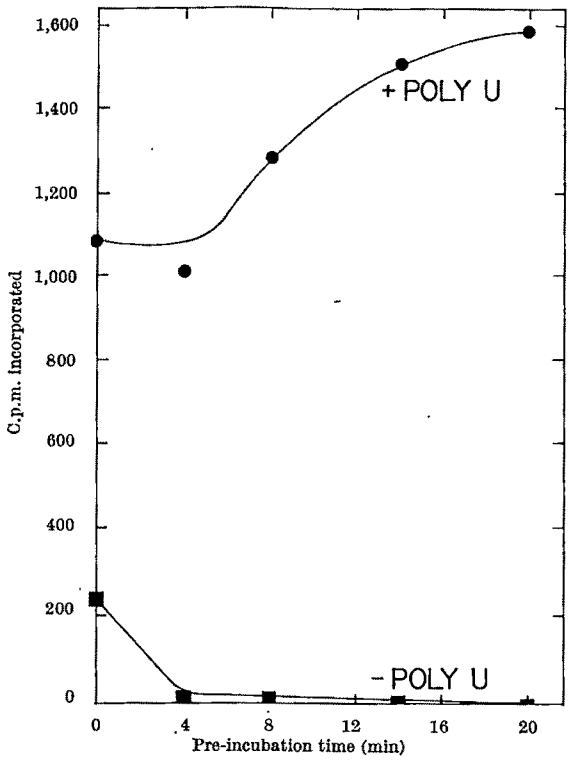


Fig. 1. Effect of pre-incubation time and polyuridylic acid on aminoacyl transfer. Approximately 0·7 mg of ribosomes was incubated at 37° for varying periods of time in the presence of 1 μmole of GTP·$MgCl_2$, 10 μmoles of glutathione, and 10 mg of *p*H 5 supernatant protein in a total volume of 1·6 ml. After 0, 4, 8, 14 and 20 min of pre-incubation, 0·08 mg (2,500 c.p.m.) of ^{14}C-phenylalanyl *s*RNA (squares, −poly U) or ^{14}C-phenylalanyl *s*RNA plus 0·2 mg of polyuridylic acid (circles, +poly U) were added and incubation, in a final volume of 2 ml. was continued for an additional 20 min

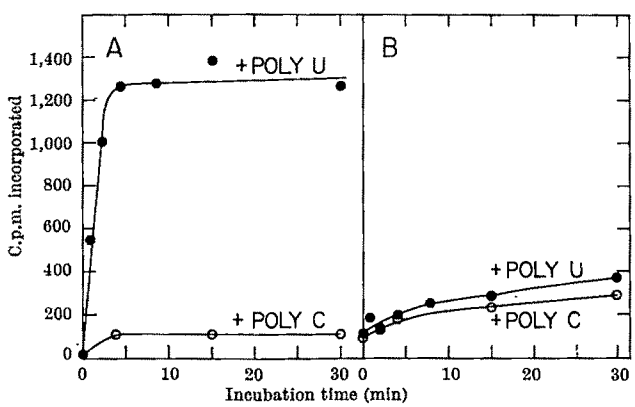


Fig. 2. Effect of polyuridylic acid on the time-dependent aminoacyl transfer to pre-incubated ribosomes. Approximately 0·7 mg of ribosomes were incubated at 37° for 8 min in the presence of GTP, glutathione and *p*H 5 supernatant (*A*) or transferases I and II (*B*) as described in Fig. 1. ^{14}C-phenylalanyl *s*RNA and 0·2 mg of polyuridylic acid (closed circles, +poly U) or 0·2 mg of polycytidylic acid (open circles, +poly C) were then added and incubated for varying periods of time as shown

acid (poly C) or in the absence of synthetic polynucleotides. Aminoacyl transfer, in experiments with ribosomes pre-incubated with combined transferases I and II (Fig. 2*B*), was not significantly stimulated on addition of poly U or poly C. Inability of purified transferases I and II to catalyse the decrease in endogenous ribosomal activity, and the subsequent failure to exhibit a significant poly U effect, are consistent with the suggestion that an additional factor is present in the *p*H 5 supernatant, responsible for the degradation of the endogenous messenger associated with ribosomes[12].

Previous results with polynucleotides varying in base composition suggested that the polynucleotide-enhanced incorporation was specific for individual amino-acids and did not involve the simultaneous transfer of several of, or all, the *s*RNA-bound amino-acids[12]. The incorporation of ^{14}C-phenylalanine from *s*RNA containing a complement of endogenous unlabelled amino-acids, in absence of poly U, probably reflects synthesis of amino-acid heteropolymers. The difference in aminoacyl transfer in presence and absence of poly U might reflect synthesis of polyphenylalanine while endogenous incorporation, in absence of poly U, probably reflects synthesis of protein. The effect of poly U was examined with ^{14}C-phenylalanine-labelled *s*RNA preparations in the presence (^{14}C-phenylalanyl *s*RNA-'*A*') or absence (^{14}C-phenylalanyl *s*RNA-'*B*') of other *s*RNA-bound ^{12}C-amino-acids. When ^{14}C-phenylalanyl *s*RNA-'*B*' was added to pre-incubated ribosomes, in absence of poly U, little incorporation occurred (Fig. 3); significant incorporation was observed with ^{14}C-phenylalanyl *s*RNA-'*A*' under similar conditions. These results are consistent with the obligatory incorporation of all amino-acids in the course of protein synthesis[17]. Addition of poly U markedly stimulated ^{14}C-phenylalanyl transfer from ^{14}C-phenylalanyl *s*RNA-'*A*' and ^{14}C-phenylalanyl *s*RNA-'*B*' preparations. Estimates on the basis of carbon-14 incorporated indicate that the poly U-stimulated incorporation of radiocarbon from both preparations is of the same order of magnitude. These results are consistent with the poly U-enhanced formation of polyphenylalanine in this system, and suggest that, in contrast to the endogenous activity exhibited by ribosomes, synthetic polynucleotide-stimulated aminoacyl transfer is specific and does not involve other amino-acids. Polyuridylic acid-enhanced incorporation of phenylalanine thus appears to reflect formation of amino-acid homopolymer while endogenous incorporation may represent formation of amino-acid heteropolymers.

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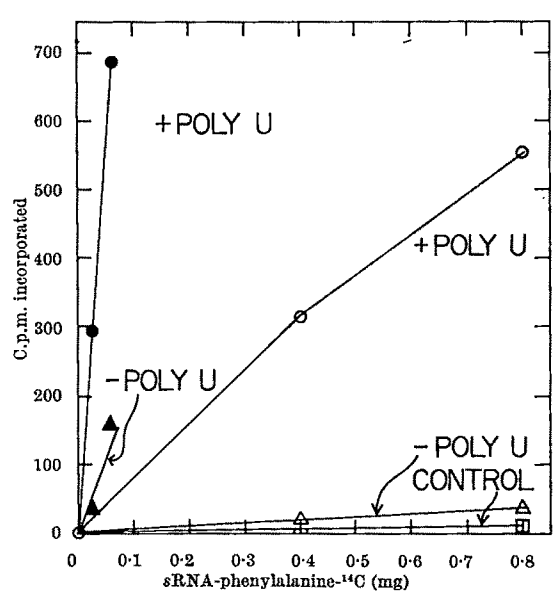


Fig. 3. Effect of polyuridylic acid on aminoacyl transfer from ^{14}C-phenylalanyl *s*RNA, in the presence and absence of other *s*RNA-bound amino-acids, to pre-incubated ribosomes. Approximately 8 mg of ribosomes were pre-incubated for 8 min as described in Fig. 1. Varying concentrations of aminoacyl *s*RNA labelled with ^{14}C-phenylalanine (^{14}C-phenylalanyl *s*RNA-'*A*', specific radioactivity approximately 32,000 c.p.m. per mg RNA) were added with (closed circles, +poly U) and without (closed triangles, −poly U) 0·2 mg of polyuridylic acid and incubated for 20 min. Similar incubations were carried out with *s*RNA, stripped of its endogenous amino-acids at *p*H 10 and containing only ^{14}C-phenylalanine (^{14}C-phenylalanyl *s*RNA-'*B*', specific radioactivity approximately 5,000 c.p.m. per mg RNA) in the presence (open circles, +poly U) and absence (open triangles, −poly U) of polyuridylic acid. The controls (open squares) represent incubations of ^{14}C-phenylalanyl *s*RNA-'*B*' with ribosomes in the absence of *p*H 5 supernatant

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PACEMAKER HIERARCHIES CONTROLLING THE BEHAVIOUR OF HYDRAS

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A GOAL of 'behavioural physiology' is to analyse the behaviour of an organism into its functional components and then to explain their integrated activity in terms of the individual activities of sense cell, nerve cell and muscle unit. The relative simplicity of the coelenterate nervous system makes these animals exceptionally favourable material for such an approach to the study of behaviour.

Rhythmically occurring impulses, varying in frequency from 1 to more than 12 per min, can be recorded from intact and essentially undisturbed hydras of several species[1]. These regular pulses are cryptic, that is to say, they are not directly correlated with any movement of the animal, and are not dependent on external sensory stimuli. They originate from any one of a number of pacemaker loci near the base in the lower column or stalk, and are conducted without decrement throughout the column to the hypostome. We have termed these impulses 'rhythmic potentials' and their originating pacemakers and conducting network the 'rhythmic potential system'[1]. Circumstantial, but incomplete, evidence suggests that the rhythmic potential system is a network of endodermal neurones.

We shall show here that these rhythmic potential pacemakers and their conducting system are responsible for a complex behavioural sequence in hydras, co-ordinating regional pacemaker centres of the tentacles and sub-hypostome. These pacemaker systems are shown in Table 1. The picture which has emerged of the mechanism of integration in hydras has served, in turn, as the basis for a new theory of the origin of the nervous system[2].

The frequency of rhythmic potential impulses in *Hydra littoralis* Hyman can be affected by electrical, mechanical or light stimuli. The initial reaction to a light stimulus of moderate intensity is an interruption of the existing rhythm (Fig. 1, above). After a short, somewhat variable period, rhythmic potential pulses re-occur at an enhanced frequency. The extent and duration of this enhancement depends on the state of dark adaptation of the animal prior to stimulation, the strength, duration and wave-length of the light stimulus, the nutritional state of the animal, and probably to other variables as well. The

Table 1. Classes of Pacemaker Systems in Hydras

Name	Locus	Function	Conducting network
Rhythmic potential (RP)	Lower column	Co-ordinated activity of organism; control other pacemaker systems	RP system; endodermal nerve-net (?)
Contraction burst	Sub-hypostome	Periodic total contraction of column with ectodermal muscles	Ectodermal nerve-net (?)
Attachment burst	Tentacle base	Initiate contraction burst in locomotor sequence	RP system (?)
Tentacle	Proximal part of tentacle	Contract tentacles. May precede contraction burst	Unknown; not conducted in column

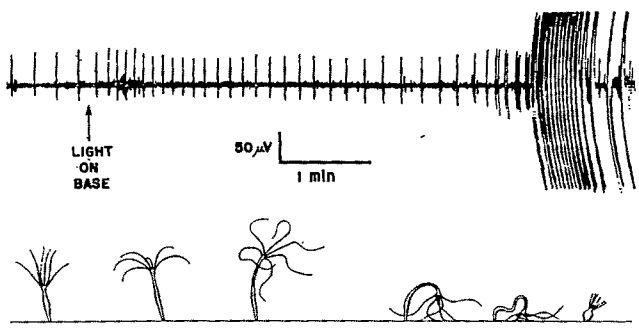


Fig. 1. Response of dark-adapted *Hydra littoralis* to localized illumination of the base. Total duration of record 7 min. Above, electrical potentials; below, behavioural response with aborted locomotion

heightened frequency usually continues for at least 3–5 min, and will last long after the stimulus is removed. Very short light flashes, such as those produced by a photographic flash-bulb, are not effective, but stimuli lasting 0·2 sec or longer regularly cause at least a transient rise in rhythmic potential frequency. Stimulation often also causes a change in the effective pacemaker with the enhanced frequency originating from a previously inactive locus[1].

Localized light stimulation of the base of the animal alone duplicates the effect of general illumination on rhythmic potential pacemakers.

The behavioural response sequence of normal *H. littoralis* to weak illumination has been investigated using time-lapse cinematography with infra-red sensitive film. When undisturbed and especially after they are put in the dark (or in light of greater than 580 mμ wave-length, 'hydra dark'), polyps usually show regular contraction bursts. Every 5–10 min they shorten into a tight ball, and then re-elongate their column and tentacles. Each shortening consists of a more or less consistent number (10–15 in *H. littoralis*) of discrete, co-ordinated contractions of the longitudinally arranged ectodermal muscles of the column. Following tentacle attachment the burst shows the maximum number of contractions[3]. Bursts can occur in a regular manner, at about 8–10 per h, or they may show a less regular pattern.

Under natural conditions, responses to light can impose a diurnal cycle of behaviour on hydras. At dawn the contraction frequency shows an abrupt rise to twice the night-time level. This frequency gradually declines during the day and then returns to the dark-level about 2 h after sunset[3]. The same response occurs on artificial illumination of the dark-adapted animal.

Each contraction of the burst is preceded by a slow, compound electrical potential which is conducted throughout the column by a separate system from that conducting the rhythmic potentials. The contraction burst potentials

(Table 1) originate in the sub-hypostomal region of the column just proximal to the tentacle insertions. Tentacle extirpation does not block contraction bursts, but decapitation of the sub-hypostome usually halts bursts completely[3,4] for an hour or more, and subsequent bursts, while normal, occur much less frequently for some hours. The localization of this pacemaker locus is confirmed by the finding that localized illumination of the sub-hypostome can interrupt an already initiated contraction burst.

The behavioural sequence following illumination of the dark-adapted, 48-h starved hydra is characteristic and quite sterotyped. For the first 5–10 sec there is no behavioural response; then a seemingly smooth elongation of the column and tentacles occurs, causing the animal to become half again as extended as is typical for the species in the dark. There then ensues a period, of variable duration, but usually lasting at least 2 min, of behaviour reminiscent of hydras initiating a feeding behaviour sequence[5]. The head and upper column sway from side to side, and the entire column may slowly swing around the attached base as in the photokinetic responses described by Haug[6]. The tentacles squirm and usually show co-ordinated lifting movements towards the central vertical axis. Sometimes the latter occur with asymmetrical contractions of the column just below the sub-hypostome, giving a combined nodding-tentacle sweeping movement that is very characteristic. Time-lapse motion pictures of this phase give the appearance of searching movements. Then tentacle activity increases and they tend to spread out on the substratum or surface film and attach to it, whereon a vigorous lengthy contraction burst occurs. Often the animal succeeds in wrenching its base loose from its attachment, initiating a locomotor step.

If the illumination is directional, the responses prior to the contraction burst often lead to the extension of the animal and tentacle attachment in the direction of the light source. The induced locomotion is then in the direction of the light. If the polyp attaches to the surface film, it will float away from its previous position. Tentacle attachment is much less characteristic of recently fed animals, so that they do not show so strong a locomotor component to their light response.

The increase in rhythmic potential frequency that follows light stimulation, and the specific and recognizable behavioural sequence after illumination of the dark-adapted hydra, together suggest a causal relationship between rhythmic potential frequency and co-ordinated activity of tentacles and columnar muscles. This hypothesis can be tested experimentally, since rhythmic potential frequency is increased by localized illumination of the base of the polyp, while leaving the major part of the column and the entire hypostome and tentacles in darkness.

The electrical activity of individual dark-adapted *H. littoralis* was recorded in the manner previously described[1], while they were observed under red light (590–1,000 mμ wave-length). The light stimulus was a 0·5-mm diameter spot of white light delivered through a polished acrylic plastic cone opaqued to the tip. Stimulation was continued until the contraction burst commenced, although the response, once initiated, will continue to completion without continuous stimulation.

The results were unequivocal: localized light stimulation of the edge of the base causes an abrupt increase in rhythmic potential frequency coincident with the illumination behavioural sequence, culminating in a contraction burst. A typical trial is shown in Fig. 1. Elongation was discernible within a few seconds of the increase in rhythmic potential frequency. Bending of the column, nodding and co-ordinated tentacle lifting all occurred. When tentacle attachment occurred, it was always accompanied by a rapid burst of 'attachment burst'[1] (Table 1) impulses. A vigorous contraction burst then ensued.

In a single trial, where localized light stimulation failed to cause an increase in rhythmic potential frequency, there was no evidence whatsoever of the behavioural response. Similarly, localized hypostomal light stimulation neither affected rhythmic potential frequency nor led to the behavioural light response. Rather, it inhibited the contraction burst pacemaker.

As a result of these findings we are able to interpret certain behaviour patterns of hydras in physiological terms. At the conclusion of the spontaneous contraction burst, the discharge rate of the rhythmic potential pacemaker loci is reset, probably as a result of the mechanical stimulation caused by the shortening of the column. This enhanced frequency gradually declines again to a 'basal' level, while the contraction burst pacemaker recovers. Eventually the latter escapes rhythmic potential inhibition, another contraction burst occurs, and the cycle is repeated. The interaction of these two centres—the rhythmic potential pacemaker showing frequency changes in the shape of a saw-toothed curve, the longer segments of which have a negative slope, and the contraction burst centre showing a curve the mirror-image of the former curve and representing potential activity—generate the spontaneous activity pattern which was first described by Trembley[7]. Such interacting systems fit the theoretical model of endogenous pacemakers suggested by Bethe[8]; they may also provide an example of the central pacemaker the spontaneous rhythm of which is frequency modulated by feed-back from an effector[9].

The 'activity potential' of the contraction burst pacemakers seems to be a physiological example of psychological 'energy'. Repeated interruption of spontaneous contraction bursts by localized hypostomal illumination becomes progressively less effective until inhibition finally fails entirely. A model system consisting of a single light-sensitive neurone with one or more pacemaker loci could account for this result.

These findings show that the rhythmic potential system controls quite complex behavioural sequences in hydras, and support the view that there are several pacemaker systems in these animals, arranged hierarchically, which together act as the central nervous system. Pacemakers and their conducting systems provide the integrating mechanism responsible for the co-ordinated behaviour of these animals. Their morphological basis remains obscure, but from their properties it seems likely that neurones are involved. But these pacemakers are not hypothetical; four different pacemaker systems have been differentiated and localized (Table 1) and more may well occur. We believe that the rhythmic potential pacemaker system is responsible for the endogenous rhythmic activity of hydras and that this system dominates the other pacemakers. However, complex behavioural sequences can occur independent of the rhythmic potential system, and sensory stimuli such as light or mechanical deformation can act directly on 'subordinate' centres. The lack of complete physiological dominance of the rhythmic potential pacemaker system emphasizes the diffuse nature of the coelenterate nerve-net. Such diffuse pacemaker activity is a primitive and fundamental property of the nervous system. It precedes, phylogenetically, the appearance of ganglionic centres[2], yet it may well persist within the central nervous systems of members of more advanced phyla.

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LETTERS TO THE EDITOR

RADIOPHYSICS

Neutral Hydrogen in the Virgo Cluster

Neutral hydrogen has recently been detected in absorption in the Virgo cluster of galaxies by measurements of the spectrum of the radio source Virgo *A* (*M*87). The observations were made using the Australian 210-ft. radio telescope and a parametric frequency-switching receiver[1].

Attempts to detect 21-cm emission from clusters of galaxies have so far proved unsuccessful[2]. On the other hand, absorption measurements on bright radio sources should be capable of detecting lower concentrations of hydrogen. However, measurements at Jodrell Bank[3] have shown that the opacity of any neutral hydrogen in front of Virgo *A* is less than 2 per cent. To measure an opacity well below this limit required a frequency-switching receiver with a low noise temperature (170° K), and a careful calibration of all unbalance effects in the receiver. The effect of any difference in the overall gain of the system between the two frequencies being compared was eliminated by determining the spectrum of Virgo *A* relative to that of the Moon. Frequency separations of up to 3 Mc/s were used, the frequencies being displaced successively to cover the frequency range 1,418 Mc/s–1,406 Mc/s (equivalent to radial velocities of +500–+2,900 km/s). The receiver bandwidth was 1·5 Mc/s.

Fig. 1*a* shows the spectrum of Virgo *A* constructed from the differential measurements, assuming that the comparison source, the Moon, is a black body. The differences measured for each frequency pair have been referred to the point at a red shift of 1,700 km/s. The errors shown are the standard deviations of each night's measurements for a given pair of frequencies. Because we have constructed the spectrum from the differences, the errors accumulate at each end of the velocity range. The expected spectral slope for a spectral index[4] of 0·83 has been drawn as a broken line in Fig. 1.

As a control experiment, a similar series of observations were made on the radio source 3*C*353, identified with a 13th magnitude galaxy in a region of the sky well away from the Virgo cluster. The results are plotted in Fig. 1*b*,

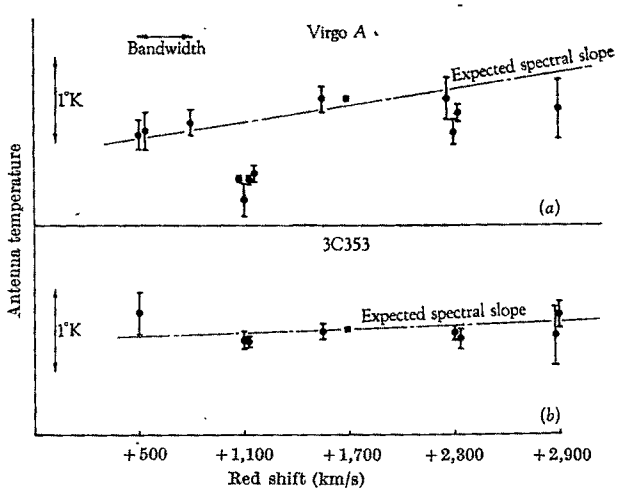


Fig. 1. Measurements of the spectra of the radio sources Virgo *A* and 3*C*353 relative to that of the Moon at wave-lengths near 21 cm. The change in antenna temperature for a 210-ft. telescope is shown as a function of the red shift

where the broken line indicates the slope corresponding to a spectral index[4] of 0·64.

The measurements on Virgo *A* show absorption amounting to 0·65° K in antenna temperature at a red shift of 1,100 km/s, close to the recession velocity of *M*87. With an antenna temperature for the source of 125° K, the corresponding opacity is 0·5 per cent. The width of the absorption feature cannot be determined accurately because of the bandwidth used (equivalent to 300 km/s), but it is not greater than 500 km/s. If the optical depth of the gas is τ, the number of ground-state hydrogen atoms per square centimetre column in the line of sight is N, and the spin temperature is T_s °K, we have (following Wild[5]):

$$\frac{N}{T_s} = 3{\cdot}85 \times 10^{14} \int_0^\infty \tau\,(\nu)\,d\nu$$

where ν is the frequency in c/s. The absorption observed corresponds to $N/T_s \doteqdot 3 \times 10^{18}$ atoms/cm² °K, if it is assumed that the absorbing gas completely covers the radio source.

If the hydrogen could be seen in emission away from Virgo *A*, we could determine the values of N and T_s separately. Attempts were made to observe emission at a velocity of 1,100 km/s at a point displaced 1° in right ascension from Virgo *A*. The telescope was driven from this point in azimuth (minimizing ground-radiation effects) to a point well outside the cluster. No emission greater than 0·2° K was detected with the bandwidth of 1·5 Mc/s.

The absence of any significant absorption in the spectrum of 3*C*353, as well as Field's failure to detect absorption in the spectrum of Cygnus *A*[6], suggest that the neutral hydrogen observed is associated with the Virgo cluster. The red shift and the relatively narrow velocity dispersion of the absorption profile also support this interpretation. Although the distribution of the hydrogen cannot be determined from the present measurements, we can consider three possibilities: the gas might be associated with *M*87 itself, or be distributed homogeneously throughout the cluster, or be in the form of a cloud in front of Virgo *A*.

Because of the intense radiation flux in the vicinity of *M*87, any gas in that galaxy will have a high spin temperature and so a low opacity[7]. The amount of hydrogen required in *M*87 to produce the observed absorption would be well above the limit determined from emission measurements on elliptical galaxies.

If the neutral hydrogen seen in absorption is assumed instead to fill the Virgo cluster homogeneously and *M*87 is near its centre, there would be emission at 21 cm with a brightness temperature of $2\,\tau\,T_s$. The lack of observable emission thus limits T_s to less than 20° K. This is somewhat lower than the value predicted by Field[8] for intergalactic, non-cluster space. With this limit on T_s, we have $N < 6 \times 10^{19}$ atoms/cm². Using de Vaucouleurs's value of 750 kiloparsec[9] for the radius of the Virgo cluster (his "Virgo *E*") leads to a density of less than 2×10^{-5} atoms/cm³ and a total mass of gas less than 10^{12} solar masses, or about 10 per cent of the mass of the galaxies in the cluster[10].

The absorption measurements show that the velocity dispersion of the gas is less than the velocity dispersion of the member galaxies of the Virgo cluster, ±550 km/s[9]. For this reason, it may be preferable to consider the hydrogen to be concentrated in a small cloud which

happens to lie directly in front of $M87$. If the cloud does not extend as far as 1° from Virgo A, we cannot exclude a higher value of T_s, and the density of the hydrogen can be increased considerably. However, the number of such clouds must be limited since no emission has been detected from them in other parts of the cluster. An examination of possible cloud models shows that the total mass of hydrogen they would contain cannot be more than about 10^{12} solar masses.

Thus whatever the distribution of the neutral hydrogen its total mass appears to be insufficient to affect the dynamical instability of the cluster suggested by application of the virial theorem to its brighter member galaxies.

Further observations are planned, using a narrower bandwidth, to determine the width of the absorption profile more accurately.

One of us (J. A. K.) holds an Australian award under the Commonwealth Scholarship and Fellowship Plan, tenable at the Australian National University, Canberra.

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Measurement of Radio Sources at Centimetre Wave-lengths

In future, the limit of detection of faint radio sources, at wave-longths of 10 cm and less, is likely to be set by the radiation from water vapour and rain in the troposphere[1,2]. The dominant factor is the minute-to-minute fluctuations in received power, which may be as much as 2° K at λ 3·7 cm. These fluctuations are greatly in excess of the receiver noise fluctuations, and may destroy all the benefit of using a modern low-noise amplifier such as a maser.

For a large radio telescope, the Rayleigh distance may be much greater than the height of the troposphere; when this is so, a very simple difference technique may be used to eliminate the water vapour fluctuations, but still detect the extra-terrestrial radio sources.

The power from a circular aperture of diameter D flows within a cylinder in the near zone, changing to a conical beam beyond the Rayleigh distance D^2/λ. Consider a dish fed by two feeds side by side in the focal plane, such that the far-zone beams are just separated. Then the near zone beams are cylinders inclined at an angle θ, equal to the beam width, which overlap throughout the near zone, becoming just separated at the Rayleigh distance. Let the receiver be made to switch between the two feeds and record the difference in power. Then an extra-terrestrial source drifting through the two beams will give an S-shaped record with amplitude double the normal amplitude; tropospheric sources are received by both feeds and produce little or no output.

As a numerical example, consider a 100-ft. dish operating at λ 3 cm. The Rayleigh distance is 30 km. In middle latitudes, tropospheric water vapour is mostly at heights below 2 km, that is, in the lowest 1/15 of the near zone. If the dish were uniformly graded, at least 20 : 1 rejection of water vapour radiation would be expected. When allowance is made both for the grading of the dish, and for the grading of water vapour with height, this figure should be increased, perhaps by a factor 2.

The proposed 2-feed system has already been used satisfactorily at longer wave-lengths (λ 50 and λ 22 cm) on the 250-ft. dish at Jodrell Bank (Conway, Long and Daintree, in preparation). The system gains in sensitivity, but loses with respect to confusion, over a normal single beam. Confusion is notoriously a hazard at metre wave-lengths, but the confusion limit at centimetre wave-lengths is below that set by receiver noise and is far below the limit set by water vapour.

The two-feed system is of little use for investigating sources the angular size of which greatly exceeds one beam width. But the argument given here would suggest that it may be the only method for investigating the discrete radio sources at short wave-lengths.

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PHYSICS

A New Instrument for Atmospheric Ozone

Recent theories of the atmospheric circulation ascribe the well-known mid-latitude spring increase in total ozone to the release into the lower stratosphere, during the breakdown of the circumpolar westerlies, of ozone which has been carried to the winter pole by air movements and stored there[1–6]. The details of this process of accumulation remain speculative, however, until many more ozone observations in the polar night become available on a routine basis. The internationally accepted standard ozone instrument, the Dobson spectrophotometer, normally uses sunlight; useful results can be obtained on the full moon[7,8], but this demands an increase in observing skill as well as imposing a serious limitation on continuity.

The idea of using stars for this purpose is by no means new[9–11], but the photographic methods which have been used require for good results an elaborate and expensive telescope. We have found it possible to construct a relatively small and inexpensive instrument with which we hope to carry out a series of observations at Resolute (lat. 74° 43′ N.) during the winter of 1963–64. As in the Dobson instrument, two wave-bands A and B in the ultra-violet, of which A is more strongly absorbed by ozone than B, are isolated by a monochromator and their relative intensities are measured. The derivation of the ozone amount, including the use of the 'long' and 'short' methods, follows exactly the procedure developed by Dobson and his associates[12]. Our method of measuring the intensities, however, is different. The two wave-bands fall on separate photomultipliers (E.M.I. type 6056) and the intensities are measured by the pulse-counting technique[13]. The telescope has an 8-in. diameter objective. The monochromator consists of a reflexion grating (Bausch and Lomb, 2,160 lines/mm, blazed for 3000 Å, ruled area 52 mm × 52 mm) in an Ebert mounting. Filters of nickel sulphate solution combined with Chance $OX.7$ glass are mounted immediately in front of the photomultipliers; these very effectively cut out stray light of greater wave-length scattered within the monochromator, and, indeed, a strong room light can be used while observations are in progress with no influence on the counting rate.

Accurate control of the wave-length setting is achieved by use of a mercury lamp, and all variations in photometric sensitivity are eliminated by the use of a standard lamp.

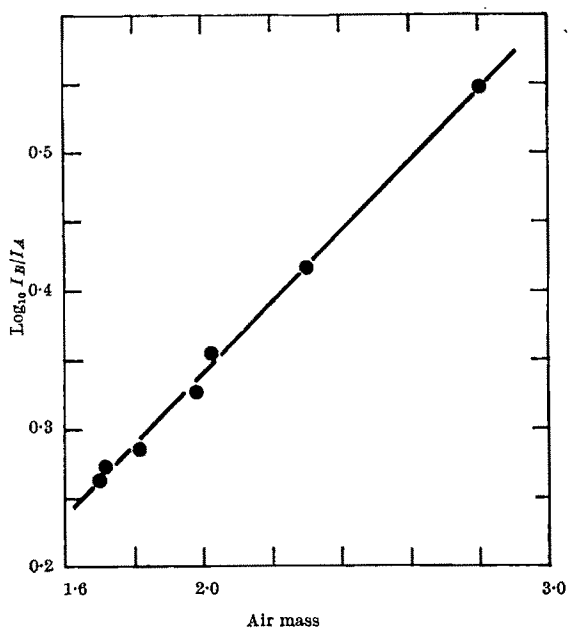


Fig. 1. Observations of ε-Ori on January 17, 1963

Observations of a number of bright stars have been made at Cambridge during the winter and spring 1962–3 in order to test the equipment. A typical example is shown in Fig. 1, in which the wave-bands were *A*: 3100–3150 Å, *B*: 3220–3250 Å. The 'exposure' time was 40 sec. The amount of ozone derived from the slope of the least-squares line shown is (402 ± 12) m atm. cm (standard error), using the data of Vigroux[14] for ozone absorption and of Cabannes[15] for Rayleigh scattering.

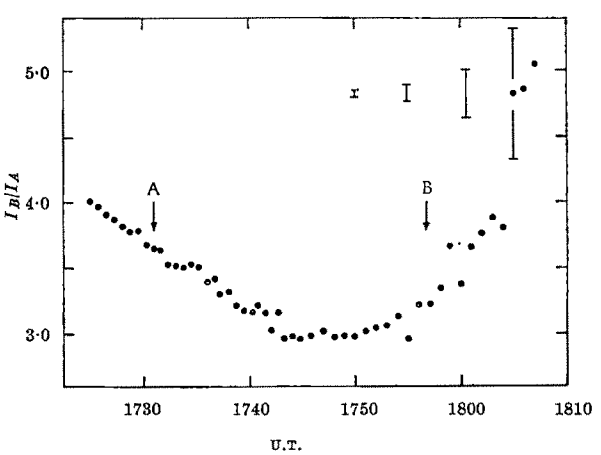


Fig. 2. Ratio of zenith sky brightnesses in the two wave-bands (ozone absorption greater in *A*) on February 26,1963. The ratio has already passed through a maximum (the normal *umkehr* or inversion point) before observations started. The minimum at approximately 1747 h is the so-called second *umkehr*. *A* indicates sunset and *B* the time at which the Sun was 4 deg below the horizon. The standard error of a single point, based on the statistical distribution of photons, is indicated by the vertical lines

If the instrument is pointed at the clear zenith sky the usual *umkehr* observation can be made as the Sun sets or rises. The necessary reduction of sensitivity can be achieved by placing perforated screens over the telescope. Fig. 2 shows the last stages of such an observation (no perforated screens) and shows clearly the 'second Umkehr' which has been reported by Ramanathan[16] and Dütsch[17]. Whether these measurements will prove useful for the determination of vertical ozone distributions in arctic regions is being investigated. We acknowledge the support received for this work from the Meteorological Office.

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METALLURGY

A Technique for producing Regular Patterns on Single Crystals

To examine the deformation of single crystals, it is often necessary to have regularly spaced marks or a grid on the surface of the specimen. In particular, the analysis of plastic strains is carried out by investigating the distortion of the grid using the intersections of the lines as reference points. There are several methods for producing a grid, but usually only one of the following two is used: (1) scratching accurately spaced lines; (2) photo-engraving the required pattern. Both are laborious and require great care in their application. All the methods known to authors subject the specimen to a certain amount of deformation. This affects only the surface of a polycrystalline specimen and can be completely disregarded except when the surface processes themselves are investigated. On the other hand, the mechanical properties of single crystals are extremely sensitive to surface effects. On this account, none of the existing methods was considered satisfactory. It is the purpose of this communication to report a technique which was developed for producing regular patterns on single crystals without subjecting the specimen to any strain.

The surface of the specimen has to be reasonably smooth to obtain distinct marks at the required spacing. While no specific directives can be given for the surface roughness, it is useful to note that satisfactory grids (Fig. 1) were produced without any mechanical or chemical surface preparation for the strain analysis of zinc single crystals, grown from the melt in graphite boats.

A silk screen, having the required pattern and stretched tautly in a light frame, was placed on the specimen surface. The screen was of the type used in the textile silk printing process. Next, the silk was covered uniformly with undiluted screen printing paint. The paint penetrated through the silk and, when the screen was lifted from the specimen, a regular pattern was left on the surface. After 24 h, when the paint was completely dry, the crystal was etched (Fig. 1). Etching was necessary because the paint could not be relied on to follow completely the deformation. The etchant attacks the metallic surface producing a relief pattern. We have found the etchants reported in Table 1 suitable for zinc and copper specimens.

Table 1

Crystal	Etchant
Zn	1_P HCl; 1_P CH_3COOH glacial
Cu	1_P HNO_3; 1_P H_2O

The depth of the marks was controlled by the etching time and for zinc and copper this was in the order of 10 sec. If the specimen is over-etched, the grid becomes irregular. Over-etching can also lift off the paint, distorting the already developed pattern. The etching can be stopped at any time by rinsing in water and resumed, if necessary, without any harmful effect.

An example of a grid produced on a zinc specimen is shown in Fig. 1. The lines are 0·3 mm apart and are estimated to be approximately 0·05 mm deep. It is believed that with this method a grid as fine as 20 lines/mm can be produced easily.

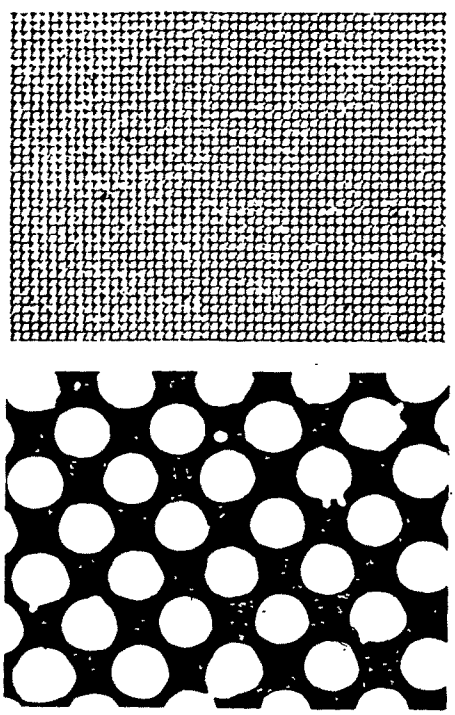


Fig. 1*a* (C × 4) and *b* (C × 30) illustrate two of the many possible patterns. A wide variety of patterns can be produced by different combinations of paint density and thickness

This technique was originally developed for the stress analysis of single crystals, since it was found that, when the strains are known, the stresses can be calculated from the differential equation:

$$\frac{\partial^3\varphi}{\partial y^3}\frac{\partial\varepsilon_x}{\partial x} - \frac{\partial^3\varphi}{\partial x\partial y^2}\frac{\partial\varepsilon_x}{\partial y} + \frac{\partial^3\varphi}{\partial x^2\partial y}\frac{\partial\varepsilon_y}{\partial x} - \frac{\partial^3\varphi}{\partial x^3}\frac{\partial\varepsilon_y}{\partial y} + \frac{\partial^3\varphi}{\partial x\partial y^2}\frac{\partial\gamma_{xy}}{\partial x} - \frac{\partial^3\varphi}{\partial x^2\partial y}\frac{\partial\gamma_{xy}}{\partial y} = 0$$

where ε_x, ε_y, γ_{xy} are the normal and shear strains in the x and y directions respectively. φ is the Airy stress function[1].

The strains were determined experimentally by using the technique described here. For this stress analysis the pattern shown in Fig. 1 was eminently satisfactory. The development of the equation will be reported elsewhere.

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CHEMISTRY

Ionization Potentials and the Structures of the Photo-ionization Yield Curves of Ethylene and its Halogenoderivatives

The problem of interpreting the differences between ionization potentials obtained by electron (E.I.) and by photon impact (P.I.) has led us to a careful examination of the structures occurring in the photo-ionization yield curves of *cis* and *trans* dichloro- and dibromoethylenes[1-3]. The regularly spaced structures found in these curves have been interpreted in terms of vibrational progressions involving the C—C valence vibrations of the ions.

Investigations have been carried out on the foregoing substances, on ethylene itself and on many halogenated derivatives by both P.I. and E.I. techniques. Ion-yield curves have been obtained over the range from the ionization thresholds up to 11·2 eV. The ionization potential by E.I. was obtained by treating the ionization curve as suggested by Warren[4]. The first differential treatment of Hurzeler, Inghram and Morrison[5] has been applied to the P.I. curves for the determination of ionization potentials and to the analysis of the structural details in these curves.

Table 1

Substance	E.I. (eV.)	P.I. (eV)	Difference
C_2H_4	10·56 ± 0·05	10·50 ± 0·02	0
C_2H_3Cl	10·10 ± 0·03	10·00 ± 0·02	0·10
C_2D_3Cl	10·10 ± 0·03	10·02 ± 0·02	0·10
C_2H_3F	10·45 ± 0·05	10·37 ± 0·02	0·08
C_2H_3Br	9·97 ± 0·05	9·82 ± 0·02	0·15
1–1$C_2H_2F_2$	10·32 ± 0·05	10·31 ± 0·02	0
cis $C_2H_2Cl_2$	9·66 ± 0·05	9·66 ± 0·02	0
trans $C_2H_2Cl_2$	9·96 ± 0·05	9·64 ± 0·02	0·36
cis $C_2H_2Br_2$	9·69 ± 0·05	9·45 ± 0·02	0·14
trans $C_2H_2Br_2$	9·54 ± 0·05	9·46 ± 0·02	0·08
cis CHF = CHCl	10·14 ± 0·03	9·86 ± 0·02	0·28
trans CHF–CHCl	10·30 ± 0·03	9·87 ± 0·02	0·43
cis CHF = CFCl	10·17 ± 0·03	9·86 ± 0·02	0·31
trans CHF = CFCl	9·96 = 0·03	9·83 ± 0·02	0·13

The results for the threshold values and also the differences between E.I. and P.I. measurements are given in Table 1. A comparison of the photo-ionization curves for C_2H_3Cl and C_2D_3Cl confirms the interpretation of the structures of these curves in terms of ionization to vibrating states of the ion involving C—C valence vibrations and provides a strong basis for similar interpretations in other compounds. There is some evidence also for the simultaneous excitation of torsional vibrations about the C—C bond.

New data have been found on spin-orbit splitting in the ions containing chlorine and bromine atoms and on electronically excited states existing between the ionization threshold and 11·2 eV. The results are summarized in Table 2.

Table 2

Substance	Doublet separation (cm^{-1})	(C—C) ion (cm^{-1})	Other frequencies (cm^{-1})	Excited electronic states (eV)
C_2H_4	—	1,463	470 (torsion)	—
C_2H_3Cl	990	1,230		10·515
C_2H_3F		1,290		10·880
C_2H_3Br	2,419	1,170	260 (torsion)	10·300
1–1$C_2H_2F_2$		1,371	605 (?)	10·890
1–2$C_2H_2Cl_2$	1,050	1,432		10·275; 10·50; 10·74
1–2CHF = CHCl	1,130	1,412		10·30
1–2CHF = CFCl	1,130	1,412		10·43; 10·82
1–2CHBr = CHBr	2,621	1,210	400	9·84

The wide photon energy range used enabled me to detect in the photo-ionization yield curves a number of sharp peaks in addition to the successive step functions characterizing vibrational excitation in the ions. These have been correlated with strong absorption bands present above the first I.P. (for example, in C_2H_3Cl) and interpreted as excited states of the molecules situated above the ionization threshold and, therefore, subject to non-radiative transitions to the ionization continuum (that is, by auto-ionization). A more detailed study of these states will be published later.

The correlation of the vibrationally and electronically excited states observed for the ions with the differences between E.I. and P.I. ionization potentials in Table 1 strengthens my previous opinion that E.I. ionization potentials correspond to the most probable transition to the ionized state (vertical I.P.). The nature of this probable transition leading to any vibrationally or electronically excited state of the ion is shown in Table 3.

Table 3

Substance	Difference E.I.—P.I. (V)	Vertical transition to:
C_2H_3Cl	0·10	v = 0 of the second level of the doublet
C_2H_3Br	0·15	v = 1 of the C—C vibration
trans CHCl=CHCl	0·36	v = 1 level of the C—C vibration of the second level of the doublet
cis CHBr=CHBr	0·14	v = 1 level of the C—C vibration
trans CHBr=CHBr	0·08	v = 1 level of the 400 cm^{-1} vibration
cis CHF=CHCl	0·28	v = 1 level of the C—C vibration of the second level of the doublet
trans CHF=CHCl	0·43	v = 0 of the first electronic excited state of 10·275 volts
cis CHF=CFCl	0·27	v = 1 level of the C—C vibration of the second level of the doublet
trans CHF=CFCl	0·11	v = 0 of the second level of the doublet

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Molecular Geometry of 7α-Bromocholesteryl Chloride

The crystal and molecular structures of 7α-bromocholesteryl chloride, $C_{27}H_{44}ClBr$ (Fig. 1), were determined by Bürki and Nowacki[1] in 1956 by single crystal X-ray diffraction analysis using a combination of heavy atom, isomorphous replacement, and Patterson superposition methods. The unit cell is monoclinic, space group $P2_1$ with cell constants of a_0 = 11·95 Å, b_0 = 8·78 Å, c_0 = 12·54 Å, β = 101·2°, and Z = 2.

The fractional atomic co-ordinates (x, y, z) obtained by Bürki and Nowacki have been converted to Cartesian co-ordinates p, q, r in Å units, where $p = xa_0 \sin \beta$, $q = yb_0$, and $r = zc_0 + xa_0 \cos \beta$, and used to calculate various intra-molecular relationships. The method of Schomaker *et al.*[2] has been used to compute a series of equations for the 'best' planes to which various atomic groupings may be referred (Table 1). The root mean square (r.m.s.) distances of atoms from these planes are listed in Table 2. Inter- and intra-ring dihedral angles are set forth in Table 3, and the perpendicular distances of individual atoms from one or more 'best' planes are given in Table 4.

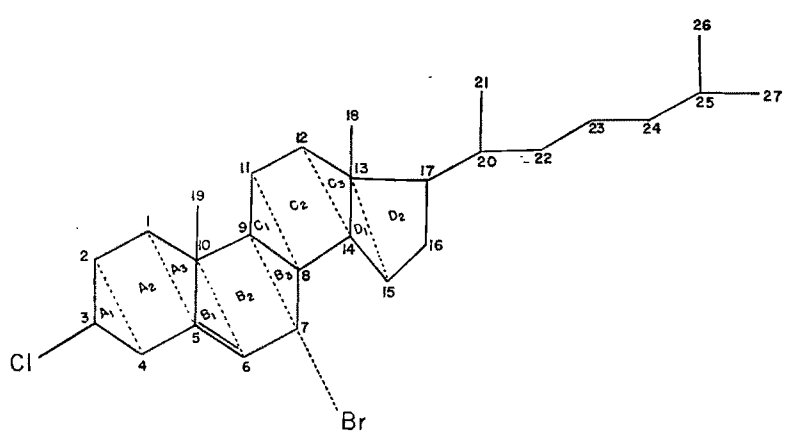


Fig. 1. 7α-Bromocholesteryl chloride

Table 1. Values of α^*, β^*, γ^*, $d\dagger$ in the Equation $\alpha x + \beta y + \gamma z = d$ of the 'Best' Planes of 7α-Bromocholesteryl Chloride

	α	β	γ	d
Entire molecule (27 carbons)	0·31	−0·86	0·41	1·32
Steroid nucleus (C_1–C_{17})	0·26	−0·90	0·35	1·07
A	0·19	−0·95	0·25	0·18
B	0·36	−0·83	0·42	1·98
C	−0·06	0·94	−0·33	0·43
D	0·36	−0·85	0·38	1·54
*A*1	0·31	0·76	−0·58	5·40
*A*2	0·41	−0·91	0·06	2·60
*A*3	0·20	0·86	−0·48	3·32
*B*1	0·59	−0·72	0·36	4·09
*B*2	0·49	0·81	0·32	3·20
*B*3	0·31	0·71	−0·63	2·64
*C*1	0·23	0·85	−0·47	2·38
*C*2	0·34	−0·92	0·18	1·23
*C*3	0·41	0·73	−0·55	2·17
*D*1	0·02	−0·74	0·67	0·46
*D*2	0·50	−0·83	0·26	1·81
*A*3*B*1	0·16	0·74	−0·65	2·64
*B*3*C*1	0·27	0·79	−0·55	2·40
*C*3*D*1	0·23	0·73	−0·65	0·81

* Direction cosines of plane.
† Perpendicular distance from origin to plane.

Table 2. Root Mean Square Distances (r.m.s.) of the Atoms of 7α-Bromocholesteryl Chloride from the 'Best' Planes

'Best' planes	r.m.s. distances (Å)
Entire molecule (27 carbons)	0·57
Steroid nucleus (C_1–C_{17})	0·28
A	0·22
*A*1	0·00
*A*2	0·03
*A*3	0·00
B	0·26
*B*1	0·00
*B*2	0·17
*B*3	0·00
C	0·23
*C*1	0·00
*C*2	0·02
*C*3	0·00
D	0·20
*D*1	0·00
*D*2	0·14
*A*3*B*1	0·23
*B*3*C*1	0·09
*C*3*D*1	0·17

Table 3. Dihedral Angles of 7α-Bromocholesteryl Chloride

Planes	Angle (deg.)	Planes	Angle (deg.)
A ∧ *B*	166	*B*2 ∧ *B*3	128
B ∧ *C*	161	*B*3 ∧ *C*1	167
C ∧ *D*	162	*C*1 ∧ *C*2	128
*A*1 ∧ *A*2	126	*C*2 ∧ *C*3	129
*A*2 ∧ *A*3	137	*C*3 ∧ *D*1	154
*A*3 ∧ *B*1	138	*D*1 ∧ *D*2	144
*B*1 ∧ *B*2	172		

The 'best' plane obtained when all 27 carbon atoms of 7α-bromocholesteryl chloride are considered has the equation: $0{\cdot}31x - 0{\cdot}86y + 0{\cdot}41z = 1{\cdot}32$. The r.m.s. distance of carbon atoms from this plane is 0·57 Å. If only the steroid nucleus, C_1 through C_{17}, is considered, the 'best' plane has the equation: $0{\cdot}26x - 0{\cdot}90y + 0{\cdot}35z = 1{\cdot}07$, with a r.m.s. distance of 0·28 Å. It is interesting to note that a similar spatial distribution of atoms is evident in cholesteryl iodide[3], where the r.m.s. distance value is 0·58 Å for the 27 carbon atom plane, and 0·28 Å for the plane of the steroid nucleus (C_1—C_{17}).

Rings *A*, *B*, *C* and *D* have a higher degree of planarity than either the entire carbon-atom molecule or the steroid nucleus, as can be seen by their r.m.s. distances of 0·22, 0·26, 0·23 and 0·20 Å respectively. Since the atomic co-ordinate standard deviation obtained by Bürki and Nowacki is approximately 0·03 Å, it is evident that the degrees of planarity of the 'best' planes representing rings *A*, *B*, *C* and *D* are approximately equal.

Table 4. PERPENDICULAR DISTANCE (Å) OF ATOMS FROM ONE OR MORE OF THE 'BEST' PLANES OF 7α-BROMOCHOLESTERYL CHLORIDE

	Entire molecule (27 carbons)	Steroid nucleus (17 carbons)	*A*	*A*2	*B*	*B*2	*C*	*C*2	*D*	*D*2	*A*3*B*1	*B*3*C*1	*C*3*D*1
C_1	0·61	0·30	0·23	0·03							−0·13		
C_2	0·11	−0·20	−0·26	−0·03									
C_3	0·35	0·15	0·25										
C_4	−0·35	−0·47	−0·20	0·03									
C_5	0·00	−0·11	0·16	−0·03	−0·14						0·36		
C_6	0·38	0·36			0·16	0·16					−0·25		
C_7	0·36	0·34			0·08	−0·17						−0·09	
C_8	−0·13	−0·24			−0·37		0·19	−0·02				0·09	
C_9	0·58	0·37			0·42	0·17	−0·12					0·09	
C_{10}	−0·06	−0·28	−0·18		−0·15	−0·16					0·03		
C_{11}	0·35	0·05					0·19	0·02				−0·09	
C_{12}	0·56	0·26					−0·23	−0·02					
C_{13}	−0·32	−0·53					0·28		−0·30	−0·10			−0·16
C_{14}	0·18	0·05					−0·31	0·02	0·23				−0·14
C_{15}	−0·06	−0·09							−0·08	0·11			0·19
C_{16}	0·00	−0·07							−0·09	−0·17			−0·17
C_{17}	0·30	0·11							0·24	+0·17			
C_{18}	−1·85	−2·03					+1·71		−1·83				
C_{19}	−1·60	−1·82	−1·76		−1·67								
C_{20}	−0·22	−0·45											
C_{21}	0·57	0·23											
C_{22}	0·34	0·15											
C_{23}	−0·37	−0·60											
C_{24}	0·28	0·09											
C_{25}	−0·22	−0·44											
C_{26}	−0·14	−0·46											
C_{27}	0·33	0·17											
Cl_3	−0·11	−0·34	−0·31	0·56									
Br_7	2·28	2·26			+1·97								

Smaller deviations from planarity occur in the *A*2, *B*2, *C*2 and *D*2 portions of the 7α-bromocholesteryl chloride molecule, which have r.m.s. distances of 0·03, 0·17, 0·02 and 0·14 Å respectively. In view of the 0·03 Å standard deviation, it is seen that in this case the degree of planarity of *A*2 equals that of *C*2, and that the degree of planarity of *B*2 equals that of *D*2.

Table 3 lists the dihedral angles of 7α-bromocholesteryl chloride (standard deviation is approximately 1·4°). Within the limits of the standard deviation, it can be seen that $B \wedge C$ equals $C \wedge D$, $A2 \wedge A3$ equals $A3 \wedge B1$, and $C1 \wedge C2$ equals $C2 \wedge C3$.

The following features of side-group geometry may be deduced from Table 4, which lists the perpendicular distance of atoms from one or more of the 'best' planes:

(1) The chlorine atom attached to C_3 is equatorial.

(2) The bromine atom attached to C_7 has an axial configuration, and is nearly perpendicular to the 'best' plane of the steroid nucleus. The evidence for this is that the perpendicular distance of the bromine to the 'best' plane of the steroid nucleus (2·26 Å) is equal to the sum of the C_7-Br bond length and the perpendicular distance of C_7 to the 'best' plane of the steroid nucleus.

(3) The bromine atom and the angular methyl groups (C_{18} and C_{19}) are on opposite sides of the steroid nucleus as can be seen from the change in sign of their respective perpendicular distances from the 'best' plane of the steroid nucleus. (Atoms lying between the origin of the unit cell and the 'best' plane have a negative value; those on the opposite side of the plane have a positive value.)

(4) The C_{18} and C_{19} angular methyl groups are axial, and almost perpendicular to the 'best' plane of the steroid nucleus. The evidence for this is that in both cases the perpendicular distance of the atom in question is equal to the sum of the appropriate methyl carbon–nuclear carbon bond length and the perpendicular distance of the appropriate nuclear carbon to the 'best' plane of the steroid nucleus.

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Recombination of Chlorine Atoms

THE rate of recombination of chlorine atoms has been investigated using a flow system. The atoms were produced in a microwave discharge, and the atom decay followed by means of a movable thermocouple probe at which recombination occurred. The reaction tube of 4-cm internal diameter was poisoned with boric oxide. Runs using chlorine only, and chlorine/argon mixtures, were carried out. Linear flow speeds in the range 30–250 cm/sec, and total pressures (measured with a sloping oil manometer) of 0·24–3 mm mercury were used.

At low chlorine pressures, a first-order decay in the chlorine atom concentration was observed. This was attributed to surface recombination. Ogryzlo[1] has previously studied this. As the chlorine pressure was raised, the order increased towards two. The following mechanism is proposed to account for these observations:

$$\mathrm{Cl} + \mathrm{wall} \xrightarrow{k_w} \tfrac{1}{2}\mathrm{Cl}_2 + \mathrm{wall}$$

$$\mathrm{Cl} + M \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Cl}M$$

$$\mathrm{Cl}M + \mathrm{Cl} \xrightarrow{k_2} \mathrm{Cl}_2 + M$$

Assuming an equilibrium concentration of the intermediate Cl*M*, the resulting rate equation can be integrated to give:

$$k_w t = \ln\left[\frac{[\mathrm{Cl}]_0}{[\mathrm{Cl}]_t}\right] + \ln\left[\frac{k_w + k[\mathrm{Cl}]_t[M]}{k_w + k[\mathrm{Cl}]_0[M]}\right] \qquad (1)$$

where

$$k = \frac{k_1 k_2}{k_{-1}}$$

The agreement between the experimental data for runs with chlorine only and this theoretical expression is demonstrated in Fig. 1. If a reasonable value is assumed for the percentage dissociation of chlorine, a value of approximately 6×10^{14} c.c.^{2} mole^{-2} sec^{-1} is derived for the *ter*molecular rate constant k_{Cl_2} defined by

$$-\frac{\mathrm{d}[\mathrm{Cl}]}{\mathrm{d}t} = k_{\mathrm{Cl}_2}[\mathrm{Cl}]^2[\mathrm{Cl}_2]$$

This constant implies that, a few centimetres from the discharge, the percentage dissociation was 1 per cent at 1 mm, 10 per cent at 0·5 mm., and 35 per cent at 0·25 mm pressure. These percentages are unlikely to differ by more than a factor of two from the actual values, so that

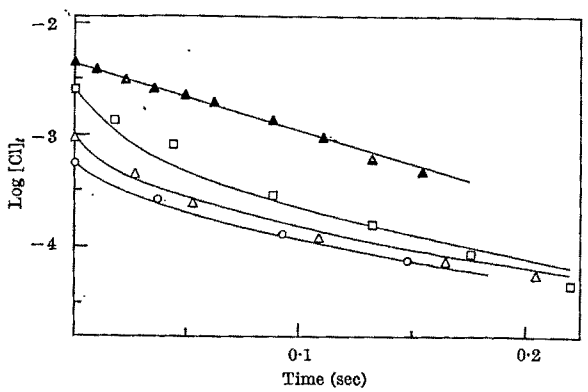


Fig. 1. Variation of log [Cl]$_t$ with time, using 100 per cent chlorine. ▲, at pressures of 0·25 mm; □, 0·5 mm; △, 0·775 mm; ○, 1·05 mm. The points are experimental; the lines are theoretical (equation (1))

the probable range of values for the rate constant is $3–12 \times 10^{14}$ c.c.^{2} mole^{-2} sec^{-1}.

From the experiments with argon/chlorine mixtures the qualitative conclusion can be drawn that argon is less efficient as a third body than chlorine for the recombination reaction; the observed reaction order remained one for all mixtures, and with particular significance for those having a high total pressure but a low partial pressure of chlorine.

The first-order rate constant, k_w, was found to vary inversely with the total pressure, implying control by radial diffusion. Using a calculated value for the diffusion coefficient[2] this would not be expected; the calculated coefficient for chlorine atoms may therefore be too high. Ogryzlo gives a value of 4×10^{-5} for γ, the surface recombination coefficient. In the present experiments, γ was probably of the order of 10^{-3}, but this could not be determined reliably since the first-order rate constant was not found to be independent of pressure. Further work is being carried out in this laboratory.

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Hydrogen – Air Fuel Cell with Homogeneous Oxidation Catalyst Redox Cycle

In fuel cells with an intermediate redox cycle, electrochemical reactants are prepared by the action of a fuel and an oxidant on a redox system. Complexes of copper in certain organic solvents present particularly interesting systems. For example, cupric acetate dissolved in pyridine oxidizes molecular hydrogen; cuprous acetate is the homogeneous catalyst as well as the reaction product[1,2]. In air, the reverse reaction proceeds with ease. Such systems may be useful in fuel cells operating on air and hydrogen as they would avoid the troublesome problem of maintaining the gas/electrolyte boundary in a specific position in the porous electrodes.

I carried out experiments to estimate the properties of such a system. Hydrogen at 95° C and atmospheric pressure reduced a 0·2 M solution of cupric acetate in pyridine without the formation of copper metal. Cells composed of platinum/copper^{II}/pyridine acetate/copper^{I}/platinum, where copper^{II} and copper^{I} stand for the solutions of the corresponding acetates in pyridine, yielded 0·21–0·24 V at 95° C. With a silver anode the potentials were 0·01–0·03 V higher. Carbon dioxide and carbon monoxide did not affect the anode potential. Hence the system should be able to utilize hydrogen from water gas, or hydrocarbon-reforming gas, without need for prior removal of carbon oxides. After polarization by drawing currents, the potential recovered readily. The potential of the cathode was 0·06 V noble versus a saturatep calomel electrode, measured by disregarding liquid junction potentials that may be associated with the pyridine acetate bridge.

Although the potential of this cell was low, compared with 0·95–1·10 V for direct hydrogen–oxygen fuel cells, other complexing and solvent media may give more favourable potentials.

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BIOCHEMISTRY

Blood Corticosterone-levels in the Rat after Administration of Amino-acids

Munro and Mukerji[1] observed that the feeding of single large doses of certain individual amino-acids, notably glycine, methionine and leucine, caused a marked stimulation of uptake of phosphorus-32 by rat-liver ribonucleic acid (RNA) during the next 18 h. In the case of methionine and leucine, but not of glycine, this action was abolished by adrenalectomy[2]. It was therefore proposed that the increased incorporation of phosphorus-32 into liver RNA after feeding methionine or leucine was due to release of adrenocortical hormones, which are known to induce changes in liver RNA metabolism[3]. The experiments reported here show that, within a 24-h period of administering methionine or leucine to rats, there is an elevation of blood corticosterone-level. Administration of other amino-acids or of casein does not have this effect. These observations would thus appear to confirm the proposed mechanism of action of methionine and leucine on liver RNA metabolism.

Male rats weighing about 150 g, trained[1] to consume food promptly, were fed a meal containing 2 g of a starch-glucose mixture[1] and 1 g of an amino-acid (glycine, methionine, leucine, alanine, aspartic acid or glutamic acid) or casein; control animals received the starch-glucose mixture without any addition. All animals were killed 24 h later by guillotine and corticosterone concentrations in the plasma and the adrenal glands were measured fluorometrically[4] with an Aminco-Bowman spectrophotofluorometer.

Table 1. Corticosteroid-levels in Plasma and Adrenal Gland 24 h After Feeding Individual Amino-acids or Casein in 1-g Quantities to Rats

Addition to carbohydrate meal	Corticosterone concentration in plasma	Corticosterone concentration in adrenal gland
Casein	86	110
Glycine	85	110
Methionine	125	139
Leucine	107	131
Alanine	80	122
Aspartic acid	82	119
Glutamic acid	76	108
By analysis of variance	$P < 0·01$	$P > 0·05$
Fiducial limits	28	—

The results are expressed as a percentage of corticosterone concentration found in plasma or gland 24 h after feeding the carbohydrate meal alone.

The control animals fed only the carbohydrate meal had high levels of corticosterone in the plasma (mean level 126 μg/100 ml. plasma) and in the adrenal glands (mean concentration 9·5 μg/100 mg adrenal tissue), compared to the values reported in the literature[4,5]. Rats taken directly

from stock cages had much lower levels, and the high concentrations observed in the experimental group can be attributed to partial undernutrition during the training period. It is thus not surprising that the addition of casein and of several of the amino-acids to the carbohydrate test meal lowered the plasma corticosterone concentration found 24 h after the meal (Table 1), since these substances would serve as sources of additional energy for the animal. In contrast, methionine and leucine raised the plasma concentration of corticosterone, and analysis of variance confirmed that the effect of these two amino-acids differed significantly from that of other amino-acids administered. Amino-acid feeding caused no depletion of adrenal corticosterone content, and even tended to raise the level, particularly after methionine and leucine administration (Table 1), though none of these increments attained statistical significance. Subsequent experiments not presented here show that prolonged administration of leucine and methionine results in considerable increases in corticosterone concentration in the gland.

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Action of Insulin on the Isolated Rat Diaphragm in the Presence of Actinomycin *D* and Puromycin

Within recent years, conclusive evidence has been obtained of a direct stimulation of transport of sugar from the extracellular to the intracellular space of the isolated perfused heart of the rat[1] and the isolated 'caged' diaphragm of the rat[2]. It is, however, difficult to explain the entire mechanism of insulin action through this effect, for clear-cut insulin-induced stimulations have been observed in the absence of extracellular or intracellular glucose: these effects include biosynthesis of proteins from labelled precursors[3], increased labelling of RNA from ^{14}C-adenine or glucose[4] and increased specific labelling of high-energy phosphates from intracellular inorganic phosphate labelled with phosphorus-32 (ref. 5). This evidence raised the question[4] whether an induced biosynthesis of messenger RNA (ref. 6), which could in turn stimulate the synthesis of specific enzymatic proteins, may participate in the hormonal action; recent work on the effects of other hormones[7,8] supports this hypothesis.

As the biosynthesis of RNA may be selectively inhibited by actinomycin *D* and inhibition of protein biosynthesis occurs with puromycin in mammalian cells, an investigation was undertaken with these inhibitors on different insulin-stimulated phenomena in the isolated rat diaphragm. Three tests were performed in the absence of glucose, namely, RNA and protein biosynthesis from ^{14}C-labelled precursors and labelling of energy-rich compounds from ^{32}P inorganic phosphate; the fourth test consisted of the measurement of glucose uptake.

The incubation method and the separation of the labelled phosphate fractions have been previously described[5]. The isolation of RNA and the estimation of its radioactivity have been performed as described by Wool[4]; the incorporation of labelled 1-^{14}C-adenine into ATP, considered to be a direct precursor of RNA, is measured in a similar manner as incorporation of ^{32}P into ATP (ref. 5). The incorporation of labelled amino-acids (U-^{14}C-protein hydrolysate or 1-^{14}C-D,L-valine) is performed according to Manchester[3]. The specific radioactivities of the extracellular and intracellular amino-acid pools are measured on the TCA-soluble extracts of the incubation medium or the diaphragm, free amino groups being determined by a ninhydrin-method, on the assumption that the average nitrogen content of these pools is 16 per cent; however, when ^{14}C-valine was used, the precursor pools are expressed as counts per unit weight of the incubation medium or the diaphragm.

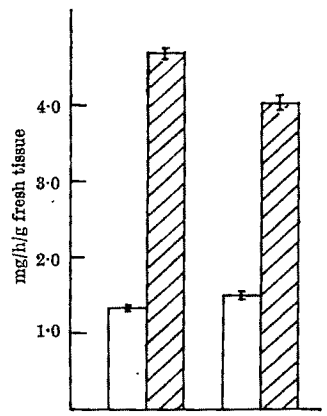


Fig. 1. Influence of puromycin on the uptake of glucose of the isolated diaphragm of the rat in absence (white) and presence (hatched) of insulin (10 μg/ml.). Left, no puromycin; right, puromycin (500 μg/ml.). Standard error of the mean indicated at the top in each case

Actinomycin *D* and puromycin dichlorhydrate are added at the beginning of the experiment and pre-incubation in the presence of these antibiotics lasted 30 min. The labelled precursors (^{32}P inorganic phosphate: 10–20 μc., ^{14}C substrates: 1 μc.) or the unlabelled glucose (2 mg/ml.) are then tipped into the incubation medium. Glucose uptake, protein and RNA labelling are measured over 2-h periods, whereas the labelling of phosphates is determined over periods of 10 min; control experiments showed that if the labelling of these phosphates is measured after pre-incubations of 2 h, the effects of insulin, puromycin and actinomycin *D* are not modified.

Table 1 shows that RNA synthesis from labelled adenine is enhanced by almost 100 per cent in the presence of insulin. Despite complete inhibition of RNA labelling by actinomycin *D*, it appears (Table 1) that labelling of protein is unchanged in presence of this antibiotic; stimulation by insulin averages 100 per cent both in its presence and in its absence. Labelling of ATP from adenine is unchanged in all cases.

Table 2 shows the effect of actinomycin *D* and puromycin on the labelling of energy-rich phosphates and its stimulation by insulin: no inhibition is detected under experimental conditions, where RNA biosynthesis is completely inhibited by actinomycin *D* (Table 1) and where the labelling of proteins is completely suppressed by puromycin (Table 3). Hence the glucose-independent metabolic stimulation by insulin, which is reflected in the turnover of these nucleotides, does not depend on protein or RNA biosynthesis.

Finally, Fig. 1 shows that enhancement of glucose uptake by insulin is independent of protein biosynthesis, as puromycin has no detectable effect on this phenomenon.

From these results, two-fold conclusions may be drawn: first, it appears that labelling of RNA and proteins from ^{14}C precursors[3,4] are sensitive to specific inhibitors and thus represent true biosynthesis of these molecules; this is apparent even when the precursor is valine, the penetration of which into the cell is not sensitive to insulin stimulation[3]. It is also noteworthy that the inhibitions by actinomycin *D* or puromycin are complete after 30 min. Hence it appears that the penetration of these compounds into the cell is not a limiting factor for their efficiency.

Table 1. Influence of Actinomycin *D* on the Insulin-induced Stimulation of the Biosynthesis of RNA and Protein in the Isolated Rat Diaphragm

Actinomycin *D* (10 μg/ml.)	0	0	+	+
Insulin (10 μg/ml.)	0	+	0	+
A Biosynthesis of RNA from (8-^{14}C) adenine				
ATP (counts/μg)	201	212	207	280
RNA fraction (counts incorporated/100 mg tissue)	1,006	2,040	26	38
B Biosynthesis of proteins from (U-^{14}C) protein hydrolysate				
Extracellular amino-acid pool (counts/μg)	319	374	327	399
Intracellular amino-acid pool (counts/μg)	66	71	69	72
Proteins (counts/mg)	316	706	395	765

Table 2. Influence of Actinomycin *D* and Puromycin on the Insulin-induced Stimulation of the Labelling of Phosphates in the Isolated Rat Diaphragm

Specific activity (percentage of extracellular or intracellular phosphorus)							
Actinomycin *D* (10 μg/ml.)		0	0	+	+	0	0
Puromycin (500 μg/ml.)		0	0	0	0	+	+
Insulin (10 μg/ml.)		0	+	0	+	0	+
Intracellular inorganic phosphorus	(*E*)	6·9	6·2	7·2	6·7	8·7	10·7
PC*	(*I*)	35·8	44·4	32·7	53·3	31·4	40·5
ATP	(*I*)	44·5	58·5	40·2	68·0	43·4	70·0
ADP	(*I*)	19·7	24·5	19·5	30·2	17·9	21·7
U + G†	(*I*)	24·4	37·9	26·2	51·5	16·7	32·5

(*E*) Percentage of extracellular inorganic phosphorus.
(*I*) Percentage of intracellular inorganic phosphorus.
* Phosphocreatine.
† Sum of uridine and guanosine phosphates.

Table 3. Influence of Puromycin on the Insulin-induced Stimulation of Protein Biosynthesis in Isolated Rat Diaphragm

A Precursor: U-^{14}C protein hydrolysate				
Puromycin (500 μg/ml.)	0	0	+	+
Insulin (10 μg/ml.)	0	+	0	+
Extracellular amino-acid pool (counts/μg)	940	740	545	518
Intracellular amino-acid pool (counts/μg)	115	120	152	131
Proteins (counts/mg)	750	1,371	24	11
B-Precursor: D,L -1^{14}C Valine				
Incubation medium (counts/100 mg)	7·800	8·800	—	8·767
Intracellular medium (counts/100 mg)	6·300	5·745	—	6·352
Proteins (counts/mg)	166	213	—	1

The second set of conclusions concerns the mutual independence of the phenomena which have been tested. This independence is particularly striking so far as protein and RNA biosynthesis are concerned: the complete suppression of the latter has no influence whatsoever on the stimulation of the former; thus it seems difficult to conclude[4] that the biosynthesis of a messenger RNA could even partially mediate the action of insulin on the isolated muscle.

Hence this action seems to consist in a general stimulation of uncorrelated metabolic events; the insulin-induced increase of glucose-uptake may, however, be inhibited by short pre-incubations with *N*-ethylmaleimide[9]. This equally holds for the labelling of energy-rich phosphates[10] and demonstrates that an interaction of insulin with specific —SH groups is a pre-requisite for its effect on the isolated muscle, the precise mechanism of which (for example, cell 'decompartmentation') remain at present a matter of speculation.

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Naphthalenes in Cigarette Smoke

In our work on the composition of the non-methanol volatile neutral fraction of cigarette smoke we have isolated and identified the naphthalenes shown in Table 1.

Table 1

Substance	Yield*
Naphthalene	0·17
2-Methylnaphthalene	0·50
1-Methylnaphthalene	0·50
2,7-Dimethylnaphthalene	0·26
2,6-Dimethylnaphthalene	0·26
1,6-Dimethylnaphthalene	1·30
1,3,6-Trimethylnaphthalene	0·70

* In μg/cigarette (average weight, 1·15 g)

The naphthalenes were isolated from chromatographic fractions of cigarette smoke condensate by precipitation as complexes with *s*-trinitrobenzene. The combined complexes were decomposed and the mixture of naphthalenes obtained was separated by preparative scale gas chromatography. The individual naphthalenes were identified by comparison of ultra-violet and infra-red spectra with those of authentic specimens and by the preparation of solid derivatives. Analytical gas chromatography showed that these were the only naphthalenes present (eluted in the order shown in Table 1) except for a small shoulder appearing on the peak corresponding to 1,6-dimethylnaphthalene. The retention volume of this shoulder corresponded to 1,8-dimethylnaphthalene and/or acenaphthene. Naphthalene, 2-methylnaphthalene, and possibly 1,8-dimethylnaphthalene have been detected already by ultra-violet spectroscopy[1,2]. Acenaphthylene, reported earlier[1], occurs in a later chromatographic fraction of the smoke condensate. The relatively large quantities of dimethyl- and trimethylnaphthalenes have escaped detection by methods used previously, but their presence gives an important lead to the understanding of the mode of formation of polycyclic hydrocarbons in cigarette smoke. The positions of the methyl groups indicate terpenoid precursors of these naphthalenes, and an extension of the suggested mode of thermal decomposition of isoprenoid polyolefines[3] can account fully for their formation.

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Boric Acid-induced Heterogeneity of Conalbumin by Starch-gel Electrophoresis

Multiple molecular forms of conalbumin[1], the iron-binding component of avian egg white, have been described by numerous investigators under a variety of experimental conditions. In the original purification of conalbumin, Longsworth *et al.*[2] identified two components, the relative proportions of which were dependent on *p*H. Afterwards, by starch-gel electrophoresis, Lush[3] identified two, and occasionally three, iron-binding proteins in native egg white, as did Williams[4] and Ogden *et al.*[5]. In each of these cases[3–5], the slower migrating of the two components was the more intense; in the latter investigations[4,5] a corresponding heterogeneity was observed in transferrin, the iron-binding protein in the serum of the animal. Thus, in the chicken, as in other species, including man[6], primates[7], cattle[8,9], bison[10], horses[11], and mice[12], a single transferrin allele appears to determine the synthesis of more than one molecular species of transferrin. Ultracentrifugal investigations on purified cattle transferrins indicate that the multiple bovine components do not represent a polymer series[13].

Ogden *et al.*[5] have also described a genetically determined variation in chicken serum transferrin which is

paralleled by a corresponding variation in the conalbumin pattern, and Williams[4] has suggested that the avian transferrin gene may determine the synthesis of both transferrin (in the liver) and conalbumin (in the oviduct). Williams[4] has also investigated the biochemical relationship between transferrin and conalbumin and has proposed that these proteins may differ only in their sialic acid content. A similar phenomenon has been described in human cerebrospinal fluid[14], where two principal transferrins have been observed, one with the full serum complement of four sialic acid residues per molecule and the other devoid of sialic acid. However, in this case the difference appears to involve more than the sialic acid moiety and may represent the absence of multiple-unit carbohydrate prosthetic groups on the transferrin molecule[15].

This communication presents evidence for an artificially induced heterogeneity of conalbumin as revealed by starch-gel electrophoresis in boric acid buffer systems. Similar results have been obtained both for conalbumin in filtered egg white and for purified conalbumin. The purification of conalbumin was accomplished by a two-step procedure involving starch-block electrophoresis and cellulose chromatography, as previously utilized for the purification of human and primate transferrins[14]. In the final step, the conalbumin fraction from 20 ml. egg white separated by preparative electrophoresis in a starch-granule block was applied to a TEAE cellulose column. In 0·015 M disodium hydrogen phosphate buffer, only iron-binding components (conalbumins) were eluted from the column. Vertical starch-gel electrophoresis was carried out according to the method of Smithies[16], and iron-binding components were detected by autoradiography after addition of iron-59 to the samples[17].

The starch-gel pattern observed for conalbumin revealed multiple iron-binding components. In *tris*-citrate (*p*H 8·8) and phosphate-citrate (*p*H 7·4) buffers, a single prominent relatively slow-migrating component was observed, together with two, and occasionally three, faint, more rapidly migrating components. This is essentially the same conalbumin pattern as described by Lush[3], Williams[4], and Ogden *et al.*[5], who each utilized the *tris*-citrate[18] buffer system.

In boric acid–sodium hydroxide buffers, however, two additional prominent components were observed the presence and relative intensity of which depended on the concentration of conalbumin and on the concentration and *p*H of the borate buffer (Fig. 1). At low protein concentrations and low boric acid concentrations (that is, at high *p*H, where a large proportion of the boric acid was neutralized by sodium hydroxide, or in dilute buffers), only a single prominent component was present which appeared to correspond to the major component observed in the *tris*-citrate and phosphate-citrate buffers. When the protein concentration or boric acid concentration was increased, the major component split into a second slower migrating component, which in turn gave rise, with considerable trailing, to a third slower-migrating component. This third component did not bind radioactive iron. The observed patterns were independent of the amount of iron added to the sample.

The two additional boric acid-induced components were present in undiluted egg white after electrophoresis in the conventional borate buffer of Smithies[16] (0·020 M metaboric acid, 0·0084 M sodium hydroxide; *p*H 8·9). At a one-eighth dilution of the egg white, only the single component was observed; reconcentration of the diluted material resulted in the reappearance of the three components (Fig. 1). The band corresponding to the slowest moving non-iron binding boric acid-induced conalbumin component was removed from an unstained slice of the starch gel and the protein was eluted by freezing and thawing. By a second electrophoresis at an appropriate dilution, this component bound radioactive iron; its mobility was increased and coincided with the single prominent conalbumin component in diluted egg white.

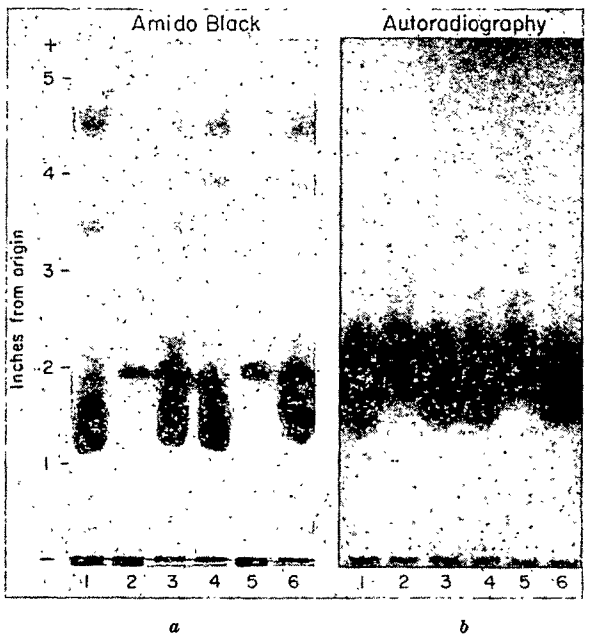


Fig. 1. Boric acid-induced heterogeneity of conalbumin in egg white. 1,4: egg white, undiluted; 2, 5: egg white, diluted 1 : 8; 3, 6: egg white, diluted and reconcentrated

(*a*) Amido black stain after vertical starch-gel electrophoresis. Gel buffer: 0·020 M H_3BO_3, 0·0084 M NaOH. The principal conalbumin components migrate in the region 1–2 in. from the origin. The trailing border of ovalbumin (not shown) has migrated 6 in. from the origin. In the region 3–4 in. from the origin is seen an additional egg white polymorphism, described by Lush (ref. 3)

(*b*) Autoradiograph from gel of Fig. 1*a*. Radioactive iron is bound to only two of the three conalbumin components observed in the amido black stain. Only one (at 2·25 in. from the origin) of the three faint more rapidly migrating iron-binding components observed in this and other buffer systems is clearly seen; the second of these components has migrated 2·75 in. and the third 3·5 in. from the origin

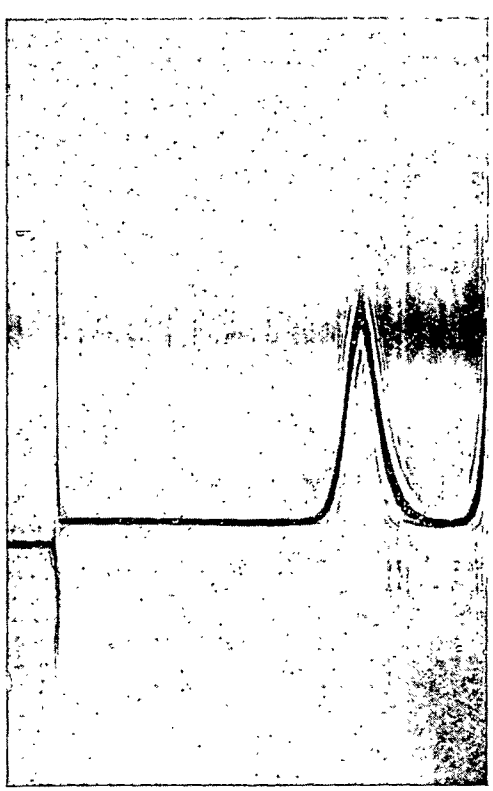


Fig. 2. Ultracentrifugal analysis of purified conalbumin in borate buffer. Protein concentration, 30 mg/ml.; solvent, 0·020 M H_3BO_3, 0·0084 M NaOH; *p*H 8·9; cell width, 3 mm; speed, 56,100 r.p.m.; time of photograph, 147 min; phase plate angle, 70° ; $s^{3\%}_{20^\circ,w} = 4{\cdot}55$

The eluted component also gave rise to faint, more rapidly migrating iron-binding components.

In a gel buffer of twice the usual concentration of boric acid and sodium hydroxide, and in buffers of the same boric acid concentration (0·020 M metaboric acid) but reduced sodium hydroxide concentration (0·0038 M, *p*H 8·57; 0·0010 M, *p*H 7·93), the proportions of the additional components were increased. However, the alterations induced in this fashion were less dramatic than those achieved by varying the conalbumin concentration. In all these experiments, the more concentrated borate buffer of Smithies[16] (0·30 M metaboric acid, 0·060 M sodium hydroxide; *p*H 8·2) was used in the electrode vessels. The three faint, more rapidly migrating components observed in the *tris*-citrate and phosphate–citrate buffers were also present in the borate buffers; no heterogeneity of these components could be detected in the boric acid system. In sedimentation velocity investigations in the ultracentrifuge (Fig. 2), a single slightly asymmetric peak was observed for purified conalbumin over the range of protein concentrations for which the boric acid effect was present in the starch gel.

The electrophoretic mobilities of the two prominent boric acid-induced components varied according to the concentration of the components. In an experiment in which egg white was diluted with isotonic saline in twelve steps to a final dilution of 1 : 8 (Fig. 3), the mobility of the slowest component gradually increased until it coincided with the intermediate component, the mobility of which also gradually increased until it coincided with the fast component. The mobility of the fast component remained constant throughout the experiment.

No definite explanation can be given for the effect of boric acid buffers on conalbumin. The concentration-dependent heterogeneity suggests the formation of complexes between boric acid and conalbumin which alter the electrophoretic mobility of the protein[19]. Boric acid complexes are well known in carbohydrate and nucleic acid chemistry[20], and a small carbohydrate moiety has been determined in conalbumin[4]. It is of interest that the postulated formation of the complex in the slowest migrating component appears to affect the capacity of the conalbumin molecule to bind iron. It is possible that the formation of the complex may also involve the carbohydrate of the gel supporting medium. The dilution and the elution experiments require that the formation of the complex be reversible, whether or not the starch gel is involved. The elution experiments also suggest that the faint, more rapidly migrating components observed in all buffer systems may represent an equilibrium with the principal conalbumin components. The sedimentation velocity experiments indicate that the effect cannot easily be attributed to aggregation of the protein. A similar phenomenon has not been observed for various concentrations of purified human transferrin or for transferrin in the serum of other animal species.

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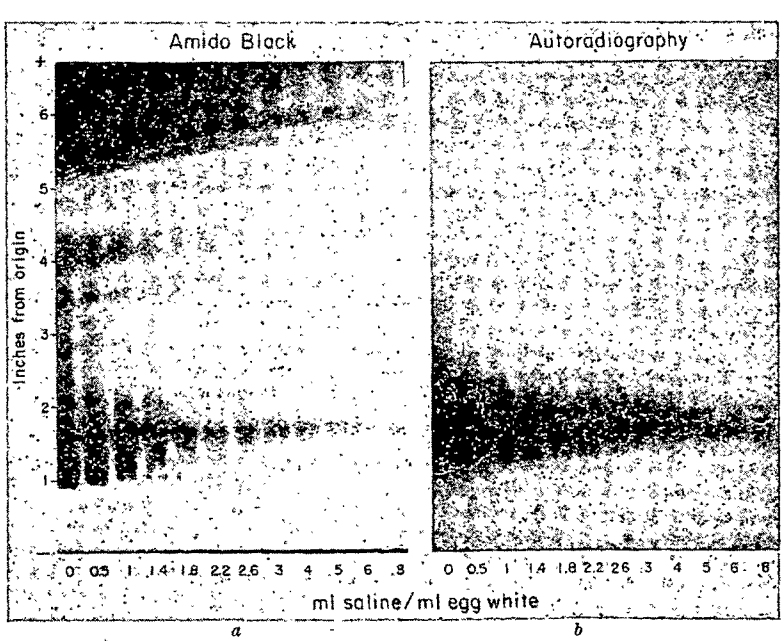


Fig. 3. Effect of dilution on electrophoretic pattern of conalbumin in egg white. Nos. (0–8) indicate ml. isotonic saline added to 1 ml. egg white prior to electrophoresis of each sample. (*a*) Amido black stain. (*b*) Autoradiograph from gel of Fig. 3*a*. The three faint iron-binding components in addition to the principal conalbumins are seen at 2, 2·25 and 3·25 in. from the origin, respectively

Reduction and Reoxidation of the Disulphide Bonds of Pepsinogen

Anfinsen and Haber have shown that the disulphide bonds of ribonuclease (RNase) can be cleaved by treatment of the native protein in 8 M urea with mercaptoethanol[1]. On exposure to molecular oxygen, reduced RNase is re-oxidized with complete regeneration of secondary and tertiary structure. The kinetics of the reaction indicate either that pairing of the correct half-cystine residues is a random process with the molecule undergoing subsequent structural rearrangement to yield the native form[2]; or, alternatively, that the reduced protein may be sufficiently structurally similar to the native enzyme to allow correct matching and reoxidation of the half-cystinyl residues, even in the absence of the disulphide bridges[3].

We have made similar observations with the enzyme precursor, pepsinogen. In a typical experiment, 40 mg Worthington chromatographically pure pepsinogen (lot 6021) was dissolved in 2·5 ml. 8·0 M urea, 0·1 M potassium chloride, 0·01 M *tris* buffer, *p*H 8·5. To this solution, 0·1 ml. of redistilled β-mercaptoethanol (Eastman Organic) was added, the vessel was flushed with nitrogen, stoppered, and the solution heated in a 37° water bath for 3 h. The reduced protein was chromatographically separated from the reducing medium at 4° on a *G*-25 medium grade 'Sephadex' (Pharmacia Company, Uppsala, Sweden) column, 1·9 × 45 cm, by elution with 0·1 M potassium chloride, 0·02 M dipotassium hydrogen phosphate–potassium dihydrogen phosphate

buffer, *p*H 5·9. The major portion of the protein was eluted in the first column volume, and all work reported in this communication pertaining to reduction was conducted on a pooled eluate of the two most concentrated fractions.

The degree of reduction of the protein, which contains 3 disulphide bonds[4], was determined by titration at *p*H 7·0 of 3·0 ml. of a sample, approximately 20 μg/ml., with either 0·8 or 0·4 mM *p*-mercuriphenylsulphonate[5]. The amount of reacted mercuriphenylsulphonate was determined spectrophotometrically by observing the change in absorption at 240 mμ. Aliquots of the solution were assayed for peptic activity after activation for 10 min at *p*H 2·0 by the hæmoglobin substrate method[6]. The percentage recovered activity was calculated by a comparison with a pepsinogen sample in the same buffer system. Re-oxidation was accomplished by allowing the solution to stand at room temperature for 20–24 h at *p*H 8·4. (The recovery of activity of reduced pepsinogen varies linearly with *p*H. For example, within the *p*H-range of 7·0–8·5, aliquots containing 9·2 μg protein/ml. recovered 44–52 per cent of theoretical activity). Fig. 1 shows the results of the changes observed with peptic activity assay and the mercurial titration.

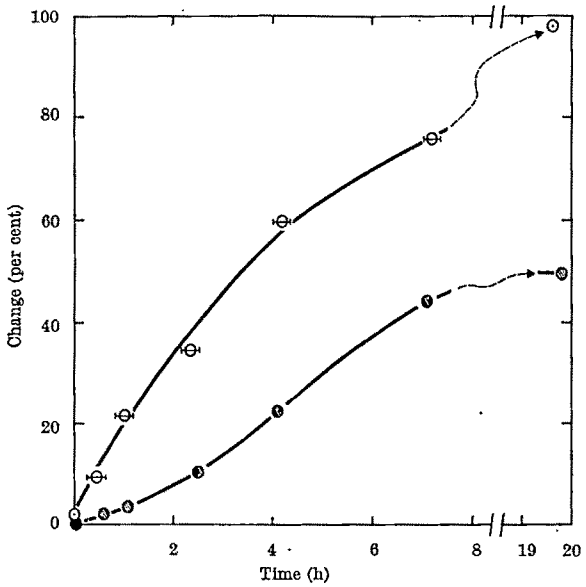


Fig. 1. Time-dependent changes in the SH groups and in the potential activity of reduced pepsinogen. Re-oxidation values are plotted as the midpoint of the time required for complete titration of a single sample. Zero time is that time when the *p*H value of the reduced protein solution was adjusted from 5·9 at 4° to 8·4° at room temperature. ○, Re-oxidation; ●, recovered activity

Three physico-chemical techniques, polarization of fluorescence[7], sedimentation, and viscosity, have been utilized for partially characterizing denatured pepsinogen and denatured-reduced pepsinogen as compared with the native protein.

Comparatively, RNase exhibits appreciable variation in ρ_h^{25} [2] between the native, denatured, and denatured-reduced states. ρ_h^{25} is defined as the mean relaxation time at 25° for a protein in a medium of known composition[7,8]. The respective values of ρ_h^{25} calculated for the foregoing states of pepsinogen as the 1-dimethylamino-naphthalene-5-sulphonyl conjugate are: $13{\cdot}0 \times 10^{-8}$, $1{\cdot}10 \times 10^{-8}$ and $0{\cdot}70 \times 10^{-8}$ sec (±20 per cent). Obviously, the protein undergoes a major loss of rigidity on denaturation; however, some secondary structure apparently persists after reduction in view of the finite value of ρ_h^{25}. Along with the 3 disulphide bonds, a phosphodiester linkage contributes to the secondary structure of the molecule, although the diester bond is not essential for potential enzymatic activity[9].

Changes in intrinsic viscosity also parallel those found with the polarization of fluorescence technique. Values of $[\eta]$ for the native and urea-denatured states of 3·7 and 28·7 ml./gm have been obtained at 27° using an Ubbelohde viscometer. The value for the reduced-denatured molecule is slightly higher than that cited for the denatured state. Urea-denatured pepsinogen used for the viscosity experiment here was dialysed for 24 h at 4° against 0·1 M potassium chloride, 0·02 M phosphate buffer, *p*H 7·0. When assayed by the hæmoglobin substrate method, practically all the theoretical activity was present.

Sedimentation diagrams of re-oxidized pepsinogen show a single boundary which has a sedimentation coefficient close to that of the native protein value, $3{\cdot}66 \times 10^{-13}$.

Both native and re-oxidized pepsinogen were subjected to starch-gel electrophoresis for 4 h at 4° using a 7·0 mM *tris*-citrate buffer, *p*H 8·4, with subsequent development of the gel slab by amido black dye. The former showed one well-defined zone with two minor components which migrated behind the major zone. Re-oxidized material gave the same major band with a broad ill-defined trailing region, the front of which moved at a rate intermediate to the two lesser components found in the native protein sample.

These experiments indicate that the tertiary and most of the secondary structure of pepsinogen can be disrupted without greatly affecting the ability of the polypeptide chain to recover some of its structure and, possibly as a consequence, some of its activity. At present, however, it would be premature to assert that the recovered active molecule is identical in all respects to native pepsinogen.

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Nitrogenous Components of Phospholipids extracted from *Penicillium chrysogenum*

Previous work carried out on the lipids of *Penicillium chrysogenum*[1,2] has implicated the phospholipids as important metabolic constituents of the mould. Subsequent analysis of these phospholipids on silicic acid impregnated paper indicated the presence of a number of compounds having R_F values similar to those obtained by Marinetti *et al.*[3] for phospholipids isolated from rat liver and heart tissue. Silberman and Gaby[4] have identified the majority of nitrogenous compounds present in the aqueous fraction of the acid hydrolysate of phospholipids extracted from the Gram-negative bacillus *Pseudomonas aeruginosa* by two-dimensional chromatography as choline, leucine, valine or methionine, ethanolamine, alanine, threonine, lysine, glycine, glutamic acid, serine, cysteine and aspartic acid.

In more recent investigations it was of interest to find that these nitrogenous compounds were also present in

Table 1. Nitrogenous Compounds present in the Phospholipid Hydrolysates of Silicic Acid Column Fractions identified by Paper Chromatography

Chloroform	Column fractions: Chloroform	Chloroform	Chloroform	
	80/20 Methanol	60/40 Methanol	20/80 Methanol	Methanol
		Alanine	Alanine	Alanine
	Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid
		Choline	Choline	
		Cysteine	Cysteine	
	Ethanolamine	Ethanolamine		
Glutamic acid	Glutamic acid	Glutamic acid	Glutamic acid	Glutamic acid
Glycine	Glycine	Glycine	Glycine	Glycine
		Leucine	Leucine	
		Lysine	Lysine	
Serine	Serine	Serine	Serine	Serine
		Threonine	Threonine	
	Valine and/or Methionine	Valine and/or Methionine	Valine and/or Methionine	Valine and/or Methionine

Table 2. Ninhydrin Positive Compounds present in the Aqueous Acid Hydrolysates of Phospholipids as determined by Analysis on the Technicon Autoanalyser

Nitrogenous compounds	μmoles/100 mg phospholipid	Percentage
Cysteic acid	*T*- 0·4	0·83
Methionine sulphoxide	*T*- 0·3	0·6
Aspartic acid*	1·4	2·9
Threonine*	*T*- 0·6	1·3
Serine*	5·0	10·0
Glutamic acid*	1·3	2·7
Glycine*	1·2	2·5
Alanine*	2·2	4·6
Glucosamine	*T*	—
Valine*	*T*- 0·8	1·7
Cysteine*	3·1	6·5
Methionine*	*T*	—
Isoleucine	0·6	1·3
Leucine*	1·0	2·1
Tyrosine	*T*	—
Phenylalanine	0·95	2·0
α-NH_2-*n*-butyric acid	*T*- 0·3	0·6
Ethanolamine*	12·7	26·4
NH_3	13·1	27·0
Ornithine	*T*	—
Lysine*	0·7	1·5
Histidine	0·9	1·9
Arginine	*T*- 2·0	4·2
? ?	*T*- 0·9	1·9

* Detected by 2-dimensional paper chromatography.
T, trace amount.

the acetone insoluble phospholipid fraction extracted from *Penicillium chrysogenum*, Ehrlich ascites cells and normal rabbit liver cells. The types and amounts of nitrogenous compounds present in the cellular phospholipids could be increased by the addition of specific amino-acids to the actively metabolizing cells[4]. While it is well known that amino-acids and other non-lipid compounds appear to follow phospholipids during fractionation procedures recent evidence would seem conclusively to establish the fact that these nitrogenous compounds are metabolic complexes and are firmly bound to the phospholipid or phospholipid-like compounds[4–9]. An attempt was made, therefore, to identify the nitrogenous compounds associated with the phospholipid fractions of the mould *P. chrysogenum* by analysing the aqueous acid hydrolysates of the acetone insoluble lipids with the aid of a Technicon autoanalyser.

The mould *P. chrysogenum*, strain 47-133*SL*5, was grown in the 3 per cent cornsteep, 3 per cent lactose, 1 per cent calcium carbonate medium previously described[1]. The lipid fraction was extracted from the washed filtered cells with a chloroform–methanol (50 : 50 v/v) mixture for 24 h at 100° in a Soxhlet extraction apparatus or by extracting the mould cells at room temperature (25°) on a rotary shaking machine for 48 h using the same chloroform–methanol solvent mixture. No significant differences in yield of phospholipids was observed when the two procedures were compared.

The chloroform–methanol extract was evaporated to dryness under vacuum and the residue extracted with petroleum ether. The petroleum ether soluble material was concentrated under partial vacuum to approximately 10 ml., and the phospholipids precipitated in 150 ml. acetone. The phospholipids were further purified by redissolving the precipitate in 10 ml. petroleum ether, washing the ether soluble phospholipids with several changes of distilled water and 0·9 per cent saline (100 ml. vol.) and reprecipitating in 150 ml. acetone. This procedure was repeated 3–5 times. The final wash contained no ninhydrin positive compounds as determined by paper chromatography. A 30–50 mg sample of the acetone insoluble lipid was hydrolysed by refluxing in 6 N hydrochloric acid for 24 h at 100°. A part of the aqueous hydrolysate (equivalent to 15 mg lipid) was placed on a 150-cm column of 'Amberlite *IR*-120' (average particle diameter of 56μ) and subjected to analysis by the automatic amino-acid analysis technique of Moore *et al.*[10]. Approximately, 50 mg of the acetone insoluble lipid in 5-ml. chloroform was placed on 10 × 455 mm columns containing 20 g silicic acid ('Unisil') and fractions eluted from the columns with 200-ml. amounts of each of the following solvents: chloroform, chloroform–methanol 80–20 v/v, 20–80 v/v, and absolute methanol in the order given. A total of 20 fractions were collected each containing 50 ml. Each fraction was concentrated under vacuum and 30 μl. or more (depending on concentration) were spotted on silicic acid impregnated paper, developed with diisobutyl ketone-acetic acid-water, and the rhodamine G, ninhydrin and choline positive areas determined[4]. The remaining portion of the fractions was hydrolysed by refluxing in 6 N hydrochloric acid for 24 h and the water-soluble nitrogenous compounds identified by two-dimensional paper chromatography.

Total recovery of the lipids eluted from silicic acid columns was 95–98 per cent. Approximately 45–55 per cent of the total weight was recovered in the 80/20 chloroform–methanol solvent fraction, 25–30 per cent recovered in the 60/40 and 10–15 per cent recovered in the 20/80 fractions. Most of the phospholipids appeared in the first 50-ml. fraction of each solvent mixture collected from the column. If the resting mould cells were incubated with an amino-acid labelled with carbon-14, that is, DL-lysine-1-^{14}C, prior to lipid extraction, the peak of specific radioactivity was eluted from the column in the first 50 ml. of the 60/40 chloroform–methanol solvent mixture. If the chloroform–methanol mixtures passed through the column were varied by 10 per cent increments, that is, 90/10, 80/20, etc., the peak of specific radioactivity appeared in the 70/30 fraction.

Each of the solvent fractions, following acid hydrolysis, was chromatographed in two dimensions on Whatman No. 1 filter paper at room temperature (25°) by the ascending technique using *n*-butanol–acetic acid–water (50 : 12 : 50 v/v/v) as the first solvent and phenol–water (80 : 20 v/v) as the second solvent. Known compounds were run separately and mixed with the hydrolysates to identify unknown spots. Identification was confirmed by repeated chromatograms substituting pyridine–acetic acid–water (50 : 35 : 15 v/v/v) for one of the original solvent mixtures. Table 1 lists the water-soluble nitrogenous compounds identified in the first 50-ml. fraction of each solvent or solvent mixture. Serine, glycine and glutamic acid were present in all of the fractions, including the chloroform fraction. Ethanolamine was the major compound present in the 80/20 fraction. Choline was detected in both the 60/40 and 20/80 chloroform–methanol solvent fractions. It is of interest to note that while the bulk of material was eluted in the 80/20 fraction the greatest number of nitrogenous compounds were eluted in the more polar solvent fractions. While it was possible to identify as many as 12 ninhydrin-positive compounds by paper chromatography it is obvious from the results presented in Table 2 that a more sensitive procedure, such as that described by Moore *et al.*[10], is required to demonstrate compounds present in minute quantities. The figures presented in Table 2 represent the averages of analysis of phospholipids obtained from three different cell preparations. The concentrations (μM) of most of the compounds varied with different cell preparations, that is, arginine varied from trace amounts to 2·0 M per 100 mg phospholipid. The percentage of each compound, however, remained remarkably constant. Duplicate analysis of each hydrolysate gave essentially identical results.

Macfarlane's[9] recent description of an acetone insoluble *O*-alanyl ester of phosphatidyl glycerol isolated from the Gram-positive bacillus *Cl. welchii* emphasizes the complexity of these compounds and supports the hypothesis that these complexes are important metabolic constituents of the cell.

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Peroxidase Isozymes in Root Nodules of Various Leguminous Plants

Multiple forms of various enzymes (isozymes) have been shown in tissues of animals[1–3] and plants[4,5] and in micro-organisms[6]. The difference in electrophoretic mobility of some isozymes obtained from different species has been reported[4,5,7]. This communication reports the existence of peroxidase isozymes which differ electrophoretically in root nodules of each of four leguminous plants. The leghæmoglobins extracted from these different nodules also differ electrophoretically.

Plants of *Galega officinalis*, *Lotus pedunculatus*, *Trifolium repens* and *Vicia sativa* were grown from seed in sterilized sand which had been inoculated with the appropriate *Rhizobia*. Nodules were collected six weeks after planting and were kept at 3° until extracted within 3 h of collecting.

Extracts were prepared by grinding 8 g of nodules in a chilled mortar with 8 ml. of 0·05 M *tris* hydrochloric acid buffer at *p*H 7·5. This method of extraction does not disrupt the *Rhizobia* which inhabit the nodules[8]. The slurry was centrifuged at 20,000*g* for 20 min and the supernatant was fractionated with ammonium sulphate at 3°. The protein precipitated between 55 and 80 per cent saturation ammonium sulphate was dissolved in 3 ml. of 0·05 M *tris* hydrochloric acid buffer at *p*H 7·5 and dialysed against 200 ml. of the same buffer at 3° for 18 h.

Starch-gel electrophoresis was carried out as described by Smithies[9] in 0·05 M *tris*-citrate buffer *p*H 8·6. After completion of the run the gel was sliced horizontally in two. One half was stained by amido black to reveal protein bands and the other half was stained to reveal the peroxidase bands by immersing it in a solution containing 0·025 per cent benzidine, 1 per cent hydrogen peroxide and 5 per cent acetic acid in 50 per cent aqueous ethanol. The parts of the starch gel containing peroxidases showed a blue coloration. Leghæmoglobins, which could be identified as brown bands prior to staining, gave the peroxidase reaction when stained with the benzidine reagent.

In both Figs. 1 and 2 leghæmoglobins are marked by the letters *A* and *B*. It will be seen that the two leghæmoglobins in the nodules of the four plants vary in electrophoretic mobility. The slowest moving bands are those of *Trifolium repens* and *Galega officinalis* followed by those of *Vicia sativa* and *Lotus pedunculatus*. The

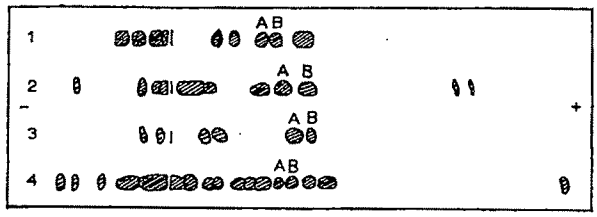


Fig. 1. Electrophoretic pattern of peroxidases in root nodule extracts. 1, *Trifolium repens*; 2, *Vicia sativa*; 3, *Lotus pedunculatus*; 4, *Galega officinalis*

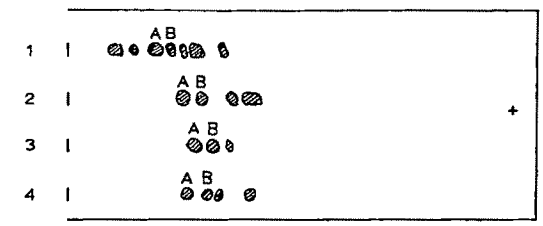


Fig. 2. Electrophoretic pattern of proteins in root nodule extracts. 1, *Trifolium repens*; 2, *Vicia sativa*; 3, *Lotus pedunculatus*; 4, *Galega officinalis*

difference in electrophoretic mobilities between leghæmoglobins of root nodules of different plant genera has a counterpart in animal kingdom, where there are differences between electrophoretic mobilities of hæmoglobins of various species of vertebrates[10].

The zymograms of peroxidases in root nodules (Fig. 1) show a characteristic pattern for each of the plants used in this work. The most outstanding differences between the peroxidase patterns are the fast sharp bands moving towards the anode in nodule extracts of both *Galega officinalis* and *Vicia sativa* which are not present in extracts of nodules of the other two plants. Although most of the peroxidase bands showed intense enzymatic reaction they did not contain enough protein to stain with amido black. In the gels that were stained with amido black no bands were observed on the cathode side of the origin (Fig. 2).

The specific role of each of the peroxidase isozymes in root nodules and the biological significance of the difference in the peroxidase pattern in nodules of different leguminous plants remain to be established.

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PHYSIOLOGY

Storage of Insecticides in French People

Storage of DDT in people without occupational exposure to the compound reflects their dietary intake[1]. Measurement of storage in a random sample of the population of the country may be used as an index of residues of other pesticides as well as those of DDT in the total food supply. We were fortunate in obtaining samples of adipose tissue from ten French people. Even though the number is small, the results are of interest because they are the first from France and because they permit a comparison with results from other countries.

Table 1. Storage of DDT in French People

Case No.	Sex	Age	Occupation	Cause of death	DDT		DDE		Total* tissue (p.p.m.)	Proportion of DDE as DDT (%)
					Tissue (p.p.m.)	Extract (p.p.m.)	Tissue (p.p.m.)	Extract (p.p.m.)		
1	F	82	None	Accident	*el*	*el*	*el*	*el*	*el*	
2	M	36	Clerk	Accident	2·1	3·3	3·2	5·1	5·7	62·4
3	M	40	Mechanic	Accident	1·1	1·7	2·4	3·5	3·8	70·3
4	M	35	Beggar	Meningeal hæmorrhage	3·7	4·6	6·9	8·7	11·4	67·4
5	F	26	None	?	*el*	*el*	*el*	*el*	*el*	
6	M	52	Restaurant operator	Stroke	3·1	3·9	5·1	6·4	8·8	64·5
7	M	41	Manufacturer	Accident	4·0	5·0	11·1	13·8	16·4	75·4
8	M	54	Carpenter	Stroke	2·7	3·4	3·0	3·7	6·0	55·6
9	M	16	Mechanic helper	Drowned	*el*	*el*	*el*	*el*	*el*	
10	M	47	Electrician	Accident	*el*	*el*	*el*	*el*	*el*	
Mean of positives					2·8	3·7	5·3	6·9	8·7	65·9
Mean of ten					1·7	2·2	3·2	4·1	5·2	

* DDT plus DDE expressed as DDT. *el*, Below the sensitivity of the analytical method.

Table 2. Concentration of DDT-derived Material in the Body Fat of People of Different Countries

Country	Exposure status	Year	No. of samples	DDT (p.p.m.)	DDE as DDT (p.p.m.)	Total as DDT (p.p.m.)	DDE as DDT (%)	Ref.
U.S.A.	General population	<1942	10	0	0	0	—	1
U.S.A.	General population	1950	75	5·3	—	5·3*	—	5
U.S.A.	General population	1955	49	7·4	12·5	19·9	62·8	6*
U.S.A.	General population	1954–56	61	4·9	6·8	11·7	58·1	1
U.S.A.	General population	1961–62	117	3·9	9·0	12·9	69·8	7
U.S.A.	Meat abstainers	1955–56	16	2·3	3·6	5·9	61·0	1
U.S.A.	Eskimos (Alaska)	1960	20	0·8	2·2	3·0	73·3	8
U.S.A.	Residents near orchards	1954–56	110	6·0	9·6	15·6	61·5	1
U.S.A.	Agricultural applicators	1954–56	30	14·0	21·1	35·1	60·1	1
U.S.A.	Formulator	1951	1	122	141	263	53·6	2
U.S.A.	Formulator	1954	1	648	483	1,131	42·7	6
Canada	General population	1959–60	62	1·6	3·3	4·9	67·0	9
Germany	General population	1958–59	60	1·0	1·3	2·3	56·5	10
England	General population	1961–62	131	—	—	2·2	—	11
France	General population	1961	10	1·7	3·5	5·2	67·3	This comm.

* Values based on original data.

The adipose tissue was taken at autopsy from people of widely different ages and occupations, but without occupational exposure to DDT. The causes of death are listed in Table 1, but in no instance was DDT involved. The tissue was weighed before being placed in 10 per cent formaldehyde (4 per cent formalin solution) for preservation during shipment. Earlier investigation has shown that this method of preservation is satisfactory for such samples[1].

Suitable portions of the samples were ground in a mortar with sodium sulphate. The ground tissue was then transferred to 500 ml. glass-stoppered bottles with the aid of carbon tetrachloride. Approximately 200 ml. of carbon tetrachloride were added to each bottle and the bottles were then mechanically shaken for 2 h. The samples were then filtered and the filtrate analysed for DDT and DDE by the Schechter–Haller method as modified by Mattson *et al.*[2]. In view of the weight of the available samples, the smallest concentration of DDT which could be detected by this method was 0·8 p.p.m.

Five of the samples—Nos. 2, 4, 6, 7 and 8—gave Schechter–Haller positive results and were large enough to permit further examination using gas chromatography. The remaining portion of each sample was extracted as described and the extract passed through a Davidow[3] column as a clean-up procedure. The carbon tetrachloride eluates were evaporated to complete dryness in test tubes in a 75° C water bath with the aid of a gentle stream of air. The tubes were then rinsed down several times with *n*-hexane and the hexane evaporated off after each rinsing to rid the samples of carbon tetrachloride. The residues were finally taken up in *n*-hexane and 100 μl. (equivalent to about 3·5 g of fat) were subjected to microcoulometric gas chromatography described by Coulson *et al.*[4]. An aluminium tube (6 ft. × 0·25 in. outer diam.) packed with 30/60 mesh acid-washed 'Chromosorb-*B*' containing 20 per cent by weight of high vacuum silicone grease was used as the partitioning column. The column temperature was maintained at 215° C and the nitrogen carrier gas was maintained at 60 c.c./min. The effluent gases were oxidatively decomposed at 800° C and titrated coulometrically. Use of the Davidow column was necessary in a search for possible metabolites of DDT about which there is current interest. Unfortunately, dieldrin cannot be eluted from this column, so any dieldrin that may have been present could not be measured and the small size of the original samples precluded other investigations.

Results obtained by the colorimetric method are shown in Table 1. It may be seen that no DDT-related material was detected in 4 of the samples. The highest concentration of DDT found in the tissue of any person was 4·0 p.p.m. and the highest concentration of DDT plus DDE expressed as DDT-equivalent was 16·4 p.p.m. The average concentration of DDT in all samples was 1·7 p.p.m. and the average concentration for DDT plus DDE as DDT-equivalent was 5·2 p.p.m. Of course, the averages were higher if only the positive values were considered.

In samples showing detectable DDT, DDE constituted 56–75 per cent, or an average of 66 per cent of the total. This proportion is similar to that found in other studies of general populations[1].

Results obtained by gas chromatography are shown in Fig. 1. Curve *A* represents standard lindane, aldrin, and the *p,p'*-isomers of DDE, DDD, and DDT appearing in that order. Curve *B*, from sample No. 8, is representative of the various peaks found in the fat of the general population of France. The first peak appeared in all French fat and is indicative of lindane. Calculated as lindane the concentration ranged from 0·66 to 1·64 p.p.m. with an average of 1·19 p.p.m. The second peak (2*b*) appeared in 4 of the 5 fat samples examined; it represents an unknown compound distinct from aldrin (2*a*). Since the unknown compound passed through a Davidow column, it would appear to be a neutral material stable to strong acids. The third peak appeared in all French fats subjected to gas chromatography and is undoubtedly *p,p'*-DDE. The DDE concentration ranged from 4·2 to 7·1 p.p.m. with an average value of 4·8 p.p.m. This value was 19 per cent

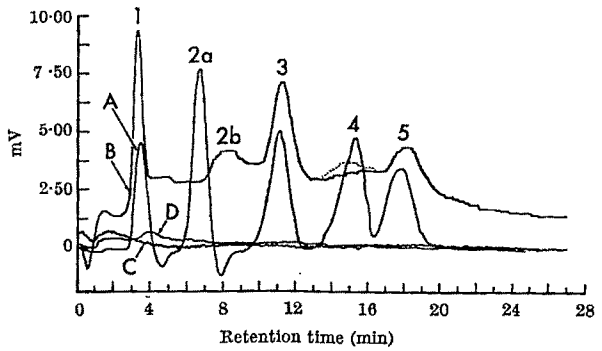


Fig. 1. Chromatograms resulting from gas-liquid separation and microcoulometric analysis of: (*A*) reference standards of (1) lindane, (2*a*) aldrin, (3) *p,p'*-DDE, (4) *p,p'*-DDD, and (5) *p,p'*-DDT; (*B*) sample No. 8 of fat from French people; (*C*) sample of fat from an American who died before 1942; and (*D*) Davidow column blank

lower than the average obtained for these same samples by the Schechter–Haller method. This difference may depend in part on the fact that these values were obtained from separate portions of the original samples. The fourth peak did not appear in the sample represented in Fig. 1, but its form is shown by a dotted insert. The fourth peak did appear in three of the fats and could be either *o,p'*-DDT or *p,p'*-DDD, or both, since standards of these two materials could not be resolved. The presence of *o,p'*-DDT in the fat would be expected, since the general population was most likely exposed to the technical form of DDT, which contains 20–25 per cent of the *o,p'*-DDT isomer. There is also the possibility that they could have been exposed to small amounts of *p,p'*-DDD and that it was stored as such in the fat. Another possibility, as will be reported elsewhere, is that *p,p'*-DDD could have been present as a metabolite of *p,p'*-DDT. The fifth peak appearing in fat was *p,p'*-DDT and was present in all samples subjected to gas chromatography. The DDT concentrations ranged from 2·3 to 5·0 p.p.m. with an average of 5·5 p.p.m., which was only 6 per cent higher than the average values obtained by the Schechter–Haller method for other portions of these same samples.

One human fat sample from the United States predating the introduction of DDT or lindane was also subjected to gas chromatography and appears as curve *C* in Fig. 1. No peaks were obtained with this fat; therefore, the compounds producing peaks in the fat of the general population were not natural constituents of fat but rather were present as a result of exposure to halogenated compounds. A Davidow column blank represented by curve *D* gave no peaks.

Table 2 permits a comparison of the results recorded here with the results of studies in other countries. Storage-levels for DDT in the general population are lower in Europe than in North America. This difference is almost certainly related to the amount of DDT used in agriculture in the different countries. It is recognized that any given crop planted in relatively small areas among other crops, in the manner generally used in Europe, is less susceptible to insect attack than is the same crop planted in extensive areas, as is frequently done in North America. Also, it seems likely that the climate of northern Europe is less conducive to insects than the climate of much of the United States.

Even though 4 of the samples contained no detectable DDT, the average concentration in the French samples was higher than that reported for West Germany or England. The results for the French samples are somewhat like those for United States samples in 1950 (ref. 5), although the range of values is smaller. It appears possible that the use of DDT in France is localized (either to certain crops or areas) in such a way that some people receive the compound in their diet while others get little or none.

In no country yet investigated do people without occupational exposure absorb as much DDT as do people who apply or formulate the compound.

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Formation of Extra Nerve-muscle Junctions in Innervated Muscle

If a foreign motor nerve is connected to a normally innervated muscle, the nerve fibres grow into it but do not establish intimate contact with muscle fibres, and are unable to transmit impulses to them. On the other hand, if the muscle fibres are deprived of their normal innervation a foreign nerve will establish functional connexions with them[1–3].

Since any part of a nerve-free muscle fibre is capable of being innervated[3–5] the observations indicate that established nerve endings exert an inhibitory influence which ronders the whole muscle fibre incapable of accepting further innervation. As Harrison[6] pointed out in 1910, there is a certain analogy between a muscle fibre and an egg cell which once it is fertilized does not accept further spermatozoa.

In the experiments recorded here the tibial nerve of the frog was dissected in the leg and reflected up to the knee. It was then attached to the sartorius muscle, leaving its existing innervation intact. The site of attachment was very near the tibial end of the muscle fibres: that is, several mm away from the nearest myoneural junctions.

It was found that connecting the nerve to a muscle, the tibial end of which has been cut at the same time, leads to formation of accessory neuromuscular junctions in this region of the muscle fibres. A few months after the operation most muscle fibres have become innervated by the foreign nerve, and many of them respond with a propagated action potential and contraction to stimulation of the nerve.

For example, Fig. 1*A* shows an intracellular action potential evoked by stimulation of the tibial nerve, some 2 months after connecting it to a cut sartorius muscle; Fig. 1*B* illustrates the action potential elicited in the same muscle fibre through stimulation of the original sartorius nerve. The larger stimulus-response delay in *A* is due partly to propagation time in the muscle fibre itself and partly to slower conduction in the regenerated nerve fibres[7].

Spontaneous sub-threshold potentials similar to those which occur at normal neuromuscular junctions[8] could be recorded from the muscle fibre if the microelectrode were inserted near the site of nerve implant. Furthermore, these regions also develop the accumulation of cholinesterase characteristic of normal end-plates.

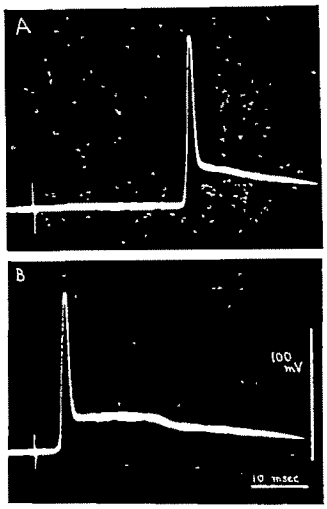


Fig. 1. Induced extra innervation in a frog's sartorius muscle fibre, 63 days after transection of the muscle and implantation of a foreign nerve (tibial) near its lower end. *A*, Intracellular action potential evoked by stimulation of the tibial nerve. *B*, action potential, recorded in the same place of the muscle fibre, after stimulation of the sartorius nerve. The recording electrode was near an original end-plate. Resting potential, 92 mV

Thus the 'barrier' which causes muscle fibres to reject additional innervation can be overcome experimentally by local injury to the muscle fibres. Under these conditions, muscle fibres will accept innervation by other motor nerves even in the presence of the original functioning myoneural synapses. It is of interest that local injury of muscle fibres has previously been found to overcome the inhibitory neural influence which reduces acetylcholine sensitivity along most of the muscle fibre[9]. It is possible that a similar procedure may help to establish experimentally new interneuronal connexions.

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In vitro Effect of Lowering Surface Energy on Esterase Activity of *Musca* Homogenates

The primary reaction between an enzyme and a substrate or a poison is essentially an adsorption reaction involving the concentration of the substance in the enzyme/water interface. Homogenates are bio-colloids where the surface energy, particularly at interfaces, is a predominating form of energy on changes of which depend certain phenomena. In aqueous preparations of enzymes, substances which lower surface energy by concentrating at the enzyme/water interface may behave as barrier between enzyme and substrate, unless the latter has a higher affinity for interfaces.

Some surface active agents have been tested for their effect on the activity of cholinesterase from *Musca* heads, using acetylcholine as substrate at the optimum concentration of $1{\cdot}5 \times 10^{-2}$ M at 37° C. Sodium cholate caused full inhibition at a concentration of 0·2 M. Sodium lauryl sulphate was extremely potent and inhibited enzyme activity completely at $2{\cdot}5 \times 10^{-4}$ M. No practical method is known by which tension at the enzyme/liquid interface can be determined, and measurements of surface tension of solutions containing these compounds at various concentrations only served to show the relation between levels of inhibition and changes in surface tension. At the foregoing concentrations, surface tension values were 49·5 and 32·5 respectively, as compared with that of water of 70·4 dynes/cm at 37° C. With each compound, the level of inhibition was greater at the concentration which gave lower surface tension value. Similar results were obtained with the aliesterase from the thorax using, in addition, sodium oleate, which gave full inhibition at 5×10^{-3} M.

N-butanol is known to activate acetyl-cholinesterase of *Musca*, possibly through activating a pro-enzyme[1]. On the other hand, it makes homogenates resistant to some degree to OP insecticides. Iso-OMPA and TEPP inhibit esterase activity at $0{\cdot}4 \times 10^{-4}$ and 10^{-3} M respectively. However, at 0·9, 1·8, 2·7, 3·6 and 4·5 per cent butanol, the levels of inhibition by these compounds are 100, 100, 73, 37 and zero per cent for iso-OMPA and 100, 100, 100, 73 and 45 per cent for TEPP. Residue analysis showed that the amount of iso-OMPA which formed salt with proteins was less in butanol-treated than in control homogenates. These results again may be explained if we take into account the effect of butanol on lowering the surface tension of water. Surface tension values at the above concentrations of the alcohol were 60, 52, 46·5, 36·5 and 29·6 dynes/cm respectively, and it follows from Gibbs's equation $C_2 = -\frac{C}{RT}\frac{d\gamma}{dC}$ that adsorption is greatest from solvents which have a high surface tension due to the fact that a greater change $d\gamma/dC$ can be brought about in a dispersions medium having a high surface tension than from one having a low surface tension value.

The toxicity of OP compounds and possibly others must be dependent in the first place on a very complex set of physico-chemical factors which determine the rate of uptake of these compounds by colloidal particles from the dispersions medium of the cytoplasm. In addition to the nature and concentration of the insecticide,' surface energy changes may be one such factor. A second factor is the degree of hydration of protein micelles. Loss of the liquid of solvation in presence of large amounts of electrolytes may, in some cases, facilitate a chemical reaction between enzyme and insecticide. For example, enzyme incubated with iso-OMPA in presence of 1 M NaCl was fully inhibited at half the dose necessary to bring about full inhibition when incubation was made in distilled water. Similar results were obtained when homogenates were flocculated with ammonium sulphate at 25 per cent saturation.

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Quantitative Aspects of Pinocytosis in Relation to Intestinal Absorption

One hypothesis to account for the way in which fat is taken up by the cells of the intestinal mucosa of mammals is that the cells engulf droplets of fat by the process of pinocytosis[1-4]. The quantitative aspects of the phenomenon of pinocytosis are of interest for the reason that the size of the vesicles that have been described in this connexion are so small that in order to engulf physiological quantities of fat the number required and the rate at which they are formed must be correspondingly large. In Table 1 are given the numbers of spherical vesicles of differing radii, of the order of size described for pinocytotic vesicles in rat intestine, that are required to be formed to engulf a volume of 1 ml. The total surface of the vesicles is also shown.

An albino laboratory rat of about 300 g body-weight will consume some 0·5–1·0 g fat per diem. Working on the basis that the absorption occurs over a period of 10 h in the upper third of the small intestine, the rate of formation of pinocytotic vesicles necessary to achieve the total absorption of the dietary fat in particulate form may be calculated. From the results of Fisher and Parsons[5], the surface area of the mucosa of the upper third of the small intestine of a 300-g rat is 150 cm^2. This figure refers to the area presented by the mucosal cells to the intestinal lumen and does not take into account any effects produced by the presence of the microvilli. If the projected area of the mucosal face of an intestinal epithelial cell of the rat is 15μ^2, the total number of absorbing cells in the upper third of the small intestine of the rat is thus 10^9. These data are used to show in Table 1 the total number of vesicles required to be formed by each intestinal mucosal cell for a volume of 1 ml. to be taken up by the absorbing surface. The rate of formation of vesicles, $cell^{-1}$ sec^{-1}, which is necessary for a total volume of 1 ml. to be engulfed is also shown. Measurements which are given by Palay and Karlin[1] indicate that the diameter of the vesicles found in the region immediately below the microvilli of the mucosal cells of the fat-absorbing rat intestine is of the order of 60 mμ. It then follows that the rate of pinocytosis necessary for 1 ml. of dietary fat to be absorbed over a period of 10 h is 250 $cell^{-1}$ sec^{-1}. Each intestinal mucosal cell appears to possess about 500 microvilli, so that with vesicles 60 mμ in diameter this rate of vesiculation is equivalent to the formation of one pinocytotic vesicle in the bordering membrane of each intermicrovillus space every 2 sec throughout the 10-h absorption period.

Table 1

Radius of vesicle (mμ)	No. of vesicles to contain 1 ml.	Vesicular surface per ml. content (cm^2)	For absorbing surface containing 10^9 cells, and for 1 ml., to be absorbed over 10 h: Total No. of vesicles to be formed by each cell	For absorbing surface containing 10^9 cells, and for 1 ml., to be absorbed over 10 h: No. of vesicles to be formed per cell per sec
5	$1·8 \times 10^{18}$	$6·0 \times 10^6$	$1·8 \times 10^9$	50,000
10	$2·4 \times 10^{17}$	$3·0 \times 10^6$	$2·4 \times 10^8$	6,700
25	$1·5 \times 10^{16}$	$1·2 \times 10^6$	$1·5 \times 10^7$	420
30	$9·1 \times 10^{15}$	$1·8 \times 10^6$	$9·1 \times 10^6$	250
50	$1·8 \times 10^{15}$	$6·0 \times 10^5$	$1·8 \times 10^6$	50
100	$2·4 \times 10^{14}$	$3·0 \times 10^5$	$2·4 \times 10^5$	7
200	$2·9 \times 10^{13}$	$1·5 \times 10^5$	$2·9 \times 10^4$	0·8

For human intestine, with 100 ml. fat absorbed over a 10-h period by the upper third of the small intestine, a similar calculation based on an estimated absorbing surface of $3·3 \times 10^4$ cm^2 (see, for example, Wilson[4]) and therefore containing 2×10^{11} absorbing cells yields an estimate of $4·5 \times 10^6$ vesicles of 60-mμ diameter to be formed per cell during the absorption period. This is equivalent to the formation of 150 $cell^{-1}$ sec^{-1} throughout the 10-h absorption period, a rate of vesiculation which is very similar to that calculated for the rat.

It is to be noticed that, for a diameter of 60 mμ, the projected area of one vesicle is 3×10^{-11} cm^2. Now with the projected area of the mucosal face of an absorbing cell as 15×10^{-8} cm^2, the area of this face which is occupied by the feet of 500 microvilli of 0·1μ diameter is $3·75 \times 10^{-8}$ cm^2, so that the remaining area which would be available for the formation of vesicles is about 10^{-7} cm^2. This area is equal to the projected area of more than 3,000 vesicles of 60-mμ diameter. In other words, there appears to be sufficient space in the cell membrane around the bases of the microvilli to accommodate the simultaneous formation of more than 3,000 pinocytotic vesicles of diameter 60 mμ.

These calculations will be in error in so much as the estimates of the parameters of the absorbing mucosal surface may be in error. The calculations are also based on the assumption that the dietary fat is converted with only trivial changes in volume by the processes of digestion into a form which is subjected to pinocytosis. If, in fact, only a fraction of the dietary fat is assimilated by pinocytosis by the mucosal cells, the rest being absorbed in some other form, then the estimates given in Table 1 will be too high. On the other hand, the calculations take no account of the presence of goblet cells in the intestinal mucosal cellular population. If these cells do not assimilate fat by pinocytosis, then the estimates given in Table 1 are somewhat too low.

Higher rates of vesiculation are required if pinocytosis is to represent a major channel for the reabsorption of other substances, for example, the digestive secretions. Thus in the case of an adult human subject, with the digestive secretions totalling 7 litres per day[6], the reabsorption of this volume of fluid entirely by pinocytotic vesiculation undertaken by all the cells of the whole small intestine would require a rate of vesicle formation of the order of 1,000 $cell^{-1}$ sec^{-1} for the whole 24 h, the vesicles having a mean diameter of 60 mμ.

Since the work done in forming a new area of surface in opposition to the interfacial tension is T ergs cm^{-2}, where T is the interfacial tension in dynes cm^{-1}, the work that has to be done by the cells in forming the surface of the vesicles appears to be very small. The data for the vesicular surface, which are given in Table 1, are numerically equal to the work done in ergs in forming the surface of vesicles of the dimensions given and with an interfacial tension of 1 dyne cm^{-1}.

Thus the absorption of 100 ml. fluid by pinocytotic vesicles of 30 mμ radius would require the expenditure of about 2 g calorie per dyne cm^{-1} of interfacial tension in the wall of the vesicles. However, the existence of an interfacial tension of 1 dyne cm^{-1} in the wall of a pinocytotic vesicle of radius 30 mμ requires that, in order to balance the force due to the tension, the intravesicular pressure should exceed the external pressure by about 200 mm mercury. On the other hand, if the pressure differential existing across the walls of vesicles of that size is as low as, say, 2 mm mercury, the interfacial tension must be 1/100 dyne cm^{-1}, and the work done in forming the new area of surface contained in the vesicles will be correspondingly smaller.

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Effect of Different Vasomotor Agents on the Visco-elastic Behaviour of Arteries of the Muscular Type

It has been assumed that two different contractile systems are present in the vascular muscle: the phasic system for transient regulatory tension and the tonic system for continuous tension. The tension of the latter seems to depend on the concentration of potassium in the muscular cells[1]. This opinion has been confirmed during physiological and biochemical investigations[2–4].

The work recorded here presents further evidence in favour of this theory by the observation of the visco-elastic behaviour of blood vessels of the muscular type. The method of quick-releases has been used[5].

Rectangular strips of the carotid from a cow, immersed in Tyrode's solution at body-temperature, are slowly and progressively stretched perpendicularly to the anatomic axis of the vessel. When a chosen tension has been reached stimulation is caused, in isometric conditions, by different vasomotor agents (potassium chloride 75–95 mM; cæsium

chloride 25–30 mM; barium chloride 5 mM; adrenaline 0·5 γ/c.c.; histamine 15–20 γ/c.c.; hypertensin 0·1 γ/c.c.; electricity 14 V, 50 cycles/sec). At maximal tension, the artery strips are allowed to shorten suddenly to various degrees (Δl = 0·75–5 per cent of the initial length). The variations of tension (ΔT) are recorded by means of an electronic transducer R.C.A. 5734 (ref. 6).

The modulus of elasticity (Young's modulus) is defined by the equation (1):

$$E = \frac{\Delta T}{\Delta l} \times \frac{\text{length}}{\text{transverse section}} \qquad (1)$$

The results of our experiments may be summarized as follows:

(1) The modulus of elasticity is not constant but rises parallel to tension (Figs. 1*A* and *B*). The relation observed between these two variables suggests an equation of the following type[7]:

$$P_2 = P_1 \times e^{-\Delta l/\lambda}$$

where P_2 is the tension after quick release; P_1 is the initial tension: λ a constant of length.

Accordingly, when Δl is plotted against log P_1/P_2, a straight line is obtained (Figs. 2 and 3).

(2) The different vasomotor agents may be grouped into two different categories:

(*a*) The first one contains potassium, cæsium and barium chlorides and histamine. The variations of the modulus of elasticity during the contraction produced by these substances are exactly equal to those measured during the rise of tension obtained by a passive elongation of the artery at rest (Figs. 1*A* and 2).

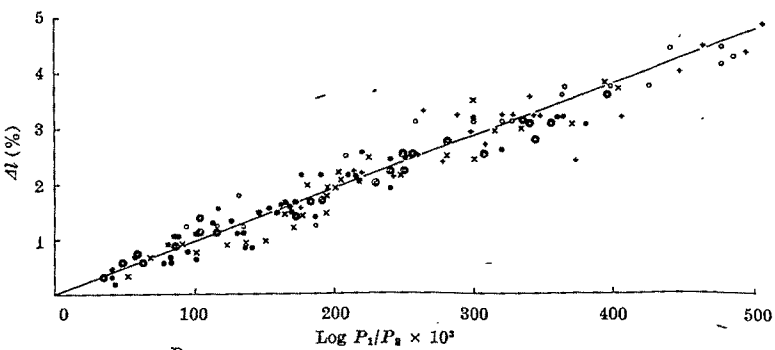


Fig. 2. Log $\frac{P_1}{P_2} \times 10^3$ versus the variations of length, Δl, expressed as a percentage of the initial length; arteries excited by potassium chloride (+ and ×), cæsium chloride (●), barium chloride (○) and histamine (⊙). Artery at rest, *. λ = 4·15 cm per cent

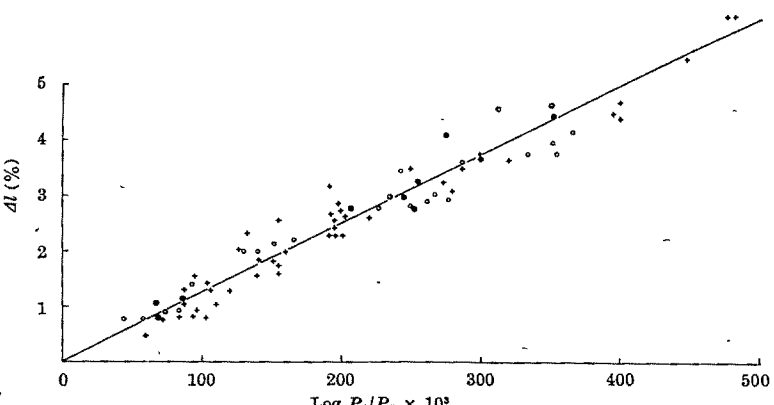


Fig. 3. Log $\frac{P_1}{P_2} \times 10^3$ versus the variations of length, Δl, expressed as a percentage of the initial length; arteries excited by adrenaline (●), electricity (○) and hypertensin (+). λ = 5·80 cm per cent

(*b*) Electricity, adrenaline and hypertensin form the second group. The contraction by these agents is accompanied by variations of the modulus of elasticity which are smaller than those observed when the substances of the first group are used (Figs. 1*B* and 3). Thus, the artery contracted by adrenaline seems to be more extensible than an artery stimulated by potassium chloride.

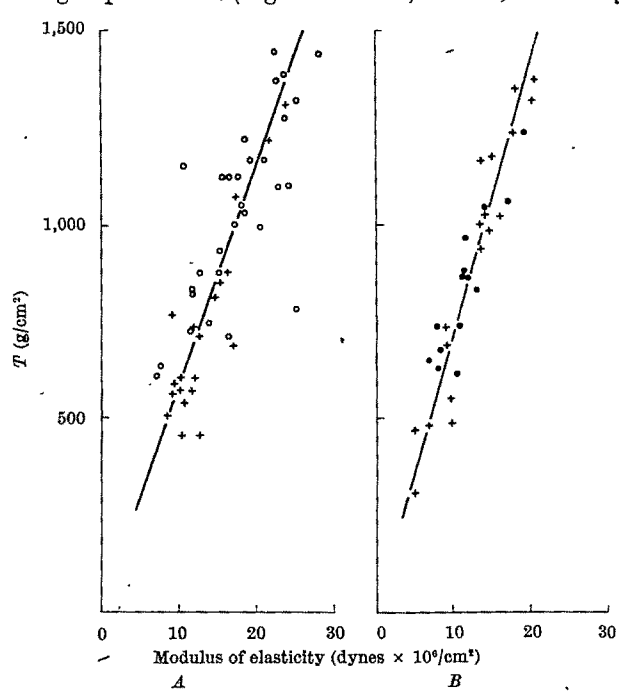


Fig. 1. Plots of Young's modulus against tension. *A*, Artery at rest (+) or excited by potassium chloride (○); *B*, artery excited by adrenaline (●) or electricity (+)

New investigations using other experimental methods give a superposable classification of these vasomotor agents. These results show that each group of substances act on a different contractile system and confirm Laszt's hypothesis[8].

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PHARMACOLOGY

Effects of Lysergic Acid Diethylamide on the Total Leukocytes and Eosinophils of the Female Rat

The hallucinogenic drug, lysergic acid diethylamide (LSD-25), produces in minute doses marked aberrations in mental functions. The psychic symptoms of LSD-25 intoxication resemble aspects peculiar to schizophrenia[1,2]. As a result, the drug has been utilized as an effective tool to explore the induction of reversible psychoses in non-psychotic subjects. It has been assumed that the similarities produced by LSD-25 could yield information pertinent to the mechanisms and pathophysiology involved in schizophrenia[2,3].

Various investigators have explored the effect of LSD-25 on the pituitary-adrenal axis using as parameters alterations in the total leukocytic[4] and eosinophilic levels[4,5]. Decreases in the frequencies of these cell types have been frequently associated with increased adreno-cortical activity[4,6]. In general, the literature contains contradictions and inconsistencies concerning the effects of LSD-25 on the white blood cells. Much of this is attributable to differences in dosage, method of administration, small samples and species variations. In addition, several investigations involved changes observed in and complicated by psychotic states. To illustrate, Forrer *et al.*[1] reported that LSD-25 induced transitory leukocytosis in schizophrenic patients. Rothlin *et al.*[7] similarly claimed leukocytotic changes in test rabbits. Although Feld *et al.*[5] observed lowered eosinophil-levels in normal and abnormal subjects, others failed to note eosinopenia either in man[8] or in rats[9]. Belsanti[10], however, analysing differential blood slides, reported that LSD-25 caused relative eosinopenia, lymphopenia and neutrophilia in schizophrenic patients. Others[4] have claimed significant eosinopenia with evidence of marked relative and absolute neutrophilia in injected guinea pigs.

The work recorded here was undertaken to determine the effects produced by prolonged administration of LSD-25 on adrenal activity by evaluating the alterations in total leukocyte, eosinophil and differential blood cell counts of female rats.

Forty-eight female Wistar rats averaging 100 g in bodyweight were divided equally into 3 groups. (All rats were housed singly in metabolism cages. The room temperature maintained by an air-conditioning system ranged from 22°–25° C. The average level of the laboratory background noise during the period of experimentation was 68 decibels during the daylight hours[19,20].) Group *A* and *B* rats were injected subcutaneously with 50 and 75 μg of LSD-25 dissolved in normal saline. Group *C* control animals received an equivalent 1 ml. dose of saline. To diminish tolerance effects, eight injections were administered over a 2-week period on alternate days. On the thirteenth and fourteenth day, the rats received consecutive daily injections. Twenty-four hours after the last injection, blood samples were obtained from the tail. Standard techniques were used for total leukocyte[11] and eosinophil[12] counts. The differential blood slides were stained with MacNeal tetrachrome solution and analyses were based on counts of 200 cells per animal.

Table 1 lists the changes produced by LSD-25 administered over a two-week period. Doses of 50 μg induced a significant 34·4 per cent decrease in the eosinophils of group *A* rats, as compared with control animals. The decrease in total leukocyte frequencies approached significance (0·09). Administration of 75 μg yielded a markedly significant decrease in leukocytic frequencies (0·01) with eosinophilic declines slightly less than that observed in group *A* rats (33·1 per cent). In both groups, comparisons of the differential data with the controls (Table 2) indicated a slight trend towards relative lymphopenia, eosinopenia and neutrophilia. Analyses of the absolute values revealed a lymphopenia ranging from 18·3 to 24·8 per cent for group *A* and *B* rats and absolute eosinopenia ranging from 41·3 to 32·0 per cent for the LSD-25 treated groups. Although group *B* data revealed a decline in the frequencies of the neutrophils, group *A* results indicated little or no change in the absolute neutrophil numbers. Depressions were noted in the lymphocyte/neutrophil ratios of group *A* and *B* rats. In general, the data are in accord with other investigations reporting induction of eosinopenia[4,5,10] and lymphopenia[10] by LSD-25.

The various leukocytic alterations thus agree with changes usually characteristic of non-specific stressors. The work recorded here consequently supports, and is in agreement with, reports that LSD-25 has a stimulatory effect on adrenal function. The study also suggests a

Table 1. Effect of Lysergic Acid on Total White Blood Cell (W.B.C.) and Eosinophil Frequencies in Female Rats

Group	No.	Dose	W.B.C./mm³	Eosin./mm³
Group *A* ± *S.E.*	16	50 μg	12,934·4 ± 830·3	65·6 ± 8·2
Group *B* ± *S.E.*	16	75 μg	11,643·8 ± 880·5	66·9 ± 10·6
Group *C* ± *S.E.*	16	—	15,228·1 ± 1,029·9	100·0 ± 13·7
% difference between *A* and *C*			−15·1%	−34·4%
P value			0·09	0·04
% difference between *B* and *C*			−23·5%	−33·1%
P value			0·01	0·07
% difference between *A* and *B*			−10·0%	2·0%
P value			0·30	>0·90

Table 2. Effect of Lysergic Acid on Relative and Absolute White Blood Cell Types

Group	W.B.C./mm³	Lymph.	Mono.	Neutro.	Eosin.
Group *A* (%)		80·13	0·75	18·78	0·34
Absolute value	12,934	10,330	99	2,462	44
Group B %		81·84	0·72	17·00	0·44
Absolute value	11,643	9,512	90	1,990	51
Group C %		82·91	0·50	16·13	0·47
Absolute value	15,228	12,650	79	2,424	75

relationship of LSD-25 intoxication responses to schizophrenia which may go beyond mere psychomimetic similarities. Various investigators have observed lowered eosinophil-levels in the blood of schizophrenic patients[13,14]. Sackler *et al.*[13,15,16] reported marked increases in the eosinophils and rises in the lymphocyte-neutrophil ratios of schizophrenic patients showing the greatest clinical improvement with sex steroid therapy. Altschule[14], similarly, reported eosinophil elevations in schizophrenics given pineal extract injections. Thus, the present evidence of a stimulatory effect on the adrenals by the hallucinogenic drug LSD-25 parallels and adds support to the Sackler *et al.* hypothesis[17,18] that relative and operative hyperadrenocorticalism with concomitant relative or absolute hormonal deficiencies, such as hypothyroidism and/or hypogonadism, may be a possible ætiologic and pathogenetic factor in at least one sub-group of schizophrenia.

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HÆMATOLOGY

Cytochemical Demonstration of Aminopeptidase Activity in Blood Platelets

A NUMBER of enzymes have been identified biochemically in platelets[1] and several of these have been localized cytochemically[1,2] as well. Aminopeptidase activity also can be demonstrated in platelets by an adaptation of Burstone's histochemical method[3,4].

Blood smears of healthy adult persons were briefly dried at room temperature and then fixed in cold acetone at 4° C for 1 h. Following this they were incubated for 22 h at room temperature in an incubating medium composed of 4 mg of alanyl-β-naphthylamide dissolved in 5 ml. of distilled water, 5 ml. of 0·1 M Sörensen's phosphate buffer at *p*H 6·8, 1 mg of magnesium sulphate and 5 mg of fast garnet *GBC*. An alternate, otherwise similar incubation medium contained 4 mg of 'Leucine' β-naphthylamide instead of alanyl beta-naphthylamide, and 1 mg of potassium cyanide instead of magnesium sulphate. After incubation in either of the foregoing solutions, the coverslips were rinsed for 10 min in tap water, briefly fixed in 10 per cent neutral formalin and mounted in glycerogel.

Microscopic examination of these preparations revealed fine granular orange red to rusty brown deposits in all platelets, an indication of considerable aminopeptidase activity (Fig. 1). A more intense reaction was seen with alanyl-β-naphthylamide, while incubation in medium from which the substrate was omitted yielded no evidence of enzymatic activity. Leukocytes also displayed aminopeptidase activity as described by Ackerman[5].

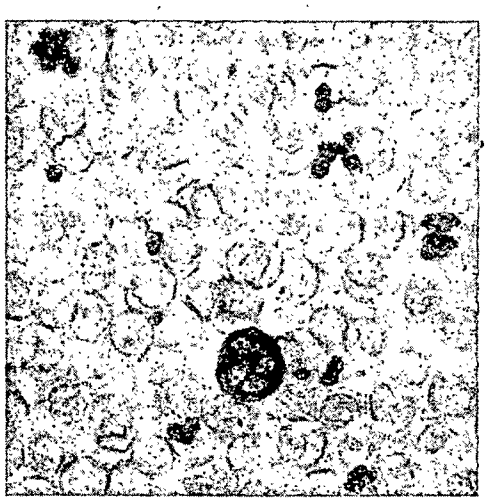


Fig. 1. Showing considerable aminopeptidase activity in human blood platelets (incubated in alanyl-β-naphthylamide). A neutrophilic leukocyte also reveals strong enzyme activity. (× 700)

The significance of the presence of aminopeptidases in platelets is uncertain, although as noted earlier their occurrence has been known for some time[6,7]. Recently Kocholaty[8], for example, has characterized several di- and tri-peptidases in normal human platelets. A more intense reaction was found with alanyl β-naphthylamide, but it is unlikely that this represents a difference in specific aminopeptidase-level in the platelets. From reports on other tissues[9,10] it would appear that more than one aminopeptidase may participate in histochemical reactions using chromogenic aminoacyl-naphthylamides, even though certain peptides may be preferentially hydrolysed. On the other hand, an increased peptidase activity has been reported in platelets during menstruation and in some hæmorrhagic disorders[11], while enzyme-levels decreased during the administration of dicoumarol[12]. These data tend to link aminopeptidase activity in platelets with blood clotting phenomena, but the true physiological role of these enzymes in platelets remains unknown.

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Department of Pathology, Harvard Medical School, and the James Homer Wright Pathology Laboratories, Massachusetts General Hospital, and the Mosher Pathology Laboratory, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts.

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Elution Procedure for the Demonstration of Methæmoglobin in Red Cells of Human Blood Smears

THE peroxidatic activity of the red blood pigment can be inhibited by cyanide in the case of methæmoglobin (Hb^{III}), but not in the case of oxyhæmoglobin (Hb^{II}) (ref. 1). It seemed promising to use this difference for a differential staining procedure of Hb^{II}- and Hb^{III}-containing red cells by first adding cyanide to the heparinized or citrated blood in question and by staining smears prepared from it by a peroxidase reaction.

The results were relatively poor. Much better results were obtained, however, with the following elution procedure:

(1) To heparinized or citrated blood 1/50 vol. of a 0·4 M solution of potassium cyanide is added. Thin blood smears are prepared.

(2) The air-dried smears are immersed into the following mixture at room temperature: 80 ml. ethanol (96 per cent); 16 ml. 0·2 M citric acid; 5 ml. hydrogen peroxide (30 per cent b.w.). The smears are agitated up and down for 1 min and then allowed to stand for 2 min.

(3) Washing first in methanol, then in water, 30 sec each.

(4) Staining for 2 min in Ehrlich's hæmatoxylin and, after rinsing with tap water, for 2 min in 0·2 per cent erythrosin (Merck). Rinsing and drying.

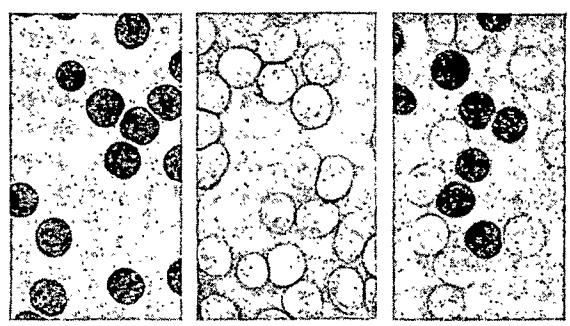


Fig. 1. Elution and staining of blood smears for the demonstration of methæmoglobin. Left, normal human adult blood; middle, same blood after preliminary conversion of the blood pigment to methæmoglobin by nitrite; right, mixture of normal and methæmoglobinæmic blood

Hb^{II}-containing red cells are stained red; Hb^{III}-containing cells are eluted and appear as ghosts (Fig. 1). The reaction is not fully understood. Since (*a*) Hb^{III} is not eluted without adding potassium cyanide to the blood; (*b*) both Hb^{II} and Hb^{III} are eluted if hydrogen peroxide is not present in the mixture; (*c*) neither Hb^{II} nor Hb^{III} is eluted without citric acid in the mixture, it seems that peroxidatic or catalatic reaction of hydrogen peroxide with the blood pigment prevents elution by citric acid. The possibility that red cell catalase could play a part was excluded, for acatalatic blood (generously provided by Prof. Takahara (Okayama) and Dr. Lejeune (Lausanne)) behaved like normal blood.

The method is not suitable for the exact estimation of methæmoglobin-levels in blood. However, it gives useful information concerning experimental oxidation and reduction of the blood pigment within the red cells.

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Bilirubin and Coagulation of Blood

Free bilirubin, dissolved in sodium carbonate, causes, if given intracardially to rabbits, a pronounced lessening of the plasmatic clotting-activity. Among other effects, prolongation of the heparin-tolerance- and recalcification-times (Howell-time) as well as lowering of the factors II and VII occur[1].

We tried to determine if *in vitro* addition of bilirubin to normal human citrated plasma also influences the clotting mechanism[2].

Free bilirubin at physiological *p*H is not very soluble in plasma. Therefore we dissolved bilirubin (50 mg bilirubin 'Homburg') in a sodium carbonate solution (0·185 g in 10 ml. water). This solution was then added to normal plasma (at a ratio of about 1 : 5). Bilirubin- and pure carbonate-plasma (control plasma) were afterwards each dialysed for 24 h, put into barbital buffer-solution *p*H 7·4 and into saline. The *p*H afterwards ranged between 7·1 and 7·5. The bilirubin concentration in the bilirubin plasma figured up to 70 mg per cent; in the control plasma it was less than 1 mg per cent. Excessive, non-albumin-bound, bilirubin was eliminated by centrifugation.

Prior to the test the bilirubin plasma was mixed with normal plasma, resulting in bilirubin concentrations of 10, 20, 30, 40, 50 and 60 mg per cent. The control plasma was added to the same normal plasma in corresponding volume parts. The activity of factors II, V, VII, VIII, IX and X was tested, also the thromboplastin- and recalcification-time as well as antithrombin II and profibrinolysin. The entire number of mixtures tested was 14, that is, bilirubin plasma in normal plasma and control plasma in normal plasma. All reaction-times were measured 10 times, and the results statistically evaluated.

Our results allow comparison between plasmas with and without added bilirubin as regards several qualities of coagulation. The reaction times measured showed no significant differences. Non-conjugated bilirubin remains in our experimental set-up even up to concentrations of 60 mg per cent without provable influence on plasmatic coagulation. Factor V is an exception. This factor displays a slight lessening in activity at concentrations of bilirubin above 20 mg per cent. The prolongation of the reaction-time, however, does not amount to more than 8 per cent.

The differing results of the experiments *in vitro* and those on the living organism demand further clarification. If animal and human plasma do not in principle react differently, one must presume that bilirubin may influence the site of production of certain coagulation factors and thereby cause a weakening of the coagulation potential.

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IMMUNOLOGY

Character and Allotypy of an Immune Globulin in Rabbit Colostrum

Rabbit serum β_2-macroglobulin and its relation to γ-globulin have been previously described[1]. This communication describes the demonstration and characterization of a third immune globulin, found in rabbit colostrum.

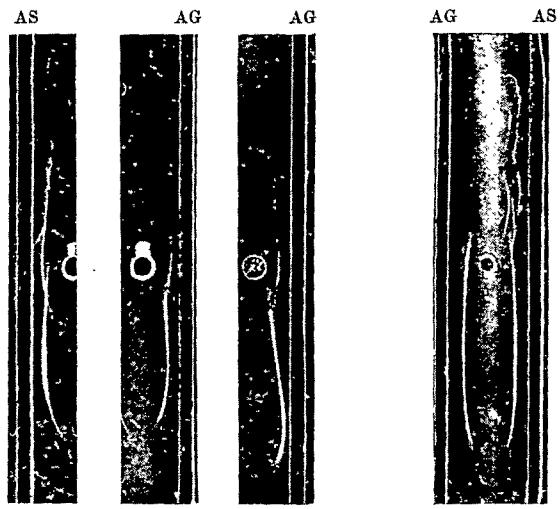


Fig. 1. Immunoelectrophoretic patterns of (*a*) rabbit colostra, (*b*) rabbit serum. Slots were filled with either anti-serum AS (goat anti-rabbit serum globulins) or AG (goat anti-rabbit γ-globulin)

When developed with a highly specific goat anti-serum (AG) to chromatographically purified rabbit γ-globulin, the immunoelectrophoretic pattern of rabbit serum showed only a single γ-globulin arc (Fig. 1*b*). Rabbit colostrum (Fig. 1*a*) gave the γ-globulin arc, but in addition a second arc with the mobility of a fast β-globulin. This immune β-globulin does not, however, precipitate all the anti-γ-globulin, since a forward spur is formed where the two arcs meet. When a less-specific goat anti-serum (AS) to rabbit serum globulins was used to develop the colostral pattern (Fig. 1*a*) the β- and γ-immune globulin arcs crossed; this shows that antibodies specific for the β- and for the γ-immune globulin were present in AS. The β-globulin was evidently present in the rabbit serum globulins used to prepare goat anti-serum AS. When the colostral pattern was developed using anti-serum AG, which had been previously absorbed with either fragment I (ref. 2) of papain hydrolysed γ-globulin or the separated *B* (light) chains[3] of rabbit γ-globulin (Fig. 2), the β-globulin arc did not develop, and only the γ-globulin arc appeared. This shows that the β-immune globulin possesses *B* chains antigenically identical with those of γ-globulin, but lacks other structural components of γ-globulin.

The β-globulin is distinguishable from β_{2M} globulin by its faster mobility[1], but might correspond to the third immune globulin, β_{2A} globulin, identified in human serum[4], particularly as this serum component has been found to

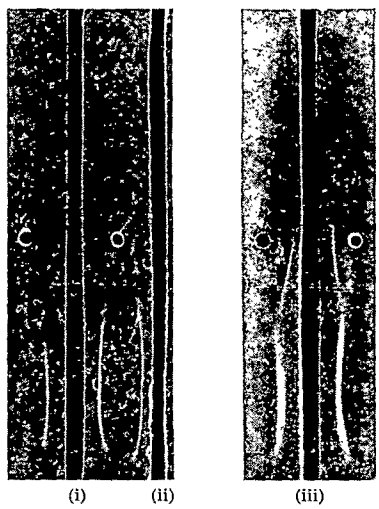


Fig. 2. Immunoelectrophoretic patterns of rabbit colostra. Slots were filled with (i) goat anti-rabbit γ-globulin serum (AG) absorbed with fragment I from papain-treated γ-globulin; (ii) AG absorbed with *B* (light) γ-globulin chains; (iii) AG untreated

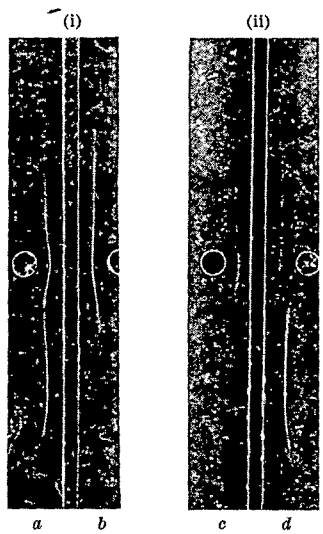


Fig. 3. Immunoelectrophoretic patterns. (*a*), (*d*), Rabbit colostrum; (*b*), (*c*), rabbit colostrum supernatant after zinc precipitation. Slots were filled with (i) anti-*A*4 allotypic anti-serum; (ii) goat anti-rabbit γ-globulin serum (AG)

be selectively concentrated into human colostrum[5]. Heremans[6] showed that γ-globulin can be selectively precipitated from human serum by zinc to give a relative enrichment of the β_{2A} component in the supernatant. The same fractionation method was applied to rabbit colostrum. Figs. 3*c* and *d* show a comparison of the immunoelectrophoretic patterns of the original colostrum and of the concentrated supernatant after zinc precipitation. The relative reduction in γ-globulin content is rather more evident when the two patterns (Figs. 3*a* and *b*) are compared after development with an allotypic anti-γ-globulin serum (anti-*A*4)[7]. This confirms the similarity between the rabbit β-globulin and human β_{2A} globulin.

The β- and γ-globulins were separated, using a column of 'Sephadex *G*200', when the β-globulin was eluted first. The immunoelectrophoretic patterns of the separated fractions are seen in Fig. 4. This fractionation, on the basis of size difference, may be successful only because, as many workers have shown with its human counterpart[6,8], the rabbit β-globulin is aggregated, or associated with other proteins. An equally complete separation was also achieved using an anionic exchanger, DEAE-cellulose.

Allotypic anti-sera, monospecific for the various genetically determined types of rabbit γ-globulin, were generously supplied by Dr. A. Kelus. Using these, the immunoelectrophoretic patterns were developed of colostra from rabbits of known allotype. Typical examples are shown in Fig. 5, exhibiting allotypic specificities *A*1 (Fig. 5*a*), *A*2 (Fig. 5*b*), *A*3 (Figs. 5*c* and *d*), *A*4 (Fig. 5*e*) and *A*5 (Fig. 5*f*). With anti-*A*4 and anti-*A*5 the β-globulin formed a precipitin line comparable in strength to that of the γ-globulin. With the specific anti-sera to *A*1 and *A*2 the line was rather weaker, and, in the case of *A*3, almost undetectable.

It is believed that the allotypic specificities *A*1, *A*2 and *A*3 are governed by allelic genes at one locus (*a*), and *A*4, *A*5 and *A*6 are governed at another locus (*b*)[7]. It is, therefore, interesting that all the specificities tested for were present in both β- and γ-globulins. Thus, although the cross-reactions with goat anti-sera revealed only that *B* (light) chain was common to both immune globulins, structural features controlled by two independent loci are now seen to be shared. Only specificities *A*4, *A*5 and *A*6 have been found on the separated *B* (light) chains[9]. It appears likely, therefore, that immune globulins, besides sharing a common light chain controlled by the (*b*) locus, may also have a common sub-unit, possibly bearing the specific antibody site, controlled by the (*a*) locus. This would be consistent with a similar finding recently reported

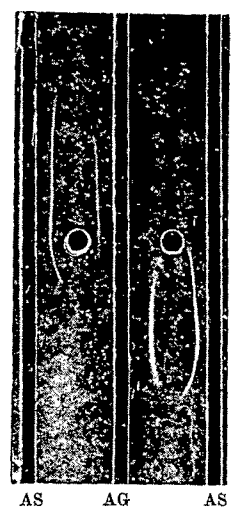


Fig. 4. Immunoelectrophoretic patterns of two rabbit colostrum fractions separated on 'Sephadex G200' gel. Slots were filled with either anti-serum AS (goat anti-rabbit serum globulins) or AG (goat anti-rabbit γ-globulin)

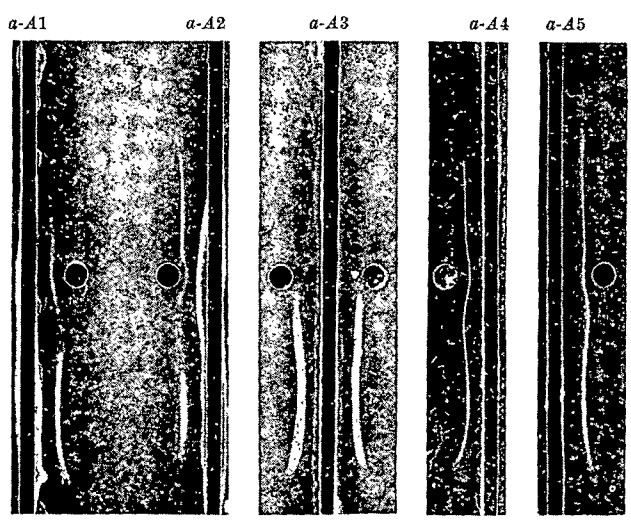


Fig. 5. Immunoelectrophoretic patterns of rabbit colostra. The patterns were developed using the following allotypic anti-sera in the slots; for (*a*) anti-*A*1 (*a*—*A*1); (*b*) *a*—*A*2; (*c*) and (*d*) *a*—*A*3; (*e*) *a*—*A*4; (*f*) *a*—*A*5

for the allotypes in rabbit serum macroglobulin preparations[10]. However, alternative theories must first be examined; for example, it will be necessary to see whether some allotypic determinants are prosthetic groups rather than part of the main polypeptide configuration.

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Immunoglobulin Abnormalities of the Thymectomized Rat

WE have shown that the adult rat, thymectomized at birth, has a global defect in delayed hypersensitive responses correlated with a depletion of small lymphocytes from blood, lymph nodes and spleen[1]. The deficiency in antibody mediated responses, in contrast, is selective. The nature of this latter defect has not been clarified. It has been shown that plasma cell and serum γ-globulin levels are normal in thymectomized rats[2].

WAG (Wistar) rats were thymectomized, or sham thymectomized, at 2–3 days of age by our usual technique[3]. At 8–12 weeks of age they were bled by cardiac puncture and the sera analysed immunoelectrophoretically. Total and differential white cell counts were done on tail blood. For the preparation of rabbit antisera, pooled normal rat serum in saline was emulsified with an equal volume of mycobacterial adjuvant (Difco) and 0·4 ml. of the mixture (1 mg protein) was given into the toe pads of all 4 feet. Six weeks later, after a precautionary subcutaneous injection of 1 mg normal rat serum, all animals were boosted intravenously with 2 mg normal rat serum in 1·0 ml. saline. Seven days later they were bled. Immunoelectrophoreses were carried out by standard methods[4]. To demonstrate more clearly the β_2M macroglobulin (*IgM*), the rabbit antisera were absorbed with a rat γ- and β-globulin fraction, the preparation of which is given elsewhere[5]. The identity of *IgM* was established by the cysteine degradation technique[6]. A pool of rat antisera against bovine serum albumin was precipitated at equivalence with bovine serum albumin and the specific precipitate washed six times. Rabbit anti-rat serum was absorbed with the specific precipitate before being reacted against normal rat serum in immunoelectrophoresis. In a separate experiment, the rabbit anti-serum was absorbed with normal new-born rat serum and then reacted against normal rat serum in immunoelectrophoresis.

Immunoelectrophoresis failed to reveal any abnormality of serum γ-globulin (*IgG*) levels in thymectomized rats.

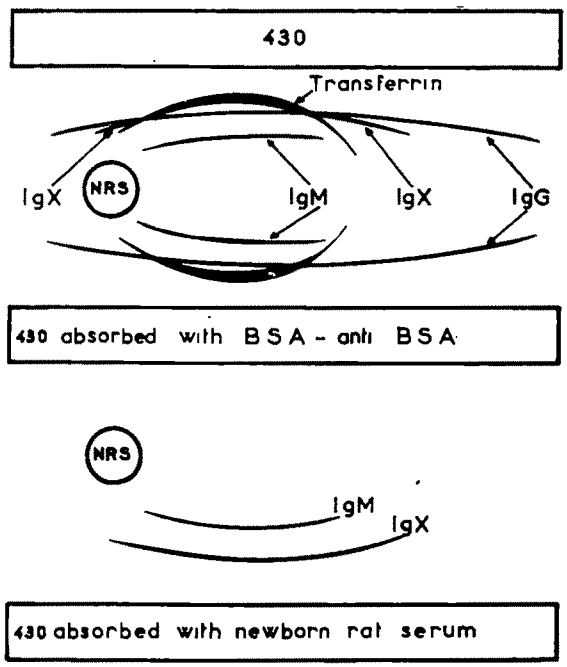


Fig. 1. Immunoelectrophoresis of normal rat serum (NRS) against rabbit – anti-rat serum (430)

IgM levels were increased in 7/8 thymectomized rats as compared to 8 sham-operated litter mate controls.

In 8/9 control rats 8–12 weeks of age, and invariably in older rats, a third immunoglobulin was detected which we have tentatively chosen to call *IgX*, and which corresponds, in all probability, to the protein designated β_{2A} by Escribano[7]. *IgX* extended cathodally into the slow β-region; anodally it extended medial to *IgG* well into the α-region (Fig. 1). Its central portion was generally hidden by the *IgG* line. It could be demonstrated in pooled normal rat serum diluted to 0·25 but not at a dilution of 0·125. In 7/14 thymectomized rats, aged 8–12 weeks, using undiluted serum, this protein line could not be detected; in a further 6, its intensity was markedly diminished. In some thymectomized rats *IgX* was still absent at 19 weeks. There was a direct correlation between the extent of lymphocyte depletion and the *IgX* deficiency (Table 1).

Rat antibody against bovine serum albumin gives a single precipitin line on diffusion in gel against bovine serum albumin. The antigen-antibody complex gives a reaction of non-identity with rat *IgG* and *IgM*. Absorption of rabbit anti-rat serum with bovine serum albumin–rat anti-bovine serum albumin specific precipitate removes the *IgX* line and no other (Fig. 1). *IgX* is not seen in sera of normal rats aged less than 6 weeks. Absorption of rabbit anti-rat serum with new-born rat serum removes all negatively migrating precipitin lines except *IgM* and *IgX* (Fig. 1).

We have shown that the thymectomized rat has a severely compromised capacity to form antibodies against bovine serum albumin. This defect would appear to be related to a deficiency in *IgX* rather than in *IgG* or *IgM*

Table 1. IMMUNOELECTROPHORETIC ANALYSIS OF *IgX* LEVELS IN THYMECTOMIZED AND SHAM-OPERATED RATS 8–12 WEEKS OF AGE

Group	*IgX* precipitin line					
	Absent		Diminished		Normal	
	No. of animals	Lymphocyte count*	No. of animals	Lymphocyte count	No. of animals	Lymphocyte count
Thymectomized	7	4,200 (2,500–5,700)	6	6,600 (5,600–7,700)	1	7,000
Sham operated	1†	15,400	0	—	8	15,800 (10,200–22,200)

* Average count—range in parentheses.
† This animal also had hypogammaglobulinæmia.

synthesis. No counterpart in the rat of human *IgA* (β_2A-globulin) has thus far been identified. Whether the *IgX* which we have found in the rat is analogous to the *IgA* of human serum remains to be established, as does a more precise delineation of its relationship to lymphocytes and to delayed sensitivity.

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Identification of a Somatic Antigen of *Staphylococcus aureus* Critical for Phagocytosis by Human Blood Leucocytes

The capacity of *Staphylococcus aureus* to produce disease resides in its ability to invade and persist in the body, and in its elaboration of toxic metabolites. Host-defensive mechanisms comprise the elaboration of antibodies which facilitate phagocytosis and intracellular destruction, and the elaboration of antitoxins. This communication reports identification of at least one antigen of the staphylococcal cell-wall which is critical for phagocytosis by human blood leucocytes.

We have identified previously[1] a non-type-specific protein agglutinogen of the staphylococcal cell-wall. The antibody homologous with this antigen can, however, be absorbed out of human serum without impairing the ability of the serum to promote phagocytosis[2]. Combination of the serum antibody with a soluble carbohydrate antigen, teichoic acid, we now find does remove the phagocytosis-promoting action of human sera.

The teichoic acid may be extracted from staphylococcal cell-walls by standard methods[3,4]. The teichoic acid used for absorption of antibody in the representative experiment shown in Fig. 1 was prepared as follows:

Undigested staphylococcal cell-walls were prepared from staphylococci of International Serotype XIII by disintegration in an 'X-press' and purification by gradient and pile centrifugation[6,7]. Teichoic acid was then extracted from the undigested cell-walls essentially as described by Morse[4]. Removal of the serum components responsible for phagocytosis and killing of staphylococcal cells by human blood leucocytes by absorption with this preparation is shown in Fig. 1.

Carbohydrates were also prepared from acetone-dried cells of *Staph. aureus* by methods in principle those of Baddiley, Armstrong and Buchanan[8]. Absorption of human sera by this teichoic acid removed phagocytosis-promoting antibody from human sera.

Baddiley, Buchanan, Martin and RajBhandary[9] and Baddiley[10] have shown that the cell-wall teichoic acid of *Staph. aureus H* is a polymer of ribitol phosphate with side-chains of *N*-acetyl-D-glucosamine attached to position 4 and D-alanine in ester linkage at position 2 or 3 of D-ribitol. Morse[4] has shown that antibody against teichoic acid of *Staph. aureus* (strain *NYH*-6) can be inhibited in the precipitation and hæmagglutination reaction by β-phenyl-N-acetylglucosamine. Sanderson, Juergens and Strominger[11] have shown that the agglutination of cell-walls of *Staph. aureus*, strain Copenhagen, can be inhibited by α-phenyl-acetylglucosaminide. (See also refs. 12 and 13.)

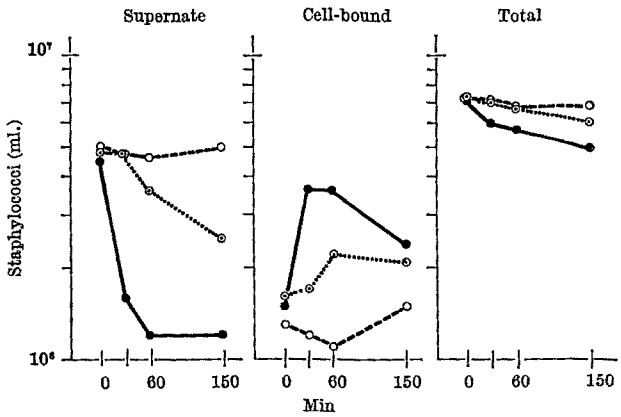


Fig. 1. Phagocytosis of staphylococcal cells (International Serotype XIII) by human blood leucocytes (Type 0) in presence of fresh human serum (Type 0) and of human serum absorbed with teichoic acid extracted from homologous staphylococcal cell walls (25 mg/ml. of 1/10 serum). Phagocytic technique adapted from Cohn and Morse (ref. 5), as described by Li, Mudd and Kapral (ref. 2). Ordinates record viable staphylococci/ml. recovered at successive times respectively from the supernatant fluid, the centrifugal sediment after lysis of leucocytes, and from the entire phagocytic system after lysis of leucocytes. First extraction: Teichoic acid obtained from first 24-h extraction with 5 per cent trichloroacetic acid in the cold. Second extraction: Teichoic acid obtained from successive 48-h extraction with 5 per cent trichloroacetic acid in the cold. Absorption removes partially or wholly both phagocytosis promoting and killing effect of fresh serum. ●, 1 per cent fresh serum; ○, 1 per cent fresh serum absorbed with teichoic acid (first extraction); ⊙, 1 per cent fresh serum absorbed with teichoic acid (second extraction)

In other investigations it has been shown that *N*-acetyl-D-glucosaminide is required for the synthesis of the muramic acid of normal cell-walls of *Lactobacillus bifidus* var. *Pennsylvanicus*[14,15]. Derivatives of *N*-acetyl glucosamine (which are growth factors for *L. bifidus* var. *Penn.*) were generously made available to us by Dr. M. C. Glick. An anomeric mixture of α-(85 per cent), β-(15 per cent) methyl-*N*-acetyl-D-glucosaminide partially inhibited the antibody in human sera promoting phagocytosis of *Staph. aureus* by human blood leucocytes (Fig. 2). A sample of β-ethyl-*N*-acetyl-D-glucosamine[16], 25 mg/ml. of 10 per cent heated human serum, also partially inhibited phagocytosis of *Staph. aureus* serotype XIII. 4-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine (*N*-acetyl lactosamine) did not inhibit phagocytosis. Our experiments with *N*-acetyl-D-glucosamine encountered complications and did not yield a clear result.

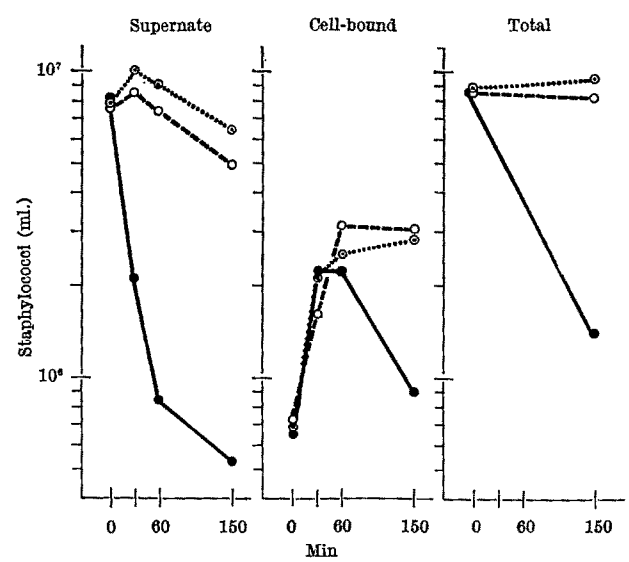


Fig. 2. Inhibition of phagocytosis and killing of staphylococcal cells (Serotype XIII) by absorption of fresh human serum with α,β-methyl-*N*-acetyl-D-glucosaminide. Type 0 serum; Type 0 cells. Solid line, 1 per cent fresh serum; broken line, 1 per cent fresh serum absorbed with 25 mg/ml. of 10 per cent serum; dotted line, 1 per cent fresh serum absorbed with 100 mg/ml. of 10 per cent serum

We have observed that human serum inactivated by heating to 56° promotes phagocytosis to a less degree than fresh serum and fails to produce intracellular killing of *Staph. aureus*[2]. In Fig. 2 it may be seen that the α,β-methyl-*N*-acetyl-D-glucosaminide inhibits killing as well as phagocytosis by human blood leucocytes. This result is compatible with the accessory killing factor being complement.

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PATHOLOGY

Induction of Melanotic Lesions of the Iris in Rats by Urethane given during the Neonatal Period

THERE have been a number of reports in recent months of experiments in which animals have been exposed to chemical carcinogenic agents during the neonatal period[1–6]. It is probable that, on the basis of dose per unit of body-weight, newly born animals are particularly sensitive to tumour-induction. For example, lung tumours may be very readily induced in mice by the neonatal injection of small doses of certain weakly carcinogenic agents[4]. Because of this high sensitivity, the use of newly born animals for screening for carcinogenicity has been suggested[3]. Of possibly even greater interest, however, is the fact that animals injected when newly born with carcinogenic agents develop varieties of neoplasms not seen when the same agents are injected into adults of the same species and strain. For example, newly born '101' strain mice injected with small doses of 9,10-dimethyl-1,2-benz-anthracene developed kidney tumours[3]. A further example of this type of phenomenon has recently come to light in an experiment in which rats were injected during the neonatal period with urethane (ethyl carbamate).

Urethane was already known to be capable of inducing neoplastic lesions in a wide variety of tissues[7–9]. The purpose of the experiment, details of which are given in Table 1, was simply to examine the effects of neonatally administered urethane in two strains of rat, the August Hooded and Wistar. The rats used were from sub-lines maintained for several years in the Institute by brother–sister mating. Fifty-one August rats (26 ♀ and 25 ♂) and 44 Wistar rats (21 ♀ and 23 ♂) constituted the two experimental groups. The former were derived from eight different litters and the latter from six. Similar numbers of litters born at about the same time were kept under observation without treatment as controls.

Table 1. INCIDENCE OF MELANOTIC LESIONS OF THE IRIS IN RATS TREATED WITH URETHANE DURING THE NEONATAL PERIOD

(1 mg/g body-weight once weekly for 8 weeks, the first injection being given during the first 24 h of life)

Strain	No. examined		Treatment with urethane*	Rats with eye lesions†	♀	♂	One eye	Both eyes
August	51	26 ♀ 25 ♂	+	20	12	8	15	5
August	50	25 ♀ 25 ♂	0‡	0	0	0	0	0
August	200§	100 ♀ 100 ♂	0	0	0	0	0	0
Wistar	44	21 ♀ 23 ♂	+	0	0	0	0	0
Wistar	50	25 ♀ 25 ♂	0‡	0	0	0	0	0

* 10 per cent w/v in sterile distilled water.
† After 9 months from the start of the experiment.
‡ Control animals were untreated.
§ These were untreated rats of between 9 and 12 months of age. In every other way they were comparable to both the preceding groups.

During the seventh month of the experiment one of the members of the staff of the animal unit, Mr. Malcolm Kipling, observed that a number of the August rats, which had been treated with urethane when young, had 'dark spots' in their eyes. Thereupon a careful examination of all the rats in the experiment was undertaken. 'Dark spots' were observed in the eyes of 11 females and 7 males of the urethane-treated August rats, both eyes being involved in 5 of the rats. None of the 50 control August rats showed lesions. Moreover, no lesions were found in 200 untreated August rats of similar or older age. No lesions were seen in rats of the Wistar strain whether treated with urethane or not. During a further two-month period of observation 2 more August rats in the urethane-treated group have developed lesions: 1 male and 1 female. Ophthalmoscopic examination showed that the lesions were located in the iris and that they were fan-shaped when the pupil was small. When the pupils were dilated, however, the lesions appeared as a thin black ring extending around a part of the periphery of the anterior chamber.

Two of the rats were killed and their eyes taken for histological examination. In each case the lesion proved to consist of pigment-containing cells situated in the connective tissue between the two epithelial surfaces of the iris. The presence of these cells increased the thickness of the iris up to three-fold (Figs. 1 and 2). The pigment could be removed by bleaching with hydrogen peroxide, indicating that it was probably melanin. Very few mitotic figures were seen and there was no evidence of invasive-

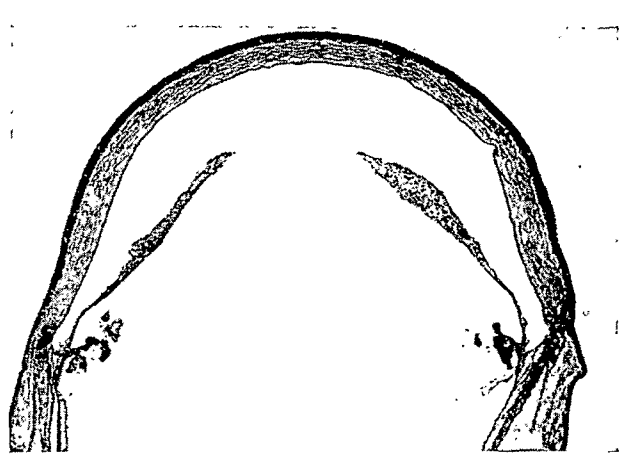


Fig. 1. Eye from seven-months-old urethane-treated August rat. The lens has been removed and the ciliary body and iris remain on either side. The thickening of the iris on the right side is associated with the presence of pigmented cells (hæmatoxylin and eosin. × 20)

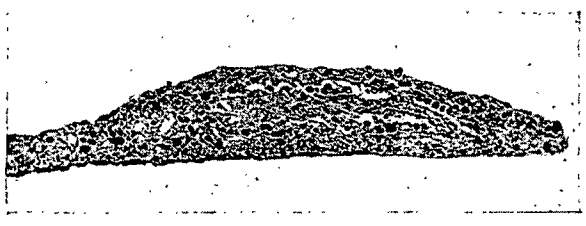


Fig. 2. Melanotic lesion of the iris from a seven-month-old August rat. (A different rat from that illustrated in Fig. 1.) Melanocytes are distributed widely in the connective tissue (hæmatoxylin and eosin. × 80)

ness. The fact that the pigmented cells were in the connective tissue and not in the epithelial layers, as well as consideration of the other features already mentioned, led us to the view that the lesions were naevi rather than melanomas, and that the pigmented cells were naevus cells (melanocytes) and not melanoblasts. We suspect, therefore, that the lesions have only a low potentiality for malignant change. A few rats have died in all the groups from lung infections, and two of the urethane-treated August rats have died from lymphatic leukæmia (malignant lymphoma of the stem cell type). No neoplasms were seen at post-mortem in any of the other rats which died or were killed. The rest of the animals in the experiment are being kept under observation.

We felt that these data were of sufficient interest to present in preliminary form for several reasons. First, there are but few examples of the experimental induction of melanotic neoplasms, though the Syrian Golden hamster appears to be especially apt to develop tumours of this type[10,11]. In 1960 Pietra and Shubik[11] reported the induction of melanotic lesions of the skin in hamsters treated with urethane in the drinking water. In their experiments the males developed a higher incidence of lesions than the females. Other reports of melanoma induction by chemical agents are mentioned by Della Porta *et al.*[10], and reviewed by Nakai and Rappaport[12]. The latter authors describe the melanotic tumours which they were able to induce in the Syrian Golden hamster by the application of 9,10-dimethyl-1,2-benzanthracene (DMBA) to the skin as consisting of dendritic melanocytes situated in the dermis. They thought the lesions resembled either cellular blue naevi or pigmented neurofibromas of man. These 'induced' lesions are quite different from the malignant melanomas which occasionally arise spontaneously in the Syrian Golden hamster[13]. The latter always involve the dermoepidermal junction. We have little doubt that the lesions seen by us were akin to these DMBA-induced tumours. We could find no record anywhere in the literature of the experimental induction of neoplastic lesions of the iris: Hartwell[14] and Shubik and Hartwell[15] list none.

Secondly, there seems to be some confusion among ophthalmic surgeons concerning the nature, malignancy potential, and best method of treatment of pigmented lesions of the iris. For example, in 1951, Reese[16] wrote, "It is certain that malignant melanomas of the iris develop from pre-existing benign melanomas. I have observed such metamorphoses, and in a number of cases in the literature (10 references given by author at this point) there is from early youth a clear history of pigmented lesions from which malignant melanomas develop". Eleven years later, Ilif and Ossofsky[17], writing on the same subject, expressed the following views: "Nevi are the most common tumors of the iris . . . The tumors never attain great size and no therapy is indicated . . . Although it is claimed that nevi are potentially malignant, it is felt that benign nevi of the iris arising in childhood rarely, if ever, undergo malignant change, and that melanomas of the iris do not arise from a pre-existing nevus but are malignant from the start". Similarly, Zimmerman[18], after describing the clinical and pathological varieties of iris tumour, wrote: "The usual iris tumors falling into the nevus-melanoma group are either completely benign nevi or relatively benign spindle cell melanomas". He, too, stresses the uncertainty among ophthalmic surgeons as to the best method of management of cases of iris tumour.

A third reason why the production of the lesions described in this communication may be important is that the eye, with its normally transparent media, provides an unusual opportunity to make direct *in vivo* observations of tumour growth. "This," says Zimmerman[18], "is particularly true in the case of tumors of the iris, which may even be examined microscopically in the living eye with the use of magnification of 8× to 40× or more."

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A Lactic Dehydrogenase Elevating Agent in Association with Human Neoplasms

The observation that a variety of murine neoplasms contain a filterable agent capable of inducing a five- to ten-fold increase in plasma lactic dehydrogenase (LDH) activity within 48 h of inoculation into normal mice was reported by Riley *et al.*[1], and confirmed by Mundy and Williams[2]. Subsequent papers described some of the characteristics of the LDH-elevating agent[3,4], reported a 50- to 1,000-fold rise in plasma LDH and an approximate five-fold increase in anaerobic glycolysis after 'infection' of 'virus-free' tumours with the LDH-elevating agent[5,6], and demonstrated elevated levels of other enzymes, in addition to LDH, in plasma from mice inoculated with this 'virus'[7,8].

So far, few studies on the host range of the LDH-elevating agent have been reported. Notkins *et al.*[9]

Table 1. Effect of Human Tumour Extracts on Mouse Serum LDH

Tumour	Tumour extract	No. of samples	Serum LDH (units/ml.)* Range	Mean
Carcinoma of breast	Homogenate	36	750–2,200	1,250
	Filtrate	35	700–2,000	1,100
Leiomyosarcoma of stomach	Homogenate	36	700–1,550	1,050
	Filtrate	36	700–1,800	1,100
Transitional cell carcinoma of kidney	Homogenate	34	600–1,650	1,000
	Filtrate	36	550–2,400	1,150
Melanoma of choroid	Filtrate	22	750–1,800	1,150
Controls		20	850–2,100	1,400

* One unit of LDH is defined as the enzyme activity in 1·0 ml. of serum which results in a decrease in optical density of 0·001 per 1 min at 32° C.

found no increase in serum LDH activity in rats bearing 6 different transplanted tumours. This is a report of attempts to demonstrate an LDH-elevating agent in association with human neoplasms.

Human tumours were obtained from a 35 years old female Negro, a 70 years old male Negro, a 53 years old male Caucasian, and a 69 years old male Caucasian. Histopathological examination showed these to be a carcinoma of the breast, a leiomyosarcoma of the stomach, a transitional cell carcinoma of the kidney, and a melanoma of the choroid, respectively.

Ten per cent suspensions of fresh tissue in Eagle's basal medium were homogenized for 1 min, at 8,000 r.p.m., in a Servall 'Omni-Mixer'. An aliquot of each homogenate was removed and the remaining material spun for 10 min at 5,000 r.p.m. The supernatants were then passed through 'Millipore' filters of 100 mμ pore size.

A total of 40 *C57BL/Fg* mice, approximately 3 months old, each received a 0·2-c.c. subcutaneous injection of either a homogenate or filtrate. Ten animals were not treated. Serial blood samples were obtained by tail bleeding and serums collected by routine laboratory procedures. Serum LDH activity was measured by the method of Henry *et al.*[10]; all results were corrected to 32° C. The animals were killed 15 weeks after treatment.

As shown in Table 1, the inoculation of mice with homogenates or filtrates of 4 human tumours did not induce a significant elevation in serum LDH activity. These findings suggest that an LDH-elevating agent was not associated with these neoplasms and, as such, plays no part in the elevation of serum or plasma LDH in patients with cancers[11]. They do not, however, exclude those possibilities, since there are many difficulties associated with attempts to transmit human viruses to animals.

Pertinent to this problem is the question of when serum LDH activity is increased after heterotransplantation of human tumours. Based on previous experiments with murine neoplasms[8,9,12,13], an early rise in enzyme activity prior to detectable tumour growth should not be found if human tumours are free of the agent. Further, in view of the suggestion that the LDH-elevating agent becomes associated with murine tumours during serial transplantation[8,9,12,13], it would be of interest to determine whether heterotransplants acquire the agent after repeated passage in the mouse. Although neoplasms were not observed in any of our experimental animals, the demonstration that human tumours can be transplanted to mice[14] and maintained in serial passage[15] indicates that these results can be obtained.

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RADIOBIOLOGY

Effect of Thyrotropin on Nucleic Acids and Protein Contents of the Thyroid

In a previous paper[1] we reported the effect of single and repeated doses of thyrotropic hormone (TSH) on the contents of protein-bound iodine-131 ($PB^{131}I$) in whole thyroids and their subcellular fractions, 1 h after administration of $Na^{131}I$. The contents of protein, ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) were analysed in thyroidal homogenates and fractions on similarly treated guinea pigs. The preliminary results of the latter investigations are reported here.

The experimental animals were male guinea pigs, weighing 200–250 g. All experiments were performed on groups, each group comprising 6 animals. A dose of TSH ('Ambinon', Organon) was given intraperitoneally once a day for 1–6 days. The animals were killed 24 h after the last injection. Different daily doses of TSH were tested, ranging from 0·2 to 2·5 I.U.

The glands of each group were pooled and homogenized in 0·44 M sucrose. The homogenate was divided into nuclear, mitochondrial, microsomal-postmicrosomal and supernatant fractions as described earlier[1].

The amounts of RNA and DNA in the homogenates and fractions were determined by Schneider's method[2]. After the extraction of nucleic acids, the protein in the precipitates was determined according to Lowry *et al.*[3].

In the controls the total RNA-content of the homogenate from six glands had a mean value of about 550 μg (Table 1). Repeated daily doses of 1·0 I.U. of TSH gave an increase of the homogenate RNA-content, which was clearly shown after one day and reached a maximum after four days' treatment (almost 2,000 μg). Administration of TSH also resulted in an increased amount of protein in the homogenate. However, this increase was less pronounced than that of RNA (Table 1). When, therefore, the

Table 1. Wet Weights and Total Amounts of RNA, DNA and Protein in Thyroid Homogenates and Fractions after Repeated Daily Doses of 1·0 I.U. of TSH. Each Value represents the Sum of a Group of Six Glands. Every Figure is a Mean Value based on at Least Three Groups

TSH treatment (No. of days)	Thyroid wet weight (mg)	Homogenate		Nuclear			Microsomal		Supernatant	
		RNA (μg)	Protein (mg)	RNA (μg)	DNA (μg)	Protein (mg)	RNA (μg)	Protein (mg)	RNA (μg)	Protein (mg)
—	185	550	17·2	250	920	4·6	40	0·8	130	10·5
1	494	820	20·3	450	910	5·2	130	1·1	200	11·6
2	545	1,440	26·1	740	1,730	11·0	270	1·8	200	11·0
4	416	1,910	26·9	1,010	1,980	14·2	250	2·6	110	6·5
6	515	1,430	30·4	770	1,640	12·4	260	3·2	130	9·1

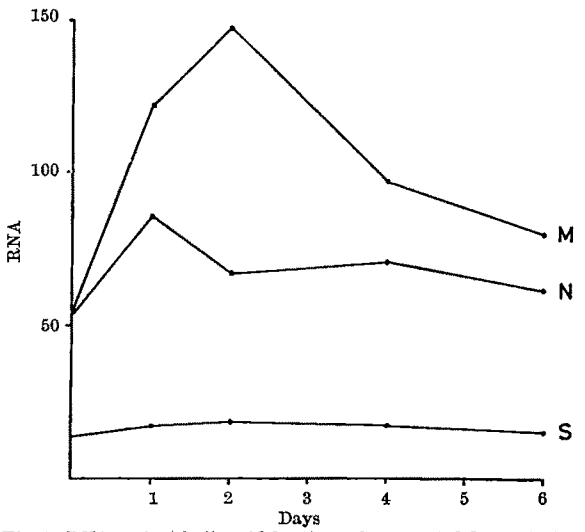


Fig. 1. RNA content in thyroid fractions after repeated doses of 1·0 I.U. of TSH. The values are expressed as μg of RNA per mg of protein in the fractions. *M*, Microsomal; *N*, nuclear ; *S*, supernatant fraction

RNA-content was calculated on protein basis it rose from 32 μg/mg of protein in the control animals to 70 μg/mg of protein after four daily doses of 1·0 I.U. of TSH. Since TSH increased the wet weight of the thyroid very rapidly, the homogenate RNA was, when calculated per mg of wet tissue, reduced after one and two days and only slightly increased after treatment with TSH for 4 days.

In the controls, the nuclear fraction had a higher content of RNA than any other fraction. It was about five times that of the microsomal fraction. However, when calculated on protein basis the nuclear and microsomal fractions showed similar values, about 50 μg/mg of protein. Administration of TSH resulted in a very rapid and marked rise in the amount of microsomal RNA, which from the second day onwards was six times that of the controls (Table 1). When calculated per mg of protein in the fraction the microsomal RNA raised from about 50 μg in the controls to about 150 μg after two days of TSH and then decreased slightly on the fourth and sixth days (Fig. 1). TSH-administration was also followed by a pronounced increase of the amount of nuclear RNA. However, it must be pointed out that the RNA-values of the nuclear fractions, both in the controls and in the TSH-treated groups, are too high owing to contamination of the nuclear fraction by unbroken cells and cytoplasmic particles.

The mitochondrial fraction contained throughout very small amounts of RNA which, judging from electron micrographs of this fraction, could be ascribed to microsomal contamination. The supernatant fraction had a fairly high RNA-content in the controls. TSH-treatment induced a slight increase after one and two days and then a reduction to, or below, control values (Table 1). The amount of supernatant protein showed fairly small fluctuations during TSH-treatment but seemed to be somewhat reduced after four days of TSH.

In the controls, the total DNA-content of the homogenate from six thyroids showed a mean value of about 1,000 μg. Most of this DNA (about 900 μg) was recovered in the nuclear fraction. One day's TSH-treatment did not significantly change the amount of nuclear fraction DNA. However, after two days' administration of TSH there was a very marked increase of nuclear DNA and after four days the amount of DNA was about twice that of the controls.

Larger and smaller doses of TSH gave results which were in principle similar to those accounted for here.

In conclusion it can be stated that administration of TSH induced in the guinea pig thyroid an increase of the RNA-content. This increase was particularly rapid and marked in the microsomal fraction and should be considered as evidence of stimulation of the protein synthesis in the gland. There was also an increased build-up of DNA which, however, was slower than that of RNA, indicating that an increase of mitotic rate is preceded by increased synthetic activity. The total protein content of the thyroid was rapidly augmented.

Further investigations, on purified fractions, are in progress.

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BIOLOGY

Effect of Thalidomide on the Development of the Chick Embryo

THE disastrous effect of thalidomide on development of the human embryo[1] has prompted us to investigate the influence of this substance on the development of the chick embryo. Although the results given here are only preliminary, we thought it advisable to publish them in a brief communication, as they may be of interest to other workers in this field.

We injected thalidomide, suspended in 0·5 ml of a 1 per cent carboxymethyl-cellulose (CMC) gel, in doses of 1 and 5 mg per egg (White Leghorn) by the yolk sac route, a method well known in virology[2]. For each dose we used six groups of 20 eggs, group 1 being inoculated one day after the beginning of the incubation (37° C), group 2 on the second day, and so on. Another six groups of 20 eggs served as controls and were injected in the same scheme with CMC only. Three days after inoculation with the substance the eggs were candled and dead ones were discarded. These, as well as any eggs not fertilized, were excluded from all groups. From then on the eggs were candled every day, and the dead were recorded and inspected. On the eighteenth day of incubation the



Fig. 1. Embryo from egg injected one day after the beginning of incubation with 5 mg thalidomide and which expired on the twelfth day of incubation, showing deformed legs and wings grown together. Histological preparations showed that the wing bones had not grown together

remaining eggs were cooled at −20° for 2 h, to kill the embryos, and inspected.

Our results can be summarized as follows: the toxicity of thalidomide for the chick embryo in the concentrations given here was not very high. Probably there is a slight difference between 1 mg and 5 mg/egg. The survival on the eighteenth day of incubation in the six groups varied in the control series from 15/16 to 9/17 (94–53 per cent, overall 69 per cent), in the 1 mg series from 16/20 to 6/12 (80–50 per cent, overall 73 per cent) and in the 5 mg series from 12/15 to 3/10 (80–30 per cent, overall 60 per cent).

In both thalidomide series we found malformations of the legs, for example, too short or twisted. In the 1 mg series, 10 of the 23 embryos (=43 per cent) that had expired before the eighteenth day of incubation, and 9 of the 61 embryos (=15 per cent) that were alive on that day, showed these kinds of abnormalities. In the 5-mg series this was the case with 22 of the 30 embryos (=73 per cent) that had expired before the eighteenth day of incubation and with 5 of the 45 embryos (=11 per cent) that were alive on that day.



Fig. 2. Embryo from egg injected one day after the beginning of incubation with 1 mg thalidomide and which expired on the tenth day of incubation, showing only one leg and wings grown together



Fig. 3. Embryo from egg injected one day after the beginning of incubation with 1 mg thalidomide and still alive on the eighteenth day of incubation, showing growing together of the wings. Röntgen photographs showed that there was no growing together of the wing bones



Fig. 4. Left: An embryo from an egg injected on the first day of incubation with 1 mg thalidomide and still alive on the eighteenth day of incubation; right, an embryo of the same age from the control group



Fig. 5. Histological preparation of wings grown together from the embryo shown in Fig. 1. (Bismarck-brown staining after Roulet, *Methoden der Pathologischen Histologie*, Springer-Verlag, 1948)

These malformations of the legs were found in the control series only on 6 of the 26 embryos (=23 per cent) that had expired before the eighteenth day of incubation and on 0 of the 58 embryos (=0 per cent) that were alive on that day.

In group 1 we observed two embryos with only one leg in the dosage series 1 mg/egg (Fig. 2). This was not found in the control series.

In total we observed eight embryos with wings grown together or deformed, that is to say five in group 1, two in group 4 and one in group 6. There were no embryos with abnormal wings in the control series. These malformations, as well as those of the legs, were mainly found in the embryos which died before the eighteenth day of incubation.

The photographs show the main malformations which were observed, namely wings grown together, one leg, and aberrant legs.

Our preliminary impression is that thalidomide gives a higher incidence of malformations in the chick embryo, especially when given very early in the incubation period. The growing together of the wings seems very important, as we have never seen this phenomenon during more than 10 years of virological work with chick embryos.

It might be of value to include this kind of test before clinical trials of drugs are started. The chick embryo seems a rather sensitive indicator of teratogenic effects, and as there is no excretion it is easy to maintain a constant level of the substance to be tested.

We thank Prof. F. Zilliken, R. C. University, Nijmegen, for a gift of thalidomide and for his advice, Miss J. C. Sekèt, Miss N. Kors and Mrs. W. C. Bouman-ten Das for assistance, and Mr. Boeve for taking the photographs.

During the preparation of this manuscript the publication of F. Kemper[3] came to our notice. This author also observed malformations, which were, however, not further described. Growing together of the wings is not mentioned by him.

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A Genus of Brackish-water Oligochæta New to Britain

During investigations into the life-cycle of *Tubifex costatus* (Claparède), 1863, in a brackish-water moat close to the shore of the River Mersey at Hale, Lancashire, a tubificid genus not so far recorded from Britain was also observed[1–4].

The worm was clearly recognizable as *Aktedrilus monospermathecus* Knöllner, 1935, from the size and shape of the animal and its chætæ, and the single spermatheca with its dorsal pore[5,6]. The worms are very thin and reach a maximum length of 1 cm, although some segments are missing posteriorly. There are very few chætæ in each bundle—a maximum of 5 in pre-clitellar bundles is much rarer than 2 or 3—and all chætæ are alike. They are all bifid crotchets with the upper teeth more or less rudimentary. The detailed structure of the male efferent ducts was not observed in this material but a careful study of whole mounts revealed that the cuticular covering of the penis was sufficiently thickened to appear as a very thin-walled penis-sheath in one specimen. This was so thin that it was distorted in whole-mount preparations and its detailed shape could not be ascertained, but it seemed to be larger than its breadth at the base and to taper distally as in *Tubifex templetoni* Southern, 1909.

Aktedrilus monospermathecus has also been recorded from the shores of Kiel Bay and Schleswig, the River Weser at Blumenthal, Vegesack, and Bremen waterworks, the Baltic in general and the Mediterranean[5–8]. The salinity preferences of the species are given as 0–18‰ and its range from 1·25–20 S‰ with a preference for 2·5–5 S‰ (ref. 8). The species was overlooked in earlier surveys of the habitat at Hale as it is very scarce; only 12 specimens were observed among 2,078 specimens of *T. costatus*. Its normal habitat is the interstitial space of sandy sea shores, but at Hale it was found in thin yellow clay overlying a thick blue-grey clay in a moat surrounding a small coppice. The moat was connected to the Mersey via an organically polluted stream which has recently been dredged and straightened to accommodate the tidal flow. The shore of the Mersey at the nearest point is a mass of foul, black mud and clay, and the nearest sandy shores are beyond Liverpool docks.

It is very probable that several other marine Tubificidae remain to be added to the British list; but many are very small and readily overlooked unless samples are sieved through a very fine mesh.

Since this note was prepared, another marine species has been recorded—(*Thalassodrilus* (*Rhyacodrilus*) *prostatus* (Knöllner), 1935—n.g. (ref. 9))—as well as *Tubifex* (*Limnodrilus*) *newaensis* (Michaelsen), 1903 (ref. 4).

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Retention of Organomercury Compounds on Dressed Grain after Washing

Farmers and seed merchants may occasionally have a surplus of grain dressed with organomercury fungicide when sowing is completed. Lindström[1] has reported that more than 50 per cent of a dry formulation (containing ethylmercury chloride) was washed off grain dressed in the laboratory after shaking for 10 min with water, thus showing that washing could reduce the potential hazard of such grain if used as part of a ration to feed livestock. In contrast, less than 5 per cent of a wet dressing (methylmercury dicyandiamide formulation) was removed. Consequently, the amount of fungicide retained after washing wheat and barley, dressed commercially, has been investigated: it was found to be considerable.

In our investigations, eight 2–3-kg samples of treated wheat or barley were taken from normal large-scale dry and wet treatments; in addition, 28 kg of wheat was dressed with a methylmercury dicyandiamide formulation at request. An interval of at least four weeks elapsed between dressing and analysis.

160 g of each sample was placed in turn in a Buchner funnel and a steady stream of cold water run over the grain for 5 min; after draining for a few minutes, each sample was divided into eight aliquots of 20 g and analysed by a slightly modified procedure of that recommended by the Joint Mercury Residues Panel[2]. As controls, 160-g samples of unwashed grain were divided into 20-g aliquots and analysed. The levels of mercury found are given in Table 1.

It was found in the case of one dry formulation, based on phenylmercury acetate and ethylmercury chloride, that half the dressing could be washed off, as described by Lindström. However, in the cases of the other three dry dressings examined, only 10–15 per cent was washed off. Neither the methylmercury dicyandiamide nor the phenylmercury acetate liquid formulations were readily removed. No evidence was obtained, therefore, in the

Table 1. Organomercury Compound on Dressed Grain before and after Washing with Water

Organomercury compounds used in dressings	Grain	Level of organomercury compound before washing expressed as p.p.m. mercury*	Level of organomercury compound after washing expressed as p.p.m. mercury*	Organomercury compound washed off	
				p.p.m. mercury	%
Methylmercury dicyandiamide (liquid formulation)	Wheat	6·3 ± 0·1	6·1 ± 0·2	0·2	3
		10·4 ± 0·1	9·2 ± 0·1	1·2	12
		15·7 ± 0·5	15·3 ± 0·7	0·4	3
	Barley	14·1 ± 0·7	12·2 ± 0·5	1·9	13
Phenylmercury acetate (liquid formulation†)	Wheat	16·5 ± 0·3	14·4 ± 0·3	2·1	12
Phenylmercury acetate and ethylmercury chloride (first dry formulation)	Wheat	21·9 ± 0·4	20·3 ± 0·7	1·6	7
	Barley	18·3 ± 0·2	17·6 ± 0·2	0·7	4
Phenylmercury acetate and ethylmercury chloride (second dry formulation)	Wheat	17·8 ± 0·5	9·4 ± 0·4	8·4	47
	Barley	26·6 ± 0·7	22·7 ± 0·5	3·9	15

* Mean ± *S.E.* of mean. † Mixed with a chlorinated hydrocarbon insecticide dressing.

investigation to suggest that appreciable amounts of a wet or dry organomercury seed dressing would normally be removed by washing dressed grain.

A subsidiary investigation showed that variances between 160 g samples from a seed treatment are significantly greater, but of the same order, as variances between 20-g sub-samples from the same 160-g sample. The standard errors of the means for eight 20-g sub-samples, quoted in Table I as an indication of the order of accuracy attained, are therefore slight under-estimations of the true errors for testing significance.

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ENTOMOLOGY

Insecticide Resistance in the Cocoa Capsid, *Distantiella theobroma* (Dist.)

THE first large-scale trials with γ-BHC against the cocoa capsids *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.) in Ghana were made in 1954[1]. The level of capsid control achieved, and the resultant crop yields of cocoa, were so outstanding that the use of this insecticide in recent years has been country-wide. Aldrin and endrin have also been used successfully and the control reported by Armstrong[2] as having been obtained with amounts of insecticide as low as ½ oz. active ingredient (a.i.) per acre is ample evidence that capsid strains, tolerant or resistant to chlorinated hydrocarbon insecticides, were unknown in Ghana in 1961. Now, however, capsids resistant to γ-BHC have been found on a plot of Amelonado cocoa at Pankese Cocoa Station, near Nkawkaw.

The plot was planted in 1956 and has since received four spray applications of γ-BHC each year, the initial spray of 4 oz. a.i. and all subsequent sprays of 1–2 oz. a.i. per acre applied in 5 gallons of water by portable, motorized mistblowers. In August 1961 it became apparent that the capsid population was building up on this plot and that the cocoa was being seriously damaged. Spraying frequency was therefore increased to once every two weeks and during December the trees were being treated individually at a rate equivalent to more than 4 oz. actual γ-BHC per acre per fortnight. However, the capsids were still not being controlled and the situation was therefore brought to the notice of the West African Cocoa Research Institute.

Using the laboratory bioassay method outlined by Armstrong[2], collections of capsids from Pankese have been compared with capsids from the Tafo area. These tests have established that the LD_{50} of γ-BHC is about 40 times higher for capsids from Pankese than it is for Tafo capsids, the decreased slope of the log. dose-probit line to 1·3 (Pankese) from 2·6 (Tafo) clearly indicating that the former have developed resistance, and not just tolerance, to γ-BHC.

A further test using aldrin, but limited in replication by the number of available capsids, has strongly indicated that the Pankese capsids are also resistant to this insecticide.

The distribution of γ-BHC-resistant capsids is thought not to be very widespread in Ghana at present, for while some cocoa at Pankese has been sprayed four times a year with γ-BHC since 1954, one of the plots in the original large-scale spraying trial being situated at this cocoa station, the other 25 sites, which also featured in the spraying trials of 1954, and which are scattered throughout the country, have similar histories of regular γ-BHC spray applications and, as yet, show no evidence of resistant strains.

The capsids at Pankese and those from Tafo, with which they were compared, were all *D. theobroma*, and it is noteworthy that this species is now the predominant cocoa capsid in Ghana, *S. singularis* being very uncommon. By contrast, in 1926, Cotterell stated that *S. singularis* was the most important cocoa pest and the population figures, given in the *Annual Reports of the West African Cocoa Research Institute*[3] and by Nicol[4], clearly show that it was once more numerous than *D. theobroma*.

S. singularis is considerably more active than *D. theobroma*, both as nymphs and adults, moving and feeding over a much larger area of the food plant[5]. The greater activity could lead to a relatively bigger pick-up of insecticide by *S. singularis* and render it more vulnerable than *D. theobroma* to toxic residues on sprayed trees. This could explain why Donald[6], using DDT, obtained 100 per cent control of *S. singularis* but not of *D. theobroma* in a trial in 1948 at Akwadum and also the recent, almost total disappearance of *S. singularis* from Ghana following the wide use of anti-capsid sprays. Thus, the sequence from about 1956 appears to be the replacement of *S. singularis* by a species less susceptible to insecticides followed by the development of insecticide resistance within that less susceptible species.

This communication is published with the permission of the Director of the Ghana Cocoa Research Institute, Tafo.

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CYTOLOGY

Karotype and Revised Chromosome Number of *Amphiuma*

A REVISED chromosome number of 28 has been established for the amphibian *Amphiuma means tridactylum* Cuvier, the congo eel, from studies of both somatic and

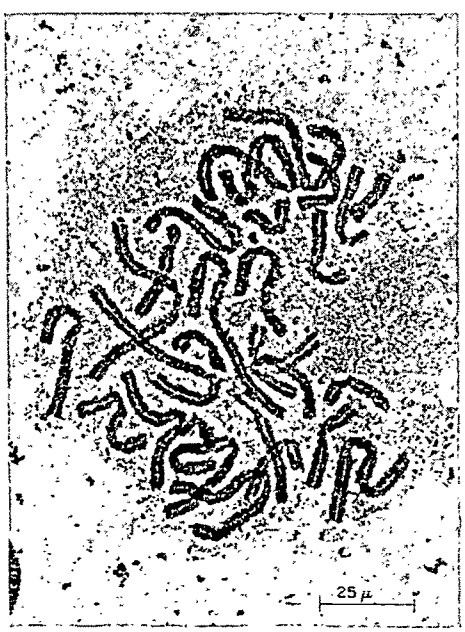


Fig. 1. Late prophase of mitosis in *Amphiuma* testis

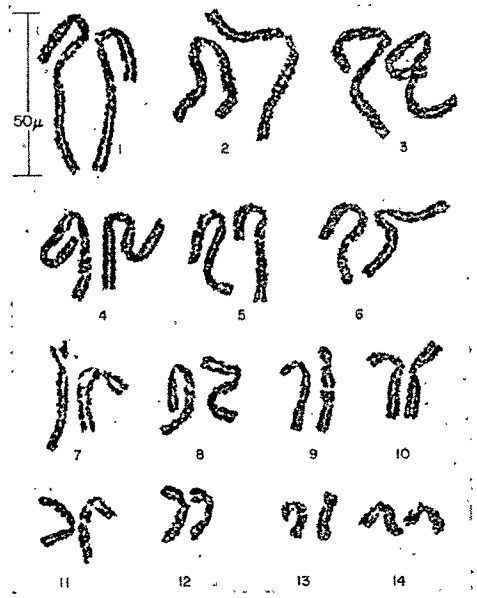


Fig. 2. Karyotype of *Amphiuma means tridactylum* constructed from Fig. 1

meiotic divisions in smears of testicular tissue. The somatic complement of 28 was clearly demonstrated in mitotic prophases and metaphases, most of which were of karyotype quality (Figs. 1 and 2). Counts derived from the various stages of meiosis, notably diakinesis (Fig. 3) and anaphase, further support the validity of the somatic count.

An examination of the karyotype (Fig. 2) reveals that the set is composed chiefly of submedian chromosomes. The largest chromosome in fully contracted metaphases has an average length of about 35μ; the smallest, a metacentric, averages about 9μ.

Heretofore, the accepted diploid number for *Amphiuma* has been 24 (refs. 1 and 2). This number is attributed to McGregor[3], who in 1899 published a study of spermatogenesis in *Amphiuma*. While McGregor did not indicate whether his material was from the two-toed or the three-toed form of *Amphiuma*, his description of an unlobed, spindle-shaped testis argues for his having studied *Amphiuma means tridactylum*[4]. It is well to note, however, that at the time of his investigation interest was in an elucidation of the process of meiosis *per se*, and not in a specific enumeration of the chromosomes.



Fig. 3. Diakinesis showing 14 bivalents

This work was carried out at the Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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GENETICS

Blood-typing of Indian Water Buffaloes with Reagents for Antigenic Factors of Cattle

More than eighty heritable antigenic factors have been detected on the erythrocytes (cells) of cattle by their reactions with blood-grouping reagents; the reagent being an antibody population made relatively specific for a particular factor by appropriate absorptions[1]. Using cattle reagents, it has been shown that at least nine genetic blood-group systems of cattle have their homologues in American bison, *Bison bison*[2–4], and Polish aurochs, *Bison bonasus*[5]. Water buffalo cells elicit antibodies in cattle which detect antigenic factors of three of the cattle blood-group systems[6]; suggesting a homology between the antigenic factors of cattle and water buffaloes. Naturally occurring agglutinins in the sera of cattle have been used to classify cells of Indian water buffaloes (*Bos bubalis*) into four types[7].

While on a visit to the Indian Veterinary Research Institute, Izatnagar, India, one of us (S. P. D.) tested cells of 40 water buffaloes of the Murrah breed with cattle blood typing reagents (*F*, *V*, *J*, and *Z*) prepared at Wisconsin. These reagents detected antigenic factors belonging to three blood-group systems. The blood typing, inhibition, titration and absorption tests were performed at 24 ± 2° C using standard techniques[8].

Neither the iso-immune *V* nor *Z* reagent, which detects factors at two independent blood-group loci (*F* and *Z*), reacted with any of the buffalo bloods. The heteroimmune *F* reagent produced slight hæmolysis with eight samples, but absorptions showed that these reactions were not specific. The *J* reagent (naturally occurring antibodies of normal cattle serum) reacted with six of the buffalo bloods; some of them reacted strongly (titres 128–256) and others weakly (titres 32–64). Absorption results suggested that the strong and weak reactions were due to quantitative variations in cellular *J* substance[9].

Sera from these six buffaloes with cellular *J*, and sera from 5 others lacking it as well as naturally occurring hæmolysins, were tested for inhibition of anti-*J*. The inhibition tests were done in duplicate using as test cells buffalo and cattle cells reactive with anti-*J*. The sera from the 6 buffaloes which had cellular *J* contained a soluble substance capable of inhibiting the anti-*J* reactions. Two of the 5 sera from the buffaloes that lacked cellular *J* did contain a *J*-inhibitory substance; the remaining three sera did not inhibit anti-*J*. The inhibition titres were slightly less using cattle rather than buffalo cells.

Sera from another 15 buffaloes lacking cellular *J* were tested for naturally occurring hæmolysins, and 11 sera did not contain them. One serum gave weak reactions that paralleled those of the *J* reagent in tests with cells from 10 buffaloes and 60 Hariana cattle. Sera from three other buffaloes contained antibodies reactive with most of the 62 cattle cells. Based on the blood types of these cells (obtained from the records of the Immunogenetics Laboratory, Indian Veterinary Research Institute), the reactions

of these 3 buffalo sera did not parallel those of any known cattle reagent. Absorptions with different cattle cells suggested that each serum contained a multiplicity of antibodies directed against unknown factors.

The reactivity of *F*, *V* and *Z* reagents with bison cells[2–4] and their failure to react with water buffalo cells suggest that serological relationship between water buffaloes and cattle is less close than between bison and cattle. Our results demonstrate that an antigenic system similar to *J* of cattle occurs in water buffaloes. This is another example of the ubiquity of the *J* blood-group substance. Whether or not the *J*-like substance of water buffaloes is identical with the *J* substance of cattle must await more data.

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Tests for Linkage of the Genes controlling Cattle Transferrins and Blood Groups

There are 11 genetically independent blood-group systems (loci) in cattle[1] which provide markers on perhaps a third of their chromosomes. Attempts to discover linkage between these blood-group genes and those controlling production traits have generally been unsuccessful[2]. Recent discoveries of various inherited biochemical polymorphisms such as transferrins, hæmoglobins, β-lactoglobulins, α-lactoalbumins, etc.[3], now provide additional markers for linkage investigations. This report presents the results of tests for linkage between the transferrin locus and seven of the blood-group loci.

Transferrin and blood-group data used in this analysis were obtained from Holstein–Friesian herds at Pabst Farms, Oconomowoc, Wisconsin; Emmons Blain, jun., Experimental Farm, Lake Mills, Wisconsin; and the Dairy Cattle Research Center, University of Wisconsin, Madison. Transferrin types were determined by vertical starch-gel electrophoresis using borate buffer[4]. The blood types were determined by the hæmolytic test[5] routinely used in our Immunogenetics Laboratory.

Linkage tests were performed by the sequential probability ratio test[6]. The heterozygote is distinguishable from the homozygote for transferrin type and for the *F* and *Z* blood-groups, but the genotypes of the other five blood-group loci had to be ascertained from the pedigree information. Individuals with doubtful genotypes were not used. Only families of sires heterozygous for both the transferrin (main locus) and blood-group loci (test loci) were used. The double and single backcrosses were the only informative matings. Lod scores were determined from the Z_1 tables computed by Morton[6].

Table 1 gives the accumulated lod scores from tests for linkage between the transferrin locus and each of the seven blood-group loci. The significance of the accumulated *Z* scores was tested by comparing ΣZ with $\log A$ and $\log B$. As recommended by Morton[6], $\log B = -2$ and $\log A = 3$ were taken as appropriate choices. Linkage of the order of 10 per cent recombination was excluded for blood-group loci *A*, *B*, *C* and *F*. For the *Z* and *S* loci, linkage at the level of 5 per cent recombination could be excluded. The data were not sufficient to exclude linkage with the *L* locus even at the level of 5 per cent recombination. Thus, there is yet no clear-cut linkage detectable between the blood-group loci and any other locus in cattle.

Table 1. Results of Sequential Probability Ratio Tests for Linkage between Transferrin Locus and Blood-group Loci

Blood-group locus	No. of sire families	No. of progeny	Accumulated lod scores (ΣZ) for different levels of recombination 0·05	0·10	0·20
A	7	26	−6·44	−3·79	−1·54
B	14	52	−9·15	−5·09	−1·80
C	13	57	−7·16	−4·12	−1·10
F	4	15	−5·05	−3·11	−1·36
Z	4	18	−3·26	−1·73	N.T.*
S	1	6	−2·16	−1·33	N.T.
L	2	6	−0·21	N.T.	N.T.

* N.T., not tested.

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Diploid-like Pairing in a Synthetic Decaploid *Avena*

In the course of investigations on species relationships within the genus *Avena*, a number of pentaploid hybrids derived from the cross *Avena abyssinica* Hochst. ($2n = 28$) × *A. sativa* L. (Black Mesdag) ($2n = 42$) were successfully treated with colchicine using the capping technique[1]. Thirty-six seeds were obtained from the doubled sectors of the treated pentaploid. Chromosome pairing was examined in four pentaploid hybrids and five decaploid plants. Twenty cells per plant were analysed and a short account of chromosome pairing at M_1 in both pentaploid and decaploid forms is given in Tables 1 and 2.

Table 1. Chromosome Pairing in the Pentaploid Hybrids

Hybrid No.	Mean No. of configurations per cell I	II	III	IV
4411 Cn	21·35	4·71	1·10	0·21
4412 Cn	22·25	4·14	1·00	0·25
4413 Cn	18·03	5·41	1·86	0·29
4415 Cn	19·22	4·86	1·77	0·18

On the basis of their investigations of chromosome morphology[2] and genome homology[3] in the *Avenae*, Rajhathy and Morrison concluded that there is only one common genome in the tetraploid and hexaploid species. They designated the genomic constitution of the hexaploid species as AACCDD and the tetraploids as AABB, and accordingly the expected theoretical pairing in the pentaploid would be 21 univalents and 7 bivalents. Some pairing between chromosomes of the A and B genome has been previously reported by Nishiyama[4] and the presence of multivalents in the pentaploid hybrids may be an indication of similar pairing.

According to the foregoing designation of the genomic relationships in the *Avenae*[2,3] the decaploid would be AAAABBCCDD with an expected chromosome pairing of up to a maximum of 7 quadrivalents and 21 bivalents. Lesik[5] reported 35 bivalents for a similar decaploid

(*A. abyssinica* × *A. sativa*). It will be observed, however, from Table 2 that although such complete pairing was not obtained the number of bivalents was far in excess of the theoretical expectation, and the number of multivalents less than expected.

Table 2. Chromosome Pairing in Synthetic Decaploids

Segregate No.	Chromosome No.	Mean No. of configurations per cell II	I	Multivalents per cell
352 Cx	68	31·70 ± 0·27	3·4 ± 0·43	0·35 ± 0·25
357 Cx	70	33·90 ± 0·16	1·9 ± 0·32	0·15 ± 0·08
358 Cx	69	30·75 ± 0·60	2·7 ± 0·33	1·15 ± 0·26
363 Cx	69	32·05 ± 0·34	4·15 ± 0·56	0·25 ± 0·10
370 Cx	70	33·35 ± 0·27	2·5 ± 0·32	0·25 ± 0·13

It is also interesting to note that two of the amphiploids 357 Cx and 370 Cx, both having the full decaploid complement, had a slightly higher number of bivalents per cell than the other decaploid plants investigated.

Since there is complete homology between the A genome of the tetraploid and hexaploid species[3,6] it would be expected that the chromosomes of the A genomes in the decaploid would behave like autotetraploids. Approximately 60 per cent of the chromosomes form multivalents in autotetraploids[7] and a comparable figure in the decaploid plants would normally be expected.

To account satisfactorily for the low number of multivalents found in decaploid plants one would have to postulate either the presence of a gene which suppresses multivalent formation between homologous chromosomes, or that the chromosomes of the A genome derived from the tetraploid species are slightly differentiated from those found in the hexaploid species. If the latter postulation is accepted, preferential pairing between A genome chromosomes derived from the same species would take place in the decaploids but, in the absence of the diploid complement in the pentaploid, chromosomes (AA) from tetraploid and hexaploid species were sufficiently homologous to form bivalents.

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VIROLOGY

An Analysis of Adenovirus Particles and Soluble Antigens produced in the Presence of 5-Bromodeoxyuridine

The addition of 30 μg/ml. of 5-bromodeoxyuridine (BUDR) to HeLa cell cultures infected with adenovirus type 5 results in a marked inhibition of the synthesis of infectious virus, but does not prevent the development of cytopathic effect nor the formation of complement-fixing antigen[1].

The work recorded here was designed to elucidate the action of BUDR on the synthesis of each of the three soluble adenovirus antigens previously described[2,3] and to investigate the electronmicroscopical and biological properties of virus materials produced in the presence of BUDR.

A concentrated suspension of HeLa cells (approximately 2×10^7 cells in 2 ml. of maintenance medium) was inoculated with 1 ml. of trypsin-digested[4] adenovirus type 5 material containing $10^{9 \cdot 0}$ TCD_{50}/ml. After contact for 1 h at room temperature the cells were sedimented by centrifugation, washed 3 times, each in 5 ml. of Gey's solution, and resuspended in 20 ml. of maintenance medium containing 30 μg/ml. of BUDR. After incubation for 70 h at 37° C the cells were collected and extracted by the technique of Gessler, Bender and Parkinson[5] using the fluorocarbon compound 'Arcton 133' (Imperial Chemical Industries, Ltd., London) as described by Pereira and Valentine[6].

The resulting material was fractionated by chromatography on a DEAE-cellulose column as described by Klemperer and Pereira[3]. Fractions were tested by complement-fixation against homologous rabbit antiserum which revealed the presence of three distinct elution peaks corresponding closely to those of antigens *A*, *B* and *C* previously described[3], except that antigen *A* produced in the presence of BUDR was eluted at slightly lower sodium-chloride molarities than the corresponding material obtained from untreated cultures.

The fractions were also tested for hæmagglutination using 1 per cent suspensions of rat erythrocytes with and without addition of a heterotypic adenovirus antiserum (type 1) at a dilution of 1/200. The results were perfectly comparable with those obtained with materials from untreated cultures[7]; fractions corresponding to antigen *A* were devoid of hæmagglutinating activity whereas antigens *B* and *C* were both capable of causing hæmagglutination, the latter requiring for this the presence of heterotypic antiserum in the system.

Samples of each of the 3 antigens fractionated by chromatography were concentrated 10 times by dialysis against 'Carbowax'[8] and tested against homotypic rabbit antiserum by gel diffusion precipitation side by side with antigens obtained in the absence of BUDR. No difference could be detected in this way between antigens produced in the presence or absence of BUDR.

HeLa cell monolayers infected with adenovirus type 5 in the presence and absence of BUDR were examined microscopically at intervals after inoculation. Techniques used were Feulgen staining for DNA after ethanol fixation, and observation by phase-contrast after osmium tetroxide fixation. The number of altered cells was similar in the two groups, but nuclear changes proceeded more slowly in the presence of BUDR, about 70 h being needed to reach the 48 h (terminal) stage of untreated cultures. Typical DNA-containing masses formed as usual, but associated columnar protein crystals were not seen in the BUDR-treated cells.

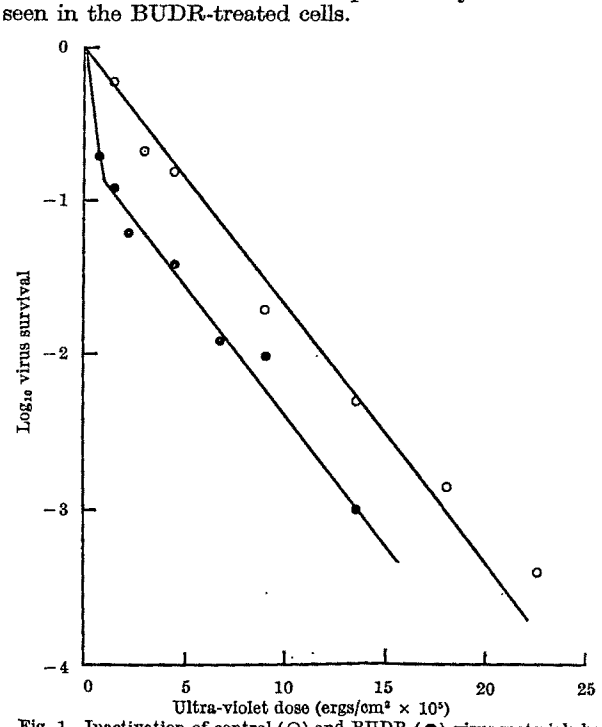


Fig. 1. Inactivation of control (○) and BUDR (●) virus materials by ultra-violet (2540 Å) radiation

Examination of the unfractionated BUDR virus material with the electron microscope revealed a relatively large number of particles with all the morphological characteristics of adenovirus particles. In preparations treated with uranyl acetate, a great majority of the particles showed a well-defined electron opaque centre surrounded by a less-opaque layer with hexagonal outline. This suggests that BUDR particles do not lack the central core of nucleic acid. Particle counts were done by the latex particle spray drop method with negative staining[9].

The infectivity titres of control and BUDR virus materials were determined by inoculating serial dilutions (0·5 $\log_{10}$ steps) into groups of 4 tubes of HeLa cells. The titration end points were read after 4 weeks' incubation with weekly fluid changes. The correlation between particle counts and infectivity titres is shown in Table 1.

Table 1. Particle Counts and Infectivity Titres of Adenovirus Type 5 from Control Cultures and from Cultures Treated with 30 μG/ML. of BUDR

Material	Particles/ml. ($\times 10^{10}$)	Infectivity doses/ml. ($\times 10^{10}$)	Particles/infectivity dose
Control	25	3·0	8
BUDR	3·5	0·0001	35,000

The low infectivity titre observed in the BUDR material could be due to residual virus from the original inoculum or, at least in part, to virus synthesized in the presence of BUDR. To test this point, we made use of the observation that incorporation of halogenated base analogues into the DNA of mammalian cells, bacteria and bacteriophages results in increased sensitivity to the inactivating action of ionizing or non-ionizing radiation[10–13]. Ultra-violet inactivation curves of control and BUDR virus materials are shown in Fig. 1. The inactivation rate of the control material is approximately constant throughout the range of ultra-violet doses included in the figure, whereas the BUDR material is at first inactivated at a rate nearly 6 times higher and afterwards at the same rate as the control preparation. From the distance between the parallel sections of the two curves it may be estimated that approximately 60 per cent of the infectivity of the BUDR material is about 6 times more sensitive to ultra-violet than the control material. From the results in Table 1, the remaining 40 per cent could be accounted for by the presence of 0·01 per cent residual virus particles from the inoculum, with normal ultra-violet sensitivity.

These results suggest that adenovirus particles containing BUDR are capable of initiating infection although the probability of this happening is considerably lower than with normal virus particles. It remains to be established whether other biological properties of the virus may be changed as a result of incorporation of BUDR.

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Adsorption *in vitro* of Bovine Enteroviruses by Non-cultured Bovine and Human Tissues

The results of some recent work[1–3] have produced evidence of the importance of specific virus cell-receptor(s) (which can be demonstrated in cell or tissue homogenates by its virus infectivity binding activity) as one of the major factors controlling the susceptibility *in vitro* of cultured cells and the species susceptibility *in vivo* to human enteroviruses, or the enteroviruses tissue tropisms *in vivo* within susceptible species.

Along the lines of work suggested by these findings, an investigation of the presence of receptors for bovine enteroviruses, in various non-cultured animal and human tissues, has been undertaken.

The preliminary results of this work, regarding the adsorption *in vitro* of bovine enteroviruses to some bovine and human tissue homogenates, are summarized in this communication.

The viruses used in this work were: poliovirus type 2 (strain *MEF*-1) and the following strains of bovine enteroviruses: *LCR*-4 (ref. 4), 47/2 and 121/1 (ref. 5).

Tissue homogenates were prepared as follows: samples of various bovine organs were obtained at the abattoir from a healthy, 4-months-old calf. The materials were sliced into small fragments, washed twice with Hanks's balanced saline solution, and ground with the aid of quartz sand in a porcelain mortar. The ground tissues were suspended at 20 per cent (w/v) in Hanks's balanced saline solution and stored at −70° C. Human tissues were obtained from the autopsy of a 21-year-old woman, who died from traumatic injuries. Tissue homogenates were prepared and stored as already described.

For the examination of adsorption, a technique very similar to that reported by Kunin and Jordan[1] was used. Briefly: 0·1 ml. of a proper virus dilution was added to a 0·4-ml volume of tissue homogenate, shaken and incubated in a 37° C water bath for 2 h. At the end of this time, 4·5 ml. of Hanks's balanced saline solution was added, the preparation thoroughly mixed, and centrifuged for 10 min at 3,000 r.p.m.

The virus assay, in suitable dilutions of supernatants, was performed in duplicate monolayers of primary culture of calf kidney cells, grown in Petri dishes, using the plaque technique of Dulbecco adapted to the bovine viruses used[5].

In the experiments with poliovirus, virus assay was performed using *KB* cell monolayers.

Virus assays were performed immediately or after a few hours of storage at +4° C.

Virus controls consisted of the same amount of virus, mixed with 0·4 ml of Hanks's balanced saline solution and treated in the same manner.

In Table 1 are summarized the results of the *in vitro* adsorption studies of bovine enteroviruses to various bovine tissue homogenates.

From these results it appeas that all the surveyed tissues contain a factor actively adsorbing the viruses. Moreover, the factor in tissues, responsible for 47/2 virus adsorption, as studied in large-intestine homogenate, was heat-labile

Table 1. Adsorption of Bovine Enteroviruses by Bovine Tissue Homogenates

Tissue homogenates	Percentage of virus adsorbed: Strain 47/2	Strain 121/1	Strain *LCR*-4
Intestine (small)	70	100	—
Intestine (large)	94‡	96§	92
Liver	60	55	—
Kidney	85	89	—
Lung	70	94	—
Brain	70	62	—
Heated* intestine (large)	0¶	—	—
Intestine (large) treated with ether†	0	—	—

* 60° C for 30 min.
† Treated with 50 per cent ether for 30 min at 37° C.
‡ Average from five experiments (values ranging from 90 to 98 per cent).
§ Average from four experiments (values ranging from 92 to 100 per cent).
¶ Results obtained in two experiments.
— Not tested

Table 2. Adsorption of Bovine Enteroviruses by Human Tissue Homogenates

Tissue homogenates	Percentage of virus adsorbed	
	Strain 47/2	Strain 121/1
Intestine (small)	93*	97
Liver	0	0
Kidney	0	0
Brain	0†	0

* Average from four experiments (values ranging from 76 to 99 per cent).
† Results obtained in two experiments.

Table 3. Adsorption of Poliovirus Type 2 by Human Intestine and by Some Bovine Tissue Homogenates

Tissue homogenates	Percentage of virus adsorbed
Human intestine (small)	98
Bovine intestine (small)	0*
Bovine intestine (large)	0†
Bovine liver	0
Bovine brain	0

* Results obtained in five experiments.
† Results obtained in two experiments.

at 60° C and inactivated by treatment with ether. This finding rules out the possibility that the binding activity towards virus infectivity, found in tissue homogenates, was due to the presence of blood antibodies or of non-antibody serum inhibitors against bovine enteroviruses[6–8].

These results, in that they demonstrate a large diffusion of a factor able to bind the bovine enteroviruses infectivity in the susceptible animal host, are very similar to those obtained for poliovirus, in monkey and human organisms, by Kunin and Jordan[1].

Moreover, the bovine enteroviruses-binding factor, in respect of heat lability and ether sensibility, behaves like the human enterovirus cell-receptor.

With this in mind, attempts were made to investigate the possible identity of the bovine enteroviruses-binding factor, which probably represents a specific virus cell-receptor, with the receptor for human enteroviruses, by studying the adsorption *in vitro* of bovine enteroviruses by human tissues, and the adsorption of a human enterovirus (namely poliovirus type 2) by some bovine tissue homogenates.

The results of the adsorption study of bovine enteroviruses, strains 47/2 and 121/1, by human tissue homogenates are reported in Table 2.

These results indicate that the two bovine enteroviruses are adsorbed by human intestine as actively as by the bovine intestine, while no adsorption could be demonstrated by the other human tissues surveyed, which to some extent demonstrates the specificity of the adsorbing activity of the human intestine, and its independence from contamination with blood which on some occasions is known to possess neutralizing activity versus bovine enteroviruses[7,8].

However, from the results of the investigation of adsorption of poliovirus type 2, by human intestine and by some bovine tissues, reported in Table 3, it appears that while the human intestine homogenate presented a strong poliovirus binding activity, none of the bovine tissues tested was able to do so.

These findings demonstrate that the bovine enteroviruses cell-receptors, although they probably have to be considered of similar chemical nature, differ from the receptors for human enteroviruses in their virus-binding specificity.

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VETERINARY SCIENCE

Environmental Temperature preferred by the Young Pig

Investigation has shown both the marked influence of environmental temperature on the young pig's metabolic rate and the modification of the effect of environment due to grouping new-born pigs together[1,2]. Other work has emphasized the importance of ambient temperature in relation to animal production in pig husbandry[3,4]. Complementary to measurements of metabolism and growth is the determination of the environmental temperature which is preferred by the animal, and observations have been made on young pigs for this purpose.

A simple form of thermocline was constructed. This was a hardboard enclosure 2·5 m long, 0·65 m high and 0·65 m deep, divided into four compartments, each approximately 0·6 m wide and provided with a 1-cm mesh wire floor. Each compartment had a door at the front, and each connected with the next through openings 25 cm high in the bottom of the dividing walls. Fans and heaters were mounted in an enclosed space over the chambers and were so adjusted that air was blown uniformly into the backs of the compartments; inside these the air movement did not exceed 15 cm sec^{-1}. Temperature increased progressively over the range 23°–37° C along the length of the thermocline.

38 pigs (Large White breed) were used in the experiments at various intervals between birth and 41 days; body-weight ranged from 0·90 to 9·68 kg. All the animals were feeding normally from the sow in pens in a farrowing house fitted with heated creeps. The air temperature in the farrowing house varied between 12° and 19° C during the four months in which the experiments took place. The pigs were left in the pen with the sow until 20 min before the beginning of the experiment, when they were introduced into the thermocline either singly or in groups. For observations on single pigs, the number of the door for entry into the thermocline was drawn by lot, the animal was introduced, and at 15-min intervals its position was noted through small inspection holes. After 45 min the animal was removed and returned to the sow. For observations on groups of 5 pigs, the order in which the pigs were to be introduced into the thermocline, as well as the doors of entry, were allocated at random by drawing lots. Positions were noted at 15-min intervals for a total of 60 min.

Table 1. Ambient Temperatures preferred by Young Pigs, both Singly and in Groups of Five

Age of pigs (days)	Ambient temperature (° C) 23–28	29–31	32–34	35–37	Mean ± *S.E.*
Single pigs					
< 1	1	2	5	2	32·3 ± 0·94
1–7	8	16	4	0	29·3 ± 0·44
8–41	16	33	11	6	29·8 ± 0·64
Groups					
2–7	2	3	3	0	30·1 ± 1·03
8–41	3	9	4	1	30·4 ± 0·66

In the case of pigs introduced singly, on 89 per cent of occasions the final compartment noted as occupied was already occupied after 30 min in the thermocline, that is, 15 min before the end of the observation period. For the groups, the final position had been taken up after 45 min (15 min before the end) on 72 per cent of occasions. All 5 pigs of the group were found together on 59 per cent of occasions, and in a further 23 per cent only 1 pig was separated from the group and occupying another compartment.

In Table 1 are shown the frequencies with which single pigs and groups occupied different compartments at the end of the periods of observation. The only significant difference in the results lies between single pigs less than 1 day old and those aged 1–7 days ($P = 0·01$). For the pigs older than 1 day there is no further significant change with age, either in the case of single pigs or of groups.

Rectal temperatures changed little in the time taken for observation. During the 45-min period in the thermocline the rectal temperatures of the pigs introduced singly showed a fall of 0·03 ± 0·03° C (mean and standard error, 104 observations). During their 60-min periods the pigs in groups showed a mean rise in rectal temperature of 0·07 ± 0·03° C (125 observations).

The higher preferred temperature for pigs less than 1 day old might be associated with their relatively lower metabolic rates and body temperatures, which were rising slowly following the initial fall at birth[1]. The critical temperature (that is, the environmental temperature at the lower limit of the thermoneutral zone) of pigs up to 1 week old is in the region of 34° C (ref. 1), which is above the preferred mean of approximately 30° C. Pigs older than 1 week, however, still preferred a mean temperature close to 30° C, although in the case of approximately half these pigs the critical temperature would be between 25° and 30° C (ref. 2). None the less, as the measurements of rectal temperature show, thermoregulation was satisfactorily achieved.

In these experiments the air and wall temperatures were similar, so that the chosen temperature referred to both air and radiant temperatures. In the pig farrowing house, in farming practice, the use of creep heaters means that the radiant temperature is higher than the air temperature, so that there will be a resultant operative temperature[5] different from either. The air temperatures used in the present experiments might apply as operative temperatures for litters of pigs in farrowing pens, and the observations made suggest that 30° C is the preferred short-term level for pigs more than 1 day old and 32° C for the first day after birth.

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SOIL SCIENCE

Examination of the Structure of Soil Humic Acids by Pyrolysis - Gas Chromatography

Zemany[1] and Harms[2] indicated that, if the conditions of pyrolysis are maintained constant, an organic substance will decompose in a specific manner. They utilized this principle for the identification of complex organic materials by pyrolysis-infra-red, or by pyrolysis-mass spectrometry techniques. A number of workers[3–10] have also used pyrolysis-gas chromatography techniques for the identification of polymers and for elucidating the structure of non-volatile organic compounds by their characteristic chromatographic patterns. The work recorded here was designed to determine the suitability of the pyrolysis-gas chromatography technique for the identification of the components of soil organic matter, and to establish the lignin or microbial origin of soil organic matter by a comparative study of the chromatographic patterns.

Four samples of soil humic acids (extracted by sodium hydroxide by the technique suggested by Lynch[11]) obtained from widely different pedological, vegetational and climatic type soils, namely, a half-bog soil from southern Delaware (Pocomoke), a prairie soil from Illinois (Sable), a red yellow podzol from North Carolina (Cecil), and a tropical Latosol from Brazil; a sample of humic acid (extracted by sodium hydroxide) obtained from *Aspergillus niger*; and a sample of commercial lignin ('Indulin', supplied by West Virginia Paper and Pulp Co.) were used for the investigation. The pyrolysis was carried out in a stream of helium carrier gas, and the pyrolysates were fed directly into the gas chromatograph.

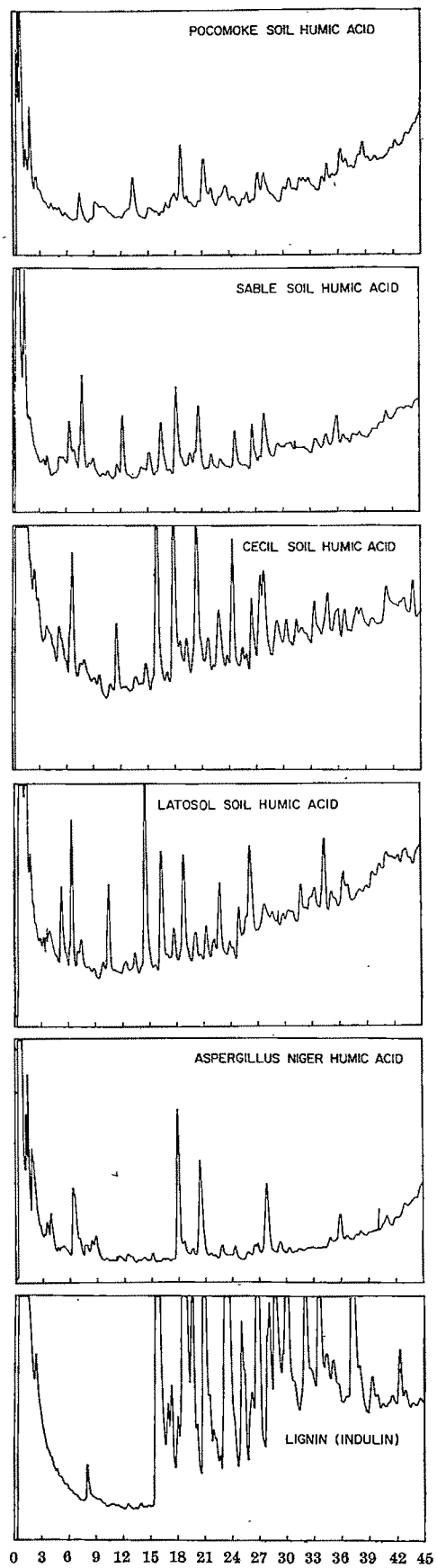


Fig. 1

Repeated experiments on the pyrolysis-gas chromatographic analysis of these six samples were conducted by varying the nature of the column, temperature of pyrolysis, sample size, nature of carrier gas and other experimental conditions. However, based on the repeated analyses, the best separation of the pyrolysates was obtained under the following experimental conditions: gas chromatograph, F. and M. Scientific Corp., U.S.A., model 609 temperature programmed instrument; column dimensions, 6 ft. × 1/8 in. (inner diam.) or 3/16 in. (outer diam.) stainless-steel coiled tube; solid support, 'Diatoports' (60–80 U.S. mesh), acid washed, base washed, siliconized; stationary phase, '*xE*-60 Nitrile Silicone' polymer (4 per cent w/w); temperatures, injections, 550° C, column: 50° C→225° C 6·4° C/min linear; carrier gas, helium at 110 ml./min; inlet pressure, 60 lb./in.^{2}: outlet pressure, atmosphere; detector, F. and M. Scientific Corp., flame ionization; recorder, Minneapolis-Honeywell Co.; 1 sec; 1/3 in./min. chart speed; sensitivity, 100 × 16; sample size, 1 mg solid (injected by F. and M. Scientific Corp. solid sample injector); analysis time, 45 min.

The chromatographic patterns obtained by the pyrolysis of the samples under aforementioned experimental conditions are shown in Fig. 1. These are representative figures based on the repeated analysis of each of the six samples. It is indicated that the pyrolysates of the Pocomoke soil humic acid contain about 10 major and 41 minor components; the pyrolysates of Sable humic acid contain about 12 major and 27 minor components; the pyrolysates of the Cecil humic acid contain about 17 major and 30 minor components; the pyrolysates of Latosol humic acid contain about 16 major and 37 minor components; the pyrolysates of *Aspergillus niger* humic acid contain about 9 major and 29 minor components; and the pyrolysates of lignin contain about 19 major and 17 minor components.

It is significant to note that the pyrolysates of lignin or *Aspergillus niger* humic acid and soil humic acid samples (Fig. 1) have quite a few common components. However, it is difficult to generalize on the quantitative aspect of the microbial or lignin origin of the soil humic acid on the basis of the present results. It is also interesting to observe that the pyrolysates of Sable humic acid and the pyrolysates of *Aspergillus niger* humic acids have about 19 similar components.

Furthermore, the present findings indicate that the pyrolysis-gas chromatography technique may provide useful results for solving the problem of the composition of soil organic matter, provided the components could be identified by infra-red, mass spectrometry, or nuclear magnetic resonance techniques.

I thank Prof. G. T. Felbeck, jun., for advice and Mr. Sam Spencer for suggestions. I am grateful also to the F. and M. Scientific Corporation, U.S.A., for providing laboratory facilities for pursuing a part of the present investigation. This communication is published with the approval of the Director of the Delaware Agricultural Experiment Station; funds for the investigation were provided by Andelot, Inc., through the University of Delaware Research Foundation.

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PSYCHOLOGY

Effect of Anxiety on Real and Ideal Vocational Aspiration among Grammar School Boys

During the course of the collection of data for an investigation of certain problems related to school-leaving behaviour among fifth-form boys in a Midland's grammar school, information was obtained about their real and their ideal expectations about their own occupation achievement. This report is concerned with an analysis of relationships among these real and ideal vocational aspirations and achievement-related anxiety as measured by a modified form of the test anxiety questionnaire (TAQ)[1] which had been prepared for use within the British school system as a 15-item self-rating scale.

One hundred and thirty-one fifth-form boys were available for the investigation. Of these 131 boys, 96 provided the data reported here. The boys were assembled in two groups of approximately equal size on subsequent days during regular school hours and the TAQ was administered along with a questionnaire containing two sets of four questions about their vocational aspirations. Each question in a set differed from the other three questions only to the extent of asking for a report on vocation aspiration for a different future date. These dates were 1, 2, 5, and 10 years hence. One set of questions had to do with real vocational aspiration and asked: "What would you most like to do or be one (two, five, ten) year(s) from now?" The other set of questions had to do with ideal vocational aspiration and asked: "What would you most like to do or be one (two, five, ten) year(s) from now?" The first set of questions about real aspiration was preceded by the instruction to report ". . . what you really think you will be spending most of your time doing; what kind of job you expect to have, or what you expect to be studying in school, for example". The second set of questions having to do with ideal vocational aspiration was preceded by the request to, "Please answer the following questions in terms of what you would most like to do or be if anything were possible".

Answers to the first set of questions were taken to be an indication of the vocational achievement to which a boy actually aspired, and answers to the second set of questions were considered to be an indication of his ideal vocational aspiration. Only those boys for whom a consistency was present within each set of questions over all four questions were included for this communication. Ninety-six of the boys met this criterion.

For purposes of statistical analysis, a numerical score was assigned to each boy's vocational aspirations by comparing his stated real and ideal vocational goals with the system of social grading of occupations developed by Hall and Jones[2]. The Hall and Jones system incorporates seven social class categories and each boy's real and ideal occupational aspirations were assigned a number between one and seven based on the social class position given to the occupations by Hall and Jones. In this system, No. 1 is given to the highest social class category, No. 2 to the next, etc. The seven categories are: (1) professionally qualified and high administrative; (2) managerial and executive; (3) inspectional, supervisory and other non-manual (higher grade); (4) inspectional, supervisory and other non-manual (lower grade); (5) routine grades of non-manual work and skilled manual; (6) manual, semi-skilled; (7) manual, routine.

The TAQ scores were treated in the following manner. A frequency distribution of the TAQ scores was prepared, and the median of the distribution was determined. The range of TAQ scores was 13·0–49·0 and the median was 29·5. Scores above the median score were considered to represent high test anxiety (high TAQ) and scores below the median score were considered to be low test anxiety (low TAQ). Forty-eight boys thus formed the high TAQ group and 48 formed the low TAQ group.

To assess the relationship between achievement-related anxiety and vocational aspiration, the high TAQ and low TAQ groups were compared on the basis of the social class assignment given to their real and ideal vocational aspirations.

Table 1 presents the results of the investigation. Ideal aspiration does not differ for the two anxiety groups ($F < 1{\cdot}0$). However, within the high anxiety group real aspiration is appreciably lower than ideal aspiration at a highly significant level ($F = 11{\cdot}54$, $df = 1$ and 94, $P < 0{\cdot}01$) and real aspiration of the high TAQ group is significantly lower than the real aspiration of the low TAQ group ($F = 5{\cdot}69$, $df = 1$ and 94, $P < 0{\cdot}05$). No statistically significant difference exists between ideal and real aspiration among the low TAQ group ($F = 1{\cdot}87$, $df = 1$ and 94, $P > 0{\cdot}10$) though the difference is in the direction of a higher ideal level of vocational aspiration for this group.

Table 1. Mean Ideal and Real Levels of Vocational Aspiration of High and Low Anxiety (TAQ) Grammar School Boys
(N = 48 high and 48 low anxiety subjects)

	Vocational aspiration		
TAQ	Ideal	Real	
High	2·19	3·02	$P < 0{\cdot}01$
Low	2·10	2·42	$P > 0{\cdot}10$
	$P > 0{\cdot}10$	$P < 0{\cdot}05$	

Atkinson and Litwin[3] have argued that the TAQ provides an index of motivation to avoid failure. The higher the TAQ score the more one is motivated to avoid situations in which failure is likely or, if it is not possible to avoid completely such situations, then to modify them as much as possible in the direction of reducing the possibility of failure. Accordingly, it is suggested here that the setting of a relatively low level of real vocational aspiration by the subjects with high TAQ scores represents an attempt by them to protect themselves from failure. By setting relatively low real aspirations they increase their probability of achieving their goals and hence they reduce the probability of experiencing failure. On the other hand, when asked to state an ideal goal, one is free to set high aspiration-levels unrestricted by the possibility of experiencing failure. Because these ideal goals will not be striven for, anxiety imposes no restraints on this type of goal setting.

This work was carried out while I was a Fulbright professor of social psychology in the University of Nottingham.

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National Science Foundation,
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Fragmentation of Patterned Targets when viewed as Prolonged After-images

When an object is viewed through some optical system which stabilizes its image on the retina a breakdown of normal vision occurs and the target may disappear[1,2]. When complex targets are viewed under these conditions disappearances may be partial[3], and the fragmentations which occur may consist of distinctly ordered, non-random segments of the original figure[4].

Most workers (for example, see refs. 5 and 6) observe, with their stabilizing apparatus, that targets disappear and reappear at short intervals when viewed for several minutes. Sometimes the whole and sometimes part of the target reappears[3]. One worker[7] suggests that all reappearances are due to a failure of the stabilization system through slippage of a contact lens, but it is difficult to explain partial reappearances in this way. Slippage would cause the entire image to shift across the retina and fall on fresh receptors. The question would be clarified if complete stabilization, without possibility of movement of the retinal image, could be achieved. It is indisputably difficult to eliminate slip of a contact lens[7] and impossible to prove that this has been done, but after-images are almost certainly stationary on the retina[8–10].

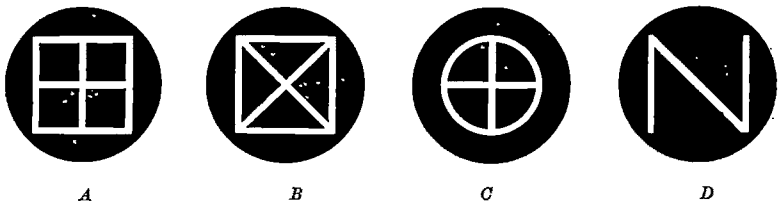


Fig. 1. Targets used in this study. The side of the square subtended 2° arc

In the present experiment we investigated the appearance of prolonged, patterned after-images. The experimental targets were drawn up on black paper out of which the outline shape of the required pattern could be cut. The black paper was backed with a sheet of tracing paper. The target was illuminated from behind by a photographic flash-bulb set in a reflector. The translucent parts of the target appeared very bright, yet evenly illuminated. With Philips '*PF*5' bulbs in a 7-in. reflector, the after-image remained visible for at least 10 min, and was sufficiently clear and positive to allow unequivocal observations to be made for at least 5 min. The duration of the flash (maker's tables) was 24 msec and the duration above half-peak intensity 12 msec. The estimated brightness of the target was about 10^6 candles m^{-2}. sec.

Five subjects were presented with the target simultaneously. Each stood about 3 m from the target, which, 10 cm in diameter, subtended an angle of approximately 2 degrees. Subjects were each instructed to fixate a particular point on the target with one eye covered. Directly the flash had occurred the experimenter switched out the lights, leaving the room in complete darkness. After 5 min observation under these conditions, subjects were asked to report on the phenomena that had occurred. Further trials were conducted after a period of at least 15 min had elapsed. Observations from trial to trial were made with alternate eyes.

The targets used were those illustrated in Fig. 1. Two trials were made with each target and all subjects reported sharp and definite after-images in each case. The general nature of the results may be summarized as follows: (*a*) After-images were of great clarity and brilliance initially, gradually losing detail but retaining sufficient positive qualities to allow reasonable observations to be made for the experimental period. (*b*) After-images faded frequently but at unpredictable intervals. Fading or disappearance was occasionally of the complete pattern, but more commonly of some part or parts of it. (*c*) There was a strong tendency for the straight lines of which a target was composed to fade as units. Typical fragmentation effects for Fig. 1*C* are illustrated in Fig. 2. (*d*) After

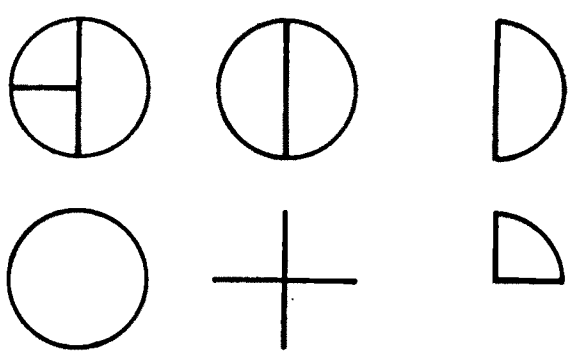


Fig. 2. Diagram showing the most frequent fragmentations observed with the after-image of Fig. 1*c*. It will be noted that entire figures are typical

fading, the image would reappear after some indefinite period of time. The nature of its reappearance was often total, that is, the image would regenerate to its normal state as a complete pattern. All subjects also reported that partial regenerations of the image might occur, and that these might feature the reappearance of single or parallel straight lines as units. (*e*) The segment of the target least likely to disappear was in the neighbourhood of the fixation point; the more peripheral the part of the pattern, the more likely it was to disappear. (*f*) After about 3 min, subjects reported a tendency for filling in of the whole of the enclosed part of the target. In due course detail was found to reappear in full. Similar results have been obtained with stabilized retinal images[5].

It has been found that, with vivid after-images of geometrical outline shapes, a range of disappearance and reappearance phenomena is experienced comparable with that reported from studies utilizing mechanical stabilizing systems. Of particular interest is the finding that disappearances may be structured, and that a large number of reappearance effects are only partial.

Yarbus[11] and Barlow[7] have suggested that a perfectly stabilized image will suffer total and lasting disappearance and that regeneration of the image must be a consequence of mechanical lens destabilization. While it is not disputed that contact lens slip will induce reappearance of an image, it now seems most likely that the partial and structured reappearances of images reported in this and other[3] studies cannot be attributed solely to such movement. Theories of retinal function that predict permanent disappearance of stabilized images will therefore need reconsideration.

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MISCELLANEOUS

Thin-layer Chromatographic Separation and Quantitative Determination of Several Free Amino-acids of Avian Liver

Thin-layer chromatographic procedures are used widely and accepted as efficient and rapid means of separating numerous organic compounds on a micro scale. The following procedure is presented to illustrate the thin-layer chromatographic separation and rapid quantitative determination of several free amino-acids of avian liver tissue.

Table 1. Recovery of Amino-Acids in a Standard Reference Solution

Amino-acid	Present	Found	Recovered
	μg/3 μl. solution		(%)
Lysine	0·30	0·30	100
Histidine	0·30	0·28	93
Arginine	0·30	0·31	103
Aspartic acid	0·30	0·32	106
Alanine	0·30	0·32	106
Valine	0·30	0·27	90
Leucines	0·30	0·28	93

Table 2. Several Free Amino-Acids of Avian Liver

Chick No.	Plate No.	No. of samples	Lysine	Histidine	Arginine	Aspartic acid	Alanine	Valine	Leucines
			← (μg/3 μl. extract) →						
20	1	6	0·41 ± 0·013*	0·29 ± 0·018	0·33 ± 0·013	0·56 ± 0·056	0·40 ± 0·039	0·28 ± 0·032	0·45 ± 0·031
20	2	6	0·43 ± 0·017	0·31 ± 0·025	0·38 ± 0·014	0·60 ± 0·049	0·41 ± 0·025	0·27 ± 0·028	0·45 ± 0·033
43	1	1	0·51	0·24	0·34	0·50	0·62	0·45	0·67
43	2	1	0·57	0·24	0·38	0·45	0·54	0·45	0·70
52	1	1	0·65	0·30	0·54	1·10	0·77	0·65	1·03
52	2	1	0·62	0·30	0·56	0·75	0·77	0·65	1·00

* Standard error of mean.

Avian liver samples are homogenized in cold distilled water (1 : 4, w/v). Free amino-acids are separated according to Awapara[1] by precipitating the proteins with absolute alcohol and separating the aqueous and organic solvent layers with chloroform.

TLC layers are prepared fresh on glass or plastic[2] plates according to standard procedures, using silica-gel *G* (Brinkmann Instruments, Great Neck, New York). When dry, the plates are placed on a heating device and warmed to 65° C and then spotted with the amino-acid solutions. A total volume of 3 μl. for each spot has been found to be appropriate. A standard reference solution of amino-acids is spotted between each of 2 unknowns. With this procedure a 20 × 20 cm plate is satisfactory for 3 standards and 6 unknowns.

Chromatographic glass tanks, of a type that can be sealed, are kept in a constant temperature atmosphere of 53° C. After the plates are spotted they are placed immediately in tanks containing a mixture of *n*-butanol-acetic acid and water (60 : 20 : 20, v/v/v). Each plate is placed in a separate tank. The solvent is allowed to run 160 mm. The plates are then removed, thoroughly dried, and re-run in the same direction under the same conditions; however, this time a 75 per cent phenol (chromatographic grade) is used as the solvent. The plates are dried again, stained with 0·25 per cent (w/v) ninhydrin (triketohydrindene hydrate) in acetone, and set in the dark for a standardized interval until the colour has developed. The chromatograms are then scanned at 525 mμ in a thin-layer chromatography densitometer (Photovolt Corp., New York).

The recovery of 7 amino-acids in a 3-μl. standard solution is shown in Table 1. Table 2 gives the content of 3-μl. extracts of several free amino-acids of avian livers. Experimental errors related to the analysis of micro quantities of biological materials are well known. Variability can be lessened and reproducibility increased if particular stress is placed on standardizing environmental conditions, preparing uniform plates, obtaining clear-cut separations and sufficient density and constancy of colour development for the particular areas to be scanned.

We thank Harry Veros for assistance. The work was supported in part by grants from the U.S. Public Health Service (E-3645) and the Photovolt Corporation, New York.

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FORTHCOMING EVENTS

Tuesday, September 24

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. P. R. Thomas: "Anionic Stereospecific Polymerization of N-distributed Acrylamides and some Vinyl Ketones".

SOCIETY OF INSTRUMENT TECHNOLOGY, AUTOMATION SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. D. B. Foster and Mr. R. I. Paterson: "Automatic Parts Handling".

Wednesday, September 25

MINERALOGICAL SOCIETY (in the Geology Department, The University, Manchester), at 2.30 p.m.—Exhibits and Scientific Papers.

BONE AND TOOTH SOCIETY (at the Institute of Orthopædics, 234 Great Portland Street, London, W.1), at 5 p.m.—Mr. L. B. H. Tarlo: "The Structure of Aspidin"; 5.30 p.m.—Mr. J. D. Currey: "Mechanical Aspects of the Structure of Bone"; 6 p.m.—Mr. J. W. Smith: "Age Changes in the Composition of Bone at the Microscopic Level".

Thursday, September 26

OIL AND COLOUR CHEMISTS' ASSOCIATION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. M. R. Mills: "The Scientific Evaluation of Unfamiliar Vegetable Oils".

Friday, September 27

OCCAM SOCIETY (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Discussion on "Life and Body" opened by Mr. A. Healey.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with a good honours degree in geography, and either a higher degree or actively engaged in research leading to a higher degree and preferably previous lecturing experience) IN GEOGRAPHY at the University College of Townsville, University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, September 27).

RESEARCH ASSISTANT AT THE TIDAL INSTITUTE AND OBSERVATORY, for a study of methods of forecasting storm surges along the East Coast—The Registrar, The University, Liverpool (September 27).

LECTURER (with experience in teaching chemistry and preferably research in education and/or experience of industry) IN EDUCATION—The Registrar, The University, Nottingham (September 28).

SENIOR LECTURER (honours graduate with wide industrial and teaching experience, and able to undertake research) IN THEORY OF MACHINES AND MACHINE DESIGN at Kingston upon Hull College of Technology—The Chief Education Officer, Guildhall, Kingston upon Hull (September 28).

SENIOR LECTURER (preferably with a research interest in a branch of mathematical analysis) IN MATHEMATICS—The Secretary, The Royal College of Science and Technology, Glasgow (September 28).

SENIOR TECHNOLOGIST (with sound training and experience in electron microscopy (RCA, EMU 3, and Philips EM 100 available) and ultramicrotomy, and preferably with a science degree or diploma) IN THE MEDICAL RESEARCH UNIT—Prof. C. E. Lumsden, Department of Pathology, School of Medicine, The University, Leeds 2 (September 28).

ASSISTANT LECTURER IN BOTANY—The Secretary, The Queen's University, Belfast, Northern Ireland (September 30).

ASSISTANT LECTURER IN THE DEPARTMENT OF VETERINARY HYGIENE AND PREVENTIVE MEDICINE, Royal (Dick) School of Veterinary Studies—The Secretary, The University, Edinburgh (September 30).

D.S.I.R. RESEARCH FELLOW (with a Ph.D. or equivalent postgraduate experience in experimental geophysics, and preferably experience in the maintenance or development of electronic equipment) IN SEISMOLOGY IN THE DEPARTMENT OF ASTRONOMY, to undertake field and laboratory studies of seismic waves and to relate their observations to earth structure and tectonics—Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh (September 30).

GENERAL SECRETARY (with considerable administrative experience and a broad interest in natural history)—The General Secretary, Council for Nature, 41 Queen's Gate, London, S.W.7 (September 30).

LECTURER IN BIOCHEMISTRY IN THE DEPARTMENT OF CHEMISTRY in the Faculty of Science—The Registrar, The University, Manchester 13 (September 30).

LECTURERS (2) IN RADIO-ASTRONOMY IN THE DEPARTMENT OF PHYSICS of the Faculty of Science, to pursue research at the Nuffield Radio-Astronomy Laboratories, Jodrell Bank, where the Mark I 250 ft. Radio Telescope is in operation and the Mark II is under construction—The Registrar, The University, Manchester 13 (September 30).

SCIENTIST (with a good honours degree in mathematics or physics or the equivalent qualifications in statistics, and experience in the application of electronic computer techniques), to design appropriate techniques of data processing and computation in relation to research and other problems in the field of radiological protection—The Director, Radiological Protection Service, Clifton Avenue, Belmont, Sutton, Surrey (September 30).

EDUCATIONAL PSYCHOLOGIST (with an honours degree in psychology or equivalent, and preferably teaching and clinical experience)—Chief Education Officer, P.O. Box 480, Education Offices, Manchester 3 (October 4).

ASSISTANT LECTURER (specializing in mycology, and preferably with interests in other aspects of cryptogamic botany and plant pathology) IN BOTANY—The Registrar, The University, Manchester 13 (October 5).

LECTURER OR ASSISTANT LECTURER (with a good honours degree in pharmaceutical chemistry or chemistry or an equivalent qualification, and preferably experience in industrial research or medicinal chemistry) IN PHARMACY—The Registrar, The University, Manchester 13 (October 5).

ASSISTANT LECTURER (with primary interests in inorganic chemistry) IN CHEMISTRY—The Registrar, University College of North Wales, Bangor, North Wales (October 7).

LECTURER (with teaching experience and a special interest in the sociology, psychology or philosophy of education) IN EDUCATION, to assist with full-time courses for experienced practising teachers studying for the Diploma in Education—The Secretary, The University, Exeter, Devon (October 7).

HORTICULTURE GRADUATES (with an honours degree in horticulture) IN THE HORTICULTURE DIVISION—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (October 10).

LECTURERS OR SENIOR LECTURERS (2) IN THE DEPARTMENT OF PHYSICS, Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 10).

STATISTICIAN (with an honours degree, and preferably research or related experience in the design and analysis of experimental and survey work) IN THE STATISTICS AND BIOMETRICS DEPARTMENT, An Foras Taluntais—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (October 11).

LECTURER (registered medical practitioner, preferably with some experience of hæmatology) IN THE DEPARTMENT OF MEDICINE—The Secretary, The University, Aberdeen (October 12).

VETERINARY PARASITOLOGIST (with a degree in veterinary science or its equivalent, together with a Ph.D. degree or research training of an equivalent standard and duration, and satisfactory evidence of postgraduate experience in a relevant field of parasitology) IN THE MCMASTER ANIMAL HEALTH LABORATORY, Commonwealth Scientific and Industrial Research Organization, Sydney, to participate in specific aspects of the programme of research concerned with the epidemiology and control of helminth infections of sheep—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 202/200 (October 12).

LECTURER OR ASSISTANT LECTURER IN PLANT BREEDING AND GENETICS in the Faculty of Agriculture, University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

SENIOR LECTURERS, LECTURERS OR ASSISTANT LECTURERS IN THE DEPARTMENT OF MATHEMATICS, University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

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CHAIR OF PHYSICAL GEOGRAPHY—The Registrar, University College of Wales, Aberystwyth (October 31).

PROFESSOR OF ANTHROPOLOGY at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

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PROFESSOR OF ELECTRONIC COMPUTATION AND DIRECTOR OF THE DIGITAL COMPUTING LABORATORY in the School of Electrical Engineering, University of New South Wales, Sydney—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Post Office Box 1, Kensington, New South Wales, Australia (November 30).

ASSISTANT PROFESSOR (with postgraduate education equivalent to the Ph.D.) IN ANIMAL NUTRITION—The Head, Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada (December 15).

BIOCHEMIST (preferably with some experience of simple statistics, immunology, and work with animals), to assist with routine and research work concerned with gonadotrophin and growth hormone in children—The Chemical Pathologist, The Hospital for Sick Children, Great Ormond Street, London, W.C.1.

CHEMICAL PHARMACOLOGIST or BIOCHEMIST (preferably with research experience), to investigate the mode of action of drugs currently used in the treatment of mental illness, using spectrophotofluorimetric techniques—Director of Clinical Research, Crichton Royal, Dumfries.

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PLANT BIOCHEMIST or BOTANIST (graduate), to undertake investigations into the fundamental and technological causes of some defects arising in fruit and vegetable products; and a BACTERIOLOGIST (graduate), to work on the microbiology of bacon with particular reference to the microbiological aspects of prepackaging in vacuum film wraps—The Director of Research, The British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

READER (with a good honours degree in physics and appropriate research experience) IN SOLID STATE PHYSICS (Ref. CST 63/47); and a PRINCIPAL LECTURER (with an honours degree in physics or equivalent qualification, appropriate teaching and research experience, administrative ability, and

preferably industrial experience) IN THE DEPARTMENT OF PHYSICS (Ref. CST 63/67)—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting appropriate Ref. No.

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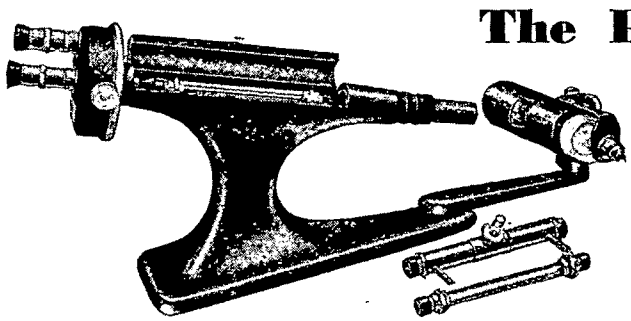
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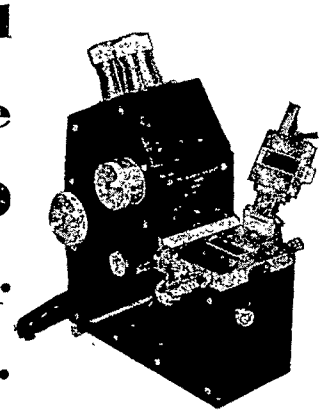
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THE NUCLEAR TEST BAN

IT is scarcely twenty years since a world, already torn apart by war, learnt from the name of a Japanese city—Hiroshima—that a major scientific discovery had created for the future a peril far greater than any that had ever been known. The late Niels Bohr, whose discoveries in the field of atomic physics had done much to provide the theoretical basis for the war-time development of atomic energy, had been in no doubts when he prophesied in a secret document penned a year or two before this happened that the inevitable emergence of nuclear weapons would "completely change all future conditions of warfare". This proposition has been at the heart of all the attempts that have been made since then to bring nuclear energy, and particularly its military applications, under international control. The successful conclusion of the recent test-ban treaty, for which the world owes much to the patient efforts of the British Prime Minister, thus provides a useful moment both to review what has gone before, and to appraise the situation which has resulted.

Over the past twenty years many eminent scientists, both inside and outside Government machines, have engaged themselves in the struggle to achieve the kind of international understanding of which the test ban is the most important sign so far. This they did because they were in a unique position to appreciate the completely new qualitative look, as opposed to quantitative transformation, which nuclear weapons had imposed on warfare. The task which they set themselves was to convince their military and political colleagues that the emergence of nuclear weapons implied nothing less than this, and that their existence threatened to outmode warfare as an instrument of major national policy. As they struggled to put this point over, usually against a tide of conventional and unheeding military thought, and often opposed by the pressure of powerful interests, their own sense of social and political responsibility inevitably took on a new meaning, and just as inevitably divided. The struggle became chaotic, and bitter differences of scientific opinion, stemming to some extent from personal animosities, helped to confuse the main issues.

The first serious effort to bring about the international control of nuclear weapons was the 1946 U.S. Baruch Plan. Its failure accelerated, and then made public, the deep divisions of opinion which were growing within the American scientific community about the way weapon technology should develop. The story is too well known to need re-telling in detail. After the U.S.S.R. had carried out its first atom bomb test, in 1949, an intense debate opened about the wisdom of proceeding with the development of an 'H-bomb'. The decision to go ahead in due course led to the removal from the nuclear stage of Dr. Robert Oppenheimer, wartime director of the U.S. Atom Bomb project, and the emergence and dominance of Dr. Edward Teller. The story of their conflict remains one of the major dramas of the post-war scientific era.

The ensuing race to perfect fusion weapons of high yield, a race in which the United Kingdom played a prominent part, inevitably increased world fears, and at the same time strengthened the intensity of the debate about the advisability of checking the proliferation of nuclear weapons. World-wide alarm about radioactive fall-out mounted rapidly after 1954, when the first big series of U.S. hydrogen bomb tests began, and had reached such proportions by 1958 that both the West and East found themselves compelled to convene a conference of scientific experts to examine the technical feasibility of monitoring an agreement to discontinue nuclear tests, in whatever medium they were carried out.

Thus little more than a decade after the first atom bomb had been used, and at a time when the arms race was accelerating fast in the pursuit of the doctrine of massive retaliation, governmental and non-governmental scientists were again brought together—this time in Geneva—to see whether a first step could be taken to bring the nuclear weapon under effective control. It was to the scientists that the political leaders of the nuclear powers, as well as the world at large, looked once again for the formulation of measures which might help reduce the dangers of world-wide destruction.

The 1958 Conference of Experts was scarcely a meeting of minds. While the Western scientific delegations urged the need to separate scientific analysis from political action, in the Russian view science, even though 'objective' in its methods, had to be subordinated to national requirements; discussion of technical issues was meaningless in the absence of political direction. With this difference of outlook, it was not surprising that, while it proved fairly easy to reach agreed conclusions about the capabilities of various methods for detecting and identifying nuclear explosions, it was all but impossible to formulate an agreed report for an integrated control system. Nevertheless, after nearly two months of debate, a joint report was produced, and was generally welcomed as representing a new and hopeful advance towards the solution of the technical and general problem of controlling what was, in effect, a major measure of disarmament.

Armed with this agreement, the nuclear powers almost immediately embarked on political negotiations which had as their aim an agreement for the cessation of nuclear weapon tests under effective international control. These negotiations have proved far more protracted than many hoped at the start. Since 1958 they have engaged the attention of more and more countries. The United Nations became their forum, while at the same time bilateral talks, both official and unofficial, continued to

be held whenever propitious. At every turn new considerations, direct and indirect, political and military, emerged to colour the issue. But finally, through what has proved both a process of national education, and a search for national advantage and security, the nations have taken a first step towards a safer world.

Although, during the more recent phase of the story, there may have been pressure from the scientists and military of both sides for new rounds of tests, it is tempting to believe that a step towards a ban could have been taken sooner if scientific and political considerations had been better separated than they appear to have been over the years since the debate began. The scientist's job was to define methods by which any nation which was party to a treaty could feel confident that no other nation was violating the agreement, and so gaining some conceivable military advantage. He had to elaborate methods of detecting tests whether carried out in the atmosphere, beyond the atmosphere, under water, or underground. He had to show how nuclear explosions could be both detected and differentiated from natural disturbances occurring at a distance. As is well known from the public record of the protracted discussions, the scientists concerned did not always interpret their task in the same way, particularly when it came to the discussion of underground tests. Taking a sophisticated view about what kind of developments in seismology were likely, as well as a practical view about the technical difficulties and costs of cheating, some felt that what the politicians wanted could soon be achieved. Others became concerned to show that, in spite of all possible seismic developments, cheating would always be possible, and to prove their point were only too ready to devise theoretical schemes of fanciful proportions which would permit of cheating. This debate could never have been settled by purely scientific considerations. At no time could scientists have guaranteed to detect at a distance every small underground nuclear explosion, or to distinguish between such an explosion and a natural earthquake or a chemical explosion of comparable size.

Had the political environment become favourable before the present year, it would have long ago been possible for the politician to remove the discussion from the scientific arena. The scientist could be expected to isolate and evaluate the technical problems concerned; but in the last analysis he could never assume the politician's responsibility for making the final political judgement, taking into account all the scientific facts which were available. But be this as it may, the world has, at long last, achieved an agreement to ban tests of nuclear weapons everywhere except underground; and even such tests are precluded if venting leads to radioactive contamination which affects countries other than the one carrying out the test.

If what has been achieved is less than some hoped for, it is nevertheless of major significance. The treaty immediately lessens public anxiety about the deleterious biological effects of contamination of the atmosphere with radioactive debris. It will also both slow down and impede further nuclear weapon development by the existing nuclear powers, and, both directly and indirectly, will inevitably impede, if not prevent, the proliferation of nuclear weapons, particularly among all those nations already party to the treaty. For the treaty specifically enjoins its signatories to: (*a*) prohibit, to prevent, and not to carry out any nuclear weapon test explosion, or any other nuclear explosion, at any place under their jurisdiction (within the prescribed environments); (*b*) refrain from causing, encouraging, or in any way participating in, the carrying out of any nuclear weapon test explosion, or any other nuclear explosion, anywhere which would take place in any of the prescribed environments.

It would, of course, have been better to have achieved the original objective of a comprehensive treaty which took in underground tests as well as those catered for in the present agreement. For although the present treaty calls for a continuation of negotiations for the discontinuance of all nuclear test explosions, it does leave open the possibility of further nuclear weapon development through experiments carried out underground. The extent to which this is either possible or useful has been very fully indicated in open debate in the U.S. Senate Committee Hearings which provided the prelude to the debate in the Senate which has just ended in the decision to ratify. But whatever the answer here, the treaty, nevertheless, contributes significantly to a reduction in international tension, and at the same time opens the door for a further move towards disarmament. As Walter Lipmann has written: "The treaty takes out of the race of armaments most of the feverish gamble for supremacy which unlimited testing invites".

The case for the agreement has been generally upheld both for this reason and because of the belief that its overall advantages outweigh whatever technical risks there may be of cheating and of any consequent military exploitation. Opposition to the treaty in the United States has focused on the possibility of a Soviet breakthrough in the field of anti-missile defence, and on the possibility that the Russians, by testing underground, might redress the balance of advantage—given this can be measured at all—which the West is at present thought to have in low-yield tactical nuclear weapons. This school of thought has been spurred on by Dr. Teller, and in it may be included many who are opposed to any attempt to achieve a real measure of disarmament. Indeed, some of President Kennedy's remarks at a recent press conference could be taken to imply that Dr. Teller was scarcely likely to be satisfied by any agreement which banned tests.

Against the kind of opposition which has been put up, it has been authoritatively maintained that the most difficult problems to be solved in developing an anti-ballistic missile system are in themselves non-nuclear and can be vigorously explored without nuclear testing. The concept of the United States forging ahead of the U.S.S.R. in anti-missile development, if all are allowed to engage in unlimited atmospheric testing, has also been described by Walter Lipmann as "a romantic form of self-deception. . . . Dr. Teller's promise that American scientists will be able to do what Soviet scientists will not be able to do is not a scientific judgement. It is a reckless pseudo-patriotic gamble on the inherent superiority of American over Russian scientists".

So far as tactical nuclear weapons are concerned, it has been cogently argued, both on scientific and military grounds, that the continuance of underground tests could never upset the deterrent balance between the major nuclear powers; this is firmly based on mutual threats of 'strategic' destruction of each other's homelands. Arguments to the contrary are bedevilled by confusions about what is 'tactical' and what is 'strategic'; too often do they discount the fact that the philosophy of limited or tactical nuclear warfare only makes sense for the

winning side, and that the loser might prefer to carry others with him into 'strategic' destruction; they ignore the fact that the physical damage caused by even a single nuclear explosion, however impressive that damage may be, is vastly augmented in significance by the psychological impact in terms of human reaction.

Now that the U.S. Senate has ratified the treaty, the world has an opportunity to exploit the political climate the agreement has helped to generate, and to press on to the next steps in disarmament. Among the possibilities being discussed are the reciprocal establishment of fixed observation posts at important rail and road junctions, harbours and airfields in NATO/Warsaw Pact countries, including the Soviet Union, so as to give warning of the mobilization of forces and surprise attacks; a non-aggression pact; the freezing of defence budgets; and the extension of the present test-ban treaty to include the underground environment. It is for the politicians to decide which step comes first, and how it is to be negotiated. In the meantime, scientists, both independent and in government employment, will continue to explore the most fruitful fields for future agreement and to isolate and evaluate relevant technical issues. It has recently been announced that a continuing study-group of American and Russian scientists has been set up to do precisely this, and it is hoped that it will not be long before British scientists are included. For of one thing we can be sure, scientists are unlikely now to disengage from the struggle to enhance the physical security of the world in which we all live, and which was brought into greater peril by the applications of their nuclear discoveries.

TO BE TASTED, NOT SWALLOWED

Problèmes Actuels d'Ophthalmologie, II

Dirigé par E. B. Streiff. (Bibliotheca Ophthalmologica, Fasc. 60.) Pp. x+218. (Basle and New York: S. Karger, 1962.) 50 Swiss francs.

Advances in Ophthalmology

Vol. 13. Edited by E. B. Streiff. (Bibliotheca Ophthalmologica, Additamenta ad Ophthalmologica, Fasc. 62.) Pp. vi+265. (Basle and New York: S. Karger, 1963.) 60 Sw. francs.

Physiology of the Eye

By Hugh Davson. Pp. vii+492. (London: J. and A. Churchill Ltd., 1963, second edition.) 65*s*.

THIS trefoil illustrates three types of writing. *Modern Problems in Ophthalmology* shows all the ardour and enthusiasm of writers who present their own work; indeed, so original are these papers that there are no references to other people's writings. *Advances in Ophthalmology* contains reviews, characterized by the eclecticism that many reviewers are bound, and some choose, to follow. The third volume, a text-book, combines the foregoing qualities and laces them with simplifications which run through the whole gamut from facilitation in presentation to undeliberate misrepresentation. The first volume contains matter discussed at an international colloquium on photocoagulation held in Lausanne as long ago as September 1959. The delay between the original presentation of the papers and the appearance of this volume is deplorable, must be disappointing to the authors, and, in view of the strides which lasers are making in this field, galling to the publishers. None the less, the book is extremely valuable to the clinician, for the colloquium assembled all the leading exponents of the art of photocoagulation. This interesting technique is discussed in all its detail, both from the technical and the clinical points of view. It is significant that its inventor, Meyer-Schwickerath, should be among those emphasizing its limitations with more vehemence than some of his followers are inclined to do. The problems covered include the use of photocoagulation in lesions of the more accessible parts of the eye, such as the lids, the conjunctiva, the cornea, and the iris. There is also a discussion of the prophylactic treatment in the case of retinal detachment together with a discussion on the value of injections of vitreous. The treatment of Eales's disease with diathermy and photocoagulation is also dealt with, as is the problem of tumours of the retina and the choroid. As Ten Doesschate pointed out, this was the first occasion on which an international conference chose to discuss the conservative treatment of these tumours. The production of the volume, a valuable addition to the clinical literature on photocoagulation, is excellent.

Advances in Ophthalmology is in its thirteenth year and has succeeded in living up to the high standard set by the preceding volumes. The reviews are of varied import. A lively article by Streiff on the gerontology and geriatrics of the fundus oculi deals not only with the incident literature but also with the author's own clinical findings. The informed reader will probably find the latter of more interest than the former, in part perhaps not quite as critical as this difficult subject demands. Nowhere is this clearer than in the discussion of the changes in retinal function, where the author failed to consider changes occurring in the rest of the eye. It may be mentioned parenthetically that the French title *Gérontologie et gériatrie* does not convey the expected distinction in English, for gerontology is the science of age and geriatrics the care for the aged, whereas Streiff uses these terms to differentiate physiological from pathological senile changes. Malbran and Dodds describe operative and post-operative eye complications in retinal detachments, a paper which forms a useful, though not exhaustive, introduction to a collection of puzzling conditions. Maeder's review of the literature on the pupil shows eclecticism at a premium; papers published, for example, in physiological or optical journals are largely missed. The volume concludes with a general review on trachoma by Bietti and Vozza which is very well documented and forms an invaluable introduction to the work and literature on this disease, the research into which has made such striking progress during the last few years.

Four classes of reader may turn to Davson's *Physiology of the Eye*: research workers, students, teachers, and examiners. The first will find the book a useful introduction to vision, the physiology of the eye, also to the elements of visual optics. They will frequently be referred to the four volumes of *The Eye* and learn that this book shares many of their advantages and also disadvantages. Students, who form the major but not the most important part of the audience of this book, will think it useful, and the first hundred pages or so positively enjoyable. They will find it greatly expanded in comparison with the first edition. A few passages—as must be true of any text-book—will leave them somewhat bewildered; for example, regarding the difficult question of false torsion they will be scarcely any wiser after than before reading the section attempting to explain it and they may well query Fig. 142, for it passeth understanding. But they will be overjoyed by the large number of good illustrations, conspicuous by their paucity only in the chapter on the protective mechanism of the eye. Teachers will want to use the book with as much avidity as circumspection. They will realize that Dr. Davson has written these pages in great awe of authority, which makes him often judicially

cautious in expressing personal views, a circumstance, by the way, that medical students will find unusual but which ought to do them a lot of good. Teachers will also probably find the anatomical parts explained with greater lucidity than the more recondite problems of visual physiology where, as is inevitable in as catholic an approach as Davson has succeeded in following, a few inconsistencies have crept in. Examiners, though the smallest but perhaps the most important group of people who may turn to this book, will be in a quandary. If they are omniscient—and they would scarcely be examiners if they were not—they will realize that this book contains heresies, and that in some respects it is already out of date. Are they going to mark a student's performance on his ability to provide answers to the questions they have set or on his ability to reproduce the gospel according to this book? They will allow their judgment to be governed by the book in matters which are well established or beyond dispute, but would be wise to tolerate an unexpected answer on the student's part if this appears reasonable.

R. A. Weale

PORPHYRIN DISORDERS

Diseases of Porphyrin Metabolism

By A. Goldberg and Prof. C. Rimington. (A Monograph in the Bannerstone Division of American Lectures in Living Chemistry.) Pp. xvi+231. (Springfield, Ill.: Charles C. Thomas, 1962.) 9.75 dollars.

DISCOVERIES in pure science usually precede their developments in applied science, and it is refreshing to encounter a topic in which both forms of science have in turn contributed equally to knowledge. This is especially true of the development of our understanding of porphyrin metabolism. Towards the end of the nineteenth century and the beginning of this, Garrod and other clinicians in Britain and in Germany observed red pigments in the urine and fæces of patients with certain diseases. The first scientific advances were made by Fischer and his colleagues, who investigated the chemistry of these porphyrin pigments and established their chemical structures and synthesized them. In 1935 Waldenstrom, a clinician, demonstrated the presence of porphobilinogen (PBG), a colourless precursor of porphyrins, in the urine of subjects with acute intermittent porphyria. This condition was thus clearly distinguished from congenital or photosensitive porphyria in which large amounts of preformed porphyrins were excreted. In 1946, Shemin and Rittenberg showed that, in the rat and in normal man, glycine was specifically incorporated into the protoporphyrin of the circulating hæmoglobin. Subsequent work by British and American groups showed that the uroporphyrin and coproporphyrin of Series I excreted by patients with congenital porphyria were synthesized from the same precursor as the protoporphyrin of the circulating hæm, even though this pigment belonged to Series III. Acetate was then found to be incorporated into hæm, and the brilliant work of Shemin involving the chemical degradation of the labelled protoporphyrin isolated from hæmoglobin after feeding ^{14}C- and ^{15}N-labelled glycine and ^{14}C-acetic acid showed the origins of all the atoms of this compound. This worker suggested that the protoporphyrin was synthesized by way of a monopyrrolic precursor formed by condensation of two molecules of an asymmetric four-carbon compound derived from glycine and acetic acid. Four molecules of the monopyrrole condensed to yield uroporphyrinogen from which coproporphyrinogen and protoporphyrin were afterwards formed. The monopyrrolic precursor was thought to be porphobilinogen and its isolation by Westall was a triumph for a clinical chemical department; progress then had to await the establishment of its structure by Cookson and Rimington and its synthesis by Jackson and Macdonald. This was soon followed by the demonstration of the enzymatic synthesis of porphobilinogen from succinyl coenzyme A and glycine via δ-aminolaevulic acid (ALA). Since then there have been many investigations on the mechanism of specific formation under physiological conditions of Series III porphyrins.

The disturbances of this biosynthetic pathway responsible for the various clinical disorders of porphyrin metabolism are now subjects of intensive study in a number of laboratories throughout the world. Although many details remain to be filled in, our knowledge has progressed considerably and has supported Garrod's concept that these inborn errors of metabolism were due to enzymatic deficiencies. The precise abnormality leading to the excessive excretion of ALA and PBG in acute porphyria, however, remains to be elucidated.

The time is ripe for a comprehensive review of our knowledge of the porphyrins and of the diseases associated with disturbances of their metabolism and we are fortunate in having this book, the senior author of which has contributed so much to this subject since he first recognized the importance of porphyrins at a time when they were little more than biochemical curiosities. Dr. Goldberg has made important contributions both to the clinical aspects of the disease and to the mechanisms of production of the experimental porphyrias. The first chapter concerns general aspects of the porphyrias, their classification and geographic distribution, and the second discusses the chemistry and biosynthesis of the pigments themselves and their distribution in the body. There follow successive chapters on congenital (erythropioetic) porphyria, acute intermittent porphyria and the various forms of cutaneous porphyria. Each is described from both the clinical and the chemical points of view. Subsequent chapters concern diseases of porphyrin metabolism other than the porphyrias, the incidence of porphyria in animals, and include an account of the various forms of experimental porphyria. It is a pity that the recent discovery of griseofulvin-induced porphyria was made too late to be included.

Nevertheless, it is difficult to praise this monograph too highly; the format and style are well up to the standard set by the series. In particular, the photographs of patients and the microphotographs of tissues, coloured when demonstrating the presence of fluorescent porphyrins, are excellent and the list of references is up to date to 1961. It is unlikely that this book will be read by all clinicians, all pathologists or all biochemists; it will be of very great interest to clinicians who have actually encountered patients with disorders of porphyrin metabolism, especially neurologists who have seen the devastating sequelæ the disease can leave in the nervous system, and dermatologists who will learn of the various forms of porphyria which can cause photosensitivity. To the pathologist the subject illustrates beautifully the way science and medicine have developed to mutual advantage, while for the biochemist the book reveals many remaining problems especially in determining the details of abnormalities in enzymatic syntheses responsible for these interesting diseases.

C. H. Gray

RARE EARTH AND ACTINIDE SPECTRA

Operator Techniques in Atomic Spectroscopy

By Prof. Brian R. Judd. (McGraw-Hill Advanced Physics Monograph Series.) Pp. ix+242. (London and New York: McGraw-Hill Book Company, Inc., 1963.) 77*s*.

IT was round about 1948 that the electron spin resonance group at Oxford began to investigate rare earth salts, and thereby created for themselves an urgent need to

know a good deal more about the spectroscopy of this comparatively unknown group of substances. Fortunately most of the basic theory was already available, in Condon and Shortley's classic book, *The Theory of Atomic Spectra*, and with an extension to take account of crystal field effects, the stage was set for rapid developments in the whole field of the spectroscopy of rare earth ions in solids. In fact, the development did not occur at all rapidly and even excursions into the actinide series did not attract a great deal of attention. Nevertheless a solid core of good work was done, which has served as the foundation for the recent very exciting developments with rare earth solid-state lasers. Much remains to be done, of course, but with many more physicists now engaged in understanding rare earth spectra there is a need to be sure that the theoretical techniques which are used are as powerful as possible.

Now the methods given in Condon and Shortley, and their crystal field extension, have served us well, but there is no doubt that they can be extremely tedious to use. Further, it commonly happens that the end result of a lengthy calculation is quite simple, and one is left wondering whether or not there was a 'better way' of doing it. Well in many cases there was, and it is the purpose of this book to explain what we should have done.

The new method is based on the work of Prof. Racah, who examined in considerable depth the structure of many-electron wave-functions and their associated operators, using, particularly, group theoretical ideas. The result was a very elegant and instructive new way of dealing with problems in atomic spectroscopy, particularly those concerned with unfilled shells of electrons of high orbital angular momenta. Unfortunately the mathematical background is outside the range of knowledge of most spectroscopists, so that having coped with Condon and Shortley they are now faced with an even less familiar field.

Prof. Judd writes extremely clearly and gives his reader every help, with lots of examples and illuminating comments. Even so there is little doubt that a typical response will be to ask whether it is really necessary. The answer will of course depend a great deal on the reader, and the time which he is prepared to devote to what is really a large subject. The average experimental spectroscopist is probably best advised to leave this topic alone, unless he is a particularly good mathematician, though he should certainly look through the book and try to obtain some feeling for the mathematical techniques which his theoretical colleagues are using. The theoretical spectroscopists, on the other hand, should delve much more deeply, for this is the modern way of dealing with many-electron atoms, and here is one of the principal exponents explaining it to them. They should, indeed, read carefully and thank Prof. Judd for leading them so smoothly into this comparatively difficult and unfamiliar branch of theoretical physics.

K. W. H. Stevens

METALLURGY, ARCHÆOLOGY AND THE BRITISH ISLES

Metallurgy in Archaeology

A Prehistory of Metallurgy in the British Isles. By Dr. R. F. Tylecote. Pp. xvi + 368 + 28 plates. (London: Edward Arnold (Publishers) Ltd., 1962.) 84*s*.

"THE original object of this book was to gather together the wealth of information on metallurgical aspects of archaeology contained mainly in the appendices to excavation reports. . . . It was not long before it became clear that the information available amounted to a prehistory of metallurgy in the British Isles," and it is to these Islands that the author's attention is mainly, though by no means exclusively, directed. It will come as somewhat of a surprise to many readers to discover how great this wealth, in fact, is. In garnering in his harvest, Dr. Tylecote has performed a most valuable service: between seven and eight hundred references to pertinent journals, many quite obscure, are provided and the mere physical labour of making this collection and visiting the museums and other sources of his material is beyond praise. In this work he pays generous tribute to the help accorded by his wife. It must not, however, be assumed that this is a mere catalogue; the information brought together has then been sifted and welded into coherency, a task for which the author, both a trained engineer and metallurgist, is unusually well qualified to perform.

Starting with the native metals, and such gold as has been recorded in England, Scotland, Wales and Ireland—a nugget weighing 21·5 oz. was found in Ireland in the eighteenth century—the story is carried on to the final development of the blast furnace and the production of molten iron around 1500 A.D. The earlier chapters, necessarily devoted to the non-ferrous metals, are concerned with copper and its alloys, the production of tin and tin alloys, lead and silver. How much work has in fact been done is demonstrated by the 22 pages of analyses of copper and bronze samples alone.

Meteoric iron of indigenous origin is practically unknown in Great Britain, the Iron Age being introduced by Hallstatt people from the Continent probably between 500 and 400 B.C. It is reasonable to assume, therefore, that these immigrants were already familiar with bellows-driven bowl furnaces, knew something of the strengthening effect of carbon on iron, but were more or less ignorant of the technique of hardening carburized iron by water-quenching. After discussing the iron industry during the Roman occupation the author has a relatively short, but very important, chapter on iron during the 'Dark Ages' and the early medieval period. His conclusion is interesting. "In conclusion, we can say that there has been no advance on the pre-Roman techniques themselves, but that the knowledge of the processes of carburizing and heat-treatment is now more widespread" and "in certain cases brought together with great effect". In the Anglo-Saxon-Viking period "it would appear that smelting and smithing techniques carried on where the pre-Roman technique left off".

In the course of his discussion of pre-Roman iron, the author has collected the available data relating to no less than 71 of the curious 'currency bars'. This information is subjected to a rough statistical analysis, the whole representing perhaps the most detailed discussion of these pieces yet available and amounting to a real and intensely interesting piece of research.

Sandwiched in between the accounts of the non-ferrous materials and iron is the longest chapter in the book on methods of fabrication. This is a section of great value and is outstandingly well done. Those whose curiosity is still unsatisfied will find, in the 151 references, food for still more intensive study. Here, as indeed throughout the book, the sketches add immensely to the descriptions. Details of more than a hundred crucibles found in the Islands, their provenance, date, shape and capacity, are given; analyses of typical slags, and descriptions of methods of manufacture of vessels are but part of the ground covered. Methods used in the manufacture of gold torcs, how the late Bronze Age sword was repaired, the way the Irish trumpet was constructed or the reasons why certain castings of a similar date had had to be scrapped are examples of the range of problem discussed.

It would be too much to expect that at all points Dr. Tylecote's views coincide completely with those of any reviewer, but that this is a thoroughly reliable account of a subject which bristles with difficulties there can be no doubt: the illustrations are excellently reproduced and the index all that the most exacting reader could need. The labour which has gone into its preparation is immense and the result a work of outstanding value and interest.

F. C. Thompson

Biophysics of the Striated Muscle

By Prof. E. Ernst. Pp. 398. (Budapest: Publishing House of the Hungarian Academy of Sciences, 1963. Second edition.) 9.00 dollars.

THIS volume is a new English edition of the author's *Die Muskeltätigkeit* published in 1958 and reviewed in *Nature* (183, 1008; 1959) by Dr. D. R. Wilkie. Prof. Ernst has taken the opportunity to add new material to each chapter, and specialists in the field should welcome the work, particularly for its comprehensive survey of the literature, some of which is not very well known to English-speaking physiologists. There are sections on muscle structure, contraction, volume changes, electrical excitability, metabolism and theories of muscle contraction.

The fullest treatment is given to work which has been done in the author's own laboratory, but he is aware of this bias and, in this connexion, he quotes Empedocles: "Each of us believes only what he has encountered during his random wanderings and nevertheless prides himself on having found the whole". Professor Ernst's wanderings have led him to an outlook which differs considerably from that current in English physiology and one valuable consequence of this is that it has led him to experiments (for example, those on volume changes) which may not otherwise have been performed.

The author is very critical of some present-day theories of excitation and contraction but, although some of the criticisms deserve careful attention, others are based on misunderstandings of the theories. Thus, in discussing excitation (p. 212), he argues that "contrary to expectation impedance drops after the onset of the action current". In fact, the delay in onset of the impedance drop is perfectly well explained, indeed required, by the Hodgkin–Huxley theory. The explanations of the action potential overshoot (p. 210) and the effects of temperature on conduction velocity and spike duration (p. 222) are also misunderstood or ignored and it is not therefore surprising that Prof. Ernst comes to the conclusion that "the excitation of muscle (and nerve) can hardly be explained on the basis of current ion and membrane theory" (p. 223). Although this book will be of value to research workers in muscle biophysics, it cannot be recommended as a general introduction to the subject.

D. Noble

A Textbook of Gynaecology and Obstetrics

By Douglas G. Wilson Clyne. Pp. xxiii+1019+83 plates. (London: Longmans, Green and Co. Ltd., 1963.) 90*s*.

THE preparation of a single volume covering the whole field of obstetrics and gynæcology is an unenviable task, particularly if the author directs his book to teachers. Inevitably some subjects are compressed into small space and there are omissions. For example, in the section on the new-born, important causes of jaundice, such as glucose-6-phosphate dehydrogenase deficiency and galactose intolerance, are not described. Much is also said about vitamin K, but there is no reference to vitamin K_1.

In the chapter comparing hospital and domiciliary confinement the author echoes the Ministry of Health with the statement that almost three-quarters of deliveries take place in "an institution". It is a pity that it is not made clear that at present only just more than half the British mothers can have full hospital facilities as shown by the recent *Perinatal Mortality Survey*, and that many deliveries occur in small units which have the disadvantages of both hospital and domiciliary confinement.

For the most part the material is well chosen and the arrangement of text and references to published work is good. This is a book full of facts and figures which should prove valuable to students.

John Murray

Three Hundred Years of Psychiatry, 1525–1860

A History Presented in Selected English Texts. By Richard Hunter and Ida Macalpine. Pp. xxvi+1107. (London: Oxford University Press, 1963.) 84*s*. net.

THE authors in their preface adopt a refreshingly sceptical attitude towards psychiatry as a science. Psychiatry, they say, "does not possess a body of exact and established knowledge on which all can agree comparable with medicine. Rather it consists of attitudes, concepts, theories and therapies from which each doctor selects what accords best with his predilections, so that one is tempted to say that there is not one body of psychiatry but many psychiatrists". Furthermore, "aetiology remains speculative, pathogenesis largely obscure, classification predominantly symptomatic and hence arbitrary and possibly ephemeral; physical treatments are empirical and subject to fashion, and psychotherapies still only in their infancy and doctrinaire". To escape from present empiricism they turn to history, believing that "the history of a science is the science itself". Their book is built up on extracts from original sources arranged chronologically, and since psychiatry extends well beyond the field of medicine as a physical science they have included "the writings of divines, philosophers, philanthropists, lawyers, men of letters, even self-accounts of patients, as well as Parliamentary Acts and reports documenting society's interests in and care for the insane". The result is a fascinating compendium, richly illustrated by the reproduction of title-pages. It can be recommended as a mine of information to all who are interested in the human mind or in society's reactions to its aberrations.

Brain

Marsilae: Botanical Monograph No. 2

By Prof. K. M. Gupta. Pp. iii+113+40 figures. (New Delhi: Council of Scientific and Industrial Research, 1962.) *Rs*. 16.00, 33*s*., 5.00 dollars.

THIS work is primarily though not exclusively concerned with formal and experimental taxonomic studies on the Indian species of *Marsilea*, and in particular with those four of the nine Indian species recognized by the author which have been available to him as live plants. The bulk of the original material included consists of the results of biometric studies on these four species grown under uniform conditions of cultivation. The main appeal of this volume will therefore be to workers concerned with the taxonomy and biosystematics of this genus. An artificial key to the Indian species of *Marsilea* is provided, and one new species, *M. rajasthanensis*, is described.

The quality of this publication is, to say the least of it, uneven. The photographs of leaflets, pedicel-petiole attachments, and sporocarps, reproduced as Figs. 14, 20 and 21 are poor by any standard, and fail to display adequately the contrasting characters concerned. Diagrams, not photographs, without indication of magnification are selected for the illustrations of chromosomes on Fig. 37. The drawings included on Figs. 29, 30 and 31 do little justice to the figures of Sharp and Delmasy-Feller on which they are acknowledgely based, and the legends provided in the original publications are excluded in their entirety, an omission which is the more deplorable since Fig. 29, purporting to show microsporogenesis in *Marsilea quadrifolia*, does in fact illustrate stages in spermatogenesis, and the illustrations on Fig. 30, described as megasporogenesis in *M. diffusa*, do in fact depict the development of the female gametophyte. Furthermore, certain of the drawings included on Fig. 31, labelled as embryogenesis in *M. diffusa*, only become explicable on reference to the original legends of Delmasy-Feller, when it is discovered that half the illustrations refer not to stages in embryogenesis, but to abnormalities in the development of the female gametophyte!

J. D. Lovis

MAN AND HIS FOOD*

By Sir WILLIAM SLATER, K.B.E., F.R.S.
Formerly Secretary, Agricultural Research Council

THE impact of science on the duration of human life has been widely discussed. It is not intended here to examine this great problem in detail, but to set out only the few facts needed to illustrate the magnitude of the task of feeding the growing millions.

The population of Europe is expanding at 6 per cent in each decade, those of Africa and Asia at 21 and 27 per cent, respectively. All these growth-rates are tending to increase. Since 1950 the world population has increased by more than the total population of Europe including the U.S.S.R., and its yearly growth is greater than the present population of Great Britain.

The only final solution to this problem is a limitation of the rate of reproduction. Unless this is achieved, the population pressure must ultimately result in famine, revolution and war.

It is generally accepted that the world population will have doubled by the end of the century, and that the food required will be twice that now available to provide a diet little or no better than that of to-day. If the diet in the poorer countries is lifted to the level in the wealthier, it is estimated that the increase required would be six-fold.

What are the chances that the lower target can be reached or even exceeded to give everyone a better standard of living? To answer these questions it is necessary to study the limiting factors in food production by orthodox farming and other sources of food which may be available in A.D. 2000.

To grow crops the farmer needs land and water, energy from the Sun, nutrients from the soil or through the foliage and carbon dioxide from the atmosphere. The land suitable for farming is limited. In 1959 there were just over eleven acres of land surface in the world for each person. Of this, about one acre was under arable cultivation or tree crops, two acres under permanent grassland, three and a half acres under forest and four and a half were waste lands. Much of the latter is too mountainous or lies too near the poles for cultivation. What is left is desert, primarily through lack of water, but in some instances due to a deficiency of plant nutrients. With double the population, all these figures will be halved, except in so far as there can be redistribution between the types of land use.

The land under forest can make little or no contribution to farming. Much of it is on mountainous hillsides or on shallow soils unsuited for agriculture. Moreover, the timber it produces is needed for man's use. The farmer must, therefore, draw on the waste lands for any additional acreage. This in practice means bringing the deserts into cultivation, which in turn involves irrigation. These lands are barren because no water is immediately available either from local rainfall or from a major river with its source in the region of higher rainfall. Water may be provided either by bringing it from long distances, by sinking deep wells into water-bearing strata, or, if the desert is near the sea, by desalinization of sea-water. The first method involves great expense, and expert knowledge in the construction of dams, pipelines or canals. The poorer nations cannot meet the expense nor have they the technically qualified staff to carry out the work; the planning and financing of these schemes must, therefore, fall on the wealthier. Moreover, major irrigation works call for co-operation between nations in apportioning the available supplies. Deep wells and the essential pumping equipment are also expensive and their siting calls for skilled advice. The desalinization of sea water is at a relatively early stage in its development; the fresh water produced is too expensive for use in field irrigation on any major scale.

If we can assume that the economic and political obstacles can be overcome, the limitation on the extension of cultivatable land by irrigation will depend on the water available in each region. Unfortunately, no precise estimates have been made for many of the most important regions.

While farming can be expected to gain from the lands which are at present waste, it will also lose land to urban development as the population grows.

Any estimate of the increase in land available for food production at the end of the century must, with so many uncertain factors, be highly speculative. It is anticipated, however, that the increase cannot be more than half the area now under arable cultivation and tree crops, which would provide no more than an additional quarter of an acre per head of population in A.D. 2000. Then the farmers of the world will be faced with producing on three-quarters of an acre of cultivated land and one acre of permanent grassland the food for one person which they now produce on one acre of cultivated land and two acres of permanent grassland.

This represents increases of 33 per cent on the cultivated acreage and 100 per cent on the permanent grassland. The additional production on the cultivated land can easily be achieved and should be exceeded. Given an adequate water supply, the chief limiting factor in crop production is the supply of the major nutrients, nitrogen, phosphorus and potash. Surveys made by the Food and Agriculture Organization have shown that there is a high correlation between crop yields and the fertilizers used. The highest returns from the use of more fertilizers will be in those areas where the present applications are lowest. Thus, in Britain, while many farmers are probably applying the optimum amounts of fertilizers which the existing crop varieties can use, others could increase their yields by higher dressings. It would not be asking too much to expect, in Britain, an overall increase in crop yields of 20 per cent if all farmers made full use of fertilizers.

The return from higher fertilizer applications will vary from country to country, but in few will it be less than that in Britain. In large areas of the world where there is most need for food, the amount of fertilizer used is the smallest; as a result, the yields are the lowest and the response to higher applications will be the greatest. On the other hand, in some large grain-growing areas such as the Canadian prairies, the rainfall is the factor limiting, yields. Here, without enormous expense on irrigation fertilizers would have little effect.

Taking the world as a whole, it would not be unduly optimistic to expect that higher fertilizer usage could increase the output of existing crop varieties by 50 per cent. If the plant breeders can produce new varieties, capable of making better use of the fertilizers available, or of absorbing still higher dressings, then this figure might well be exceeded.

In countries such as India where every scrap of vegetable material is eaten by man or animals and the animal dung is dried and used as fuel, the soil has become denuded of organic matter, and as a result has lost its capacity to

* Substance of the Sanderson-Wells lecture delivered at Senate House, University of London, on May 21.

hold water or plant nutrients. If yields are to be obtained commensurate with the fertilizer used, steps must be taken to replace this lost organic matter. This will involve outside help in making available alternative fuel supplies or supplementary food to enable the farmers to grow green crops either to plough in or to feed to stock the dung of which can be returned to the land.

It is well established that once farmers begin to use more fertilizers and obtain heavier crops, they take steps to protect them against pests and diseases. Thus, the first increases in the amount of fertilizer used, where the applications have previously been low, evoke a greater response than could be anticipated from experimental evidence.

How much this awareness of the need to protect crops and the willingness and ability to do so will contribute to increased yields is again a matter of guesswork. To obtain a major contribution to the world's food supplies by crop protection it will be necessary to overcome ignorance, prejudice and superstition. Some gain, however, must result, and this can be taken into account in any final estimate.

The problem of assessing the potential production from the permanent grasslands is even more difficult than that of the cultivated land. The term 'permanent grassland' covers every type from the famous fattening pastures of Leicestershire to the virtually barren lands of India which carry a poor grass sward for a short time after the rains, or, again, from the lush valleys of the Alps to the sparse vegetation on the hills of the Middle East. Much of the permanent grassland is already giving the maximum yield of fodder that can be expected. The outstandingly good pastures can scarcely be improved, and many of the worst will not repay treatment. Between these extremes there is a wide range which can be improved by irrigation, the use of fertilizers and re-seeding with superior strains of grasses and legumes.

Grasslands produce human food only through the ruminant. The proper management of the sward, so that the animals grazing it get the maximum amount of keep from a given area and the ability of the stock to yield the greatest quantity of human food from the food they themselves eat are equally as important as the productivity of the sward.

By improving every stage in the production of human food from permanent grasslands, there is little doubt that the output from some part of the area could be doubled; in specially favoured places this increase might well be exceeded. What is not known is how the total permanent grassland is spaced out along the broad spectrum from the one extreme where there is no possibility of increasing the yield, to the other where twice the yield or even more can be obtained. On this depends the average increase throughout the world. The chance that the overall yield of the world's permanent grasslands can be doubled by A.D. 2000 is, however, remote.

Much of the human food obtained from grassland is the high-quality animal protein which is so seriously lacking in the diets of many people. A failure to double the output will mean less milk and meat per head of population. This danger may be, to some degree, avoided if part of the world's grasslands were brought under the plough, to form part of an arable rotation. The three-quarters of an acre of cultivated land per head of population, which could be available by A.D. 2000, would yield, with proper management, enough arable crops to meet human needs, so that the produce of any additional land won from permanent grass could be fed to non-ruminant pigs and poultry or used to supplement the grazing intake of cattle. Except where the most productive grassland was taken, there would be some gain in overall productivity.

Taking all these factors into account, it is difficult to believe that, however great the effort to produce more from the land, the food available from this source for each human mouth in A.D. 2000 will be as much as to-day. In so far as the effort falls short of the maximum, the gap will be widened.

We must, therefore, look for other means of increasing food supplies. One of the most promising of these is the elimination of waste between the farms and the urban centres of population. In the better developed countries this wastage is being eliminated by the prevention of damage by animal and insect pests and the control of spoilage by micro-organisms. More food is being processed on the farm and in the factory, where the non-edible portions can be fed to stock or returned to the land. In the less-developed countries there is a high percentage of waste in store; for some crops in tropical countries this is placed as high as 30 per cent. An attack on this loss of human food could do much to help to close the gap left by the inability of the farm lands to meet the world's growing needs.

Another source of additional food can be sought in the sea and inland waters; the increased supply of fish would do much to meet the shortage of high-quality protein. We are already in trouble with our fishing on the shallow continental shelves of the northern hemisphere. There is, however, a possibility of developing fishing in the deeper waters of the oceans and in some areas of the southern hemisphere. We know all too little of these possibilities and no time should be lost in examining them. We can underestimate them and so overlook a valuable source of food or we can exploit them unwisely and so destroy an important potential food supply.

The cultivation of fish in ponds also holds out hope of more high quality food. Fish culture will, however, occupy land and require a supply of water and so compete with agriculture except where it can be combined with crop production as in the paddy fields.

Another source of protein is the direct extraction of this material from green crops, which would otherwise have to be converted by ruminants with considerable loss before it was available as human food. Processes for the preparation of edible material from this source are being developed and show promise for the future.

Finally, another unusual form of food production is in the experimental stage. It consists of the growing of micro-organisms on by-products of the petroleum industry and inorganic nitrogen. It is too early yet to estimate the significance of this new venture, but it may in the future prove to be of great importance.

One factor, which is common to all aspects of the problem of feeding the doubled population at the end of the century, is the lack of definite knowledge of the facilities available. More information is needed about the world's land surface, and in particular about the nature and distribution of areas capable of development for cropping. We must know more about the land classified as permanent grassland. At the same time, we need to study all sources of water supply and relate them to the areas where they can be economically used. Much more research is needed on the potential fish supplies in the deeper oceans and on the methods suitable for their economic exploitation. Finally, we must work on and assess the possibilities of unorthodox methods of food production.

Can all our efforts in food production meet the world's needs in A.D. 2000? No one can answer this question with absolute certainty, but most of those who have investigated the problem believe that they could and that some contribution could be made to the relief of hunger and malnutrition. But to achieve this, there will have to be an awareness of the problem throughout the world, a willingness to give up superstitions and prejudices often deeply ingrained and sincerely held, a determination by all nations to put this problem first on their list of priorities and a degree of co-operation among the nations which has so far not emerged.

OCEANOGRAPHY AT THE SMITHSONIAN INSTITUTION

By Dr. I. E. WALLEN
Assistant Director for Oceanography, Museum of Natural History, Smithsonian Institution, Washington

THE Smithsonian Institution, chartered for the "increase and diffusion of knowledge among men", has traditionally been deeply involved in research on marine organisms. However, since the Institution operates no ships, its potential contribution to the National Oceanographic Program was not fully realized in the initial phases of the development of that Program. It soon became evident that, of all federal agencies, the Smithsonian Institution has the outstanding collections of marine specimens, as well as exceptional staff capabilities in the area of marine systematic biology. Therefore, the Interagency Committee on Oceanography was prompt to recommend that the Institution consider expanding its basic staff and facilities in this area and thus increase its contribution to the national oceanographic effort.

As a result of the increased international and national effort in oceanography. many biological and geological collections are expected to be brought to the Smithsonian Institution. During the International Indian Ocean Expedition, which is now proceeding, at least 44 ships from 27 nations will collect specimens in the area of the Indian Ocean. The two phases of the International Co-operative Investigations of the Tropical Atlantic will include 30 oceanographic cruises, involving 16 vessels from at least 10 nations. Twenty-two U.S. Coast Guard vessels occupy ocean stations in the Atlantic and Pacific Oceans and many make marine collections. Nine oceanographic institutions along the coasts of the United States utilize more than 21 ships to collect oceanic information. Ships of the Coast and Geodetic Survey, the Naval Oceanographic Office, the Bureau of Commercial Fisheries, and other federal agencies gather oceanographic materials and data as a part of their missions.

Many of the ships taking part in the foregoing cruises make net, dredge, trawl, and coring collections of natural history materials, especially plankton and sediments, to study the productivity of the sea, to examine the organisms in relation to the physical and chemical environment, to study the evolution of the Earth, and for other purposes. Although much remains to be developed in the way of sampling techniques and interpretation of marine populations, it is relatively easy to collect specimens. On the other hand, it is extremely time-consuming to identify and count the members of the populations.

Background of Smithsonian Institution Oceanography

Before the founding of the Smithsonian Institution in 1846, at least seven principal exploring expeditions had been conducted by the U.S. Government, including the first oceanographic expedition. During 1838–42, Captain Charles Wilkes led a round-the-world expedition to investigate the commercial whaling industry in the Pacific. His staff included naturalists, geologists, botanists, mineralogists, taxidermists and a philologist. The extensive collections which resulted were transferred to the Smithsonian Institution in 1857.

In his book *Sons of Science* (Henry Schuman, 1949), Paul H. Oehser gives the following account of the Smithsonian's early involvement in expeditions, as reported by the then assistant secretary Spencer F. Baird:

"In his 1954 Report, Baird described twenty-six important explorations undertaken during the preceding two years, including six Pacific Railroad surveys. Of the Smithsonian's part of these he wrote:

" 'With scarcely an exception, every expedition of any magnitude has received more or less aid from the Smithsonian Institution. This has consisted in the supplying of instructions for making observations and collections in meteorology and natural history, and of information as to particular desiderata; in the preparation, in part, of the meteorological, magnetical, and natural history outfit, including the selection and purchase of the necessary apparatus and instruments; in the nomination and training of persons to fill important positions in the scientific corps; and in the reception of the collections made and their reference to individuals competent to report upon them; and in employing skilful and trained artists to make accurate delineations of the new and unfigured species. Much of the apparatus supplied to the different parties was invented or adapted by the Institution for this special purpose, and used for the first time, with results surpassing the most sanguine expectations'."

During his years as assistant secretary of the Smithsonian Institution, Dr. Baird spent several summers on the Atlantic coast collecting fishes. As a result of his efforts the United States Fish Commission was established by Congressional action in 1871 with headquarters at Woods Hole, Massachusetts. The initial voyages of the *Albatross*, *Fish Hawk* and other vessels were oceanic exploratory cruises in search of commercial fishery populations. The work on the systematics of fishes, as well as continued studies on other marine vertebrates, invertebrates and algae, was left within the Smithsonian Institution, although the responsibility for commercial fishes and for fishing explorations which might lead to commercial development was transferred to the Fish Commission in the Department of Commerce in 1903, and later to the Department of Interior.

Within the Smithsonian Institution, the principal effort in oceanography is concentrated in its largest bureau, the U.S. National Museum, which is further divided into a Museum of History and Technology and a Museum of Natural History. In the latter administrative unit the position of assistant director for oceanography has recently been established. The designated responsibilities of this assistant director include: (1) aiding Museum staff members in their marine research; (2) planning, developing and operating for the Institution a Sorting Center for marine biological and geological materials; (3) maintaining liaison with ocean-going vessels and scientists to collect biological materials; (4) helping to recruit outstanding marine sediment and taxonomic scientists; (5) bringing the Smithsonian oceanographic programme to the attention of scientists; (6) representing the Smithsonian Institution on various committees and councils concerned with oceanography.

Staff members of the Museum of Natural History who are involved in the oceanographic programme are located in the Departments of Zoology, Botany and Geology. As described here, the Smithsonian Oceanographic Sorting Center is a new separate unit, reporting directly to the assistant director for oceanography.

Oceanographic Research

Since it was obvious that the number of marine scientists on the existing staff was insufficient to meet the predicted need for the oceanographic programme, additional specialists are being employed to permit critical study of marine

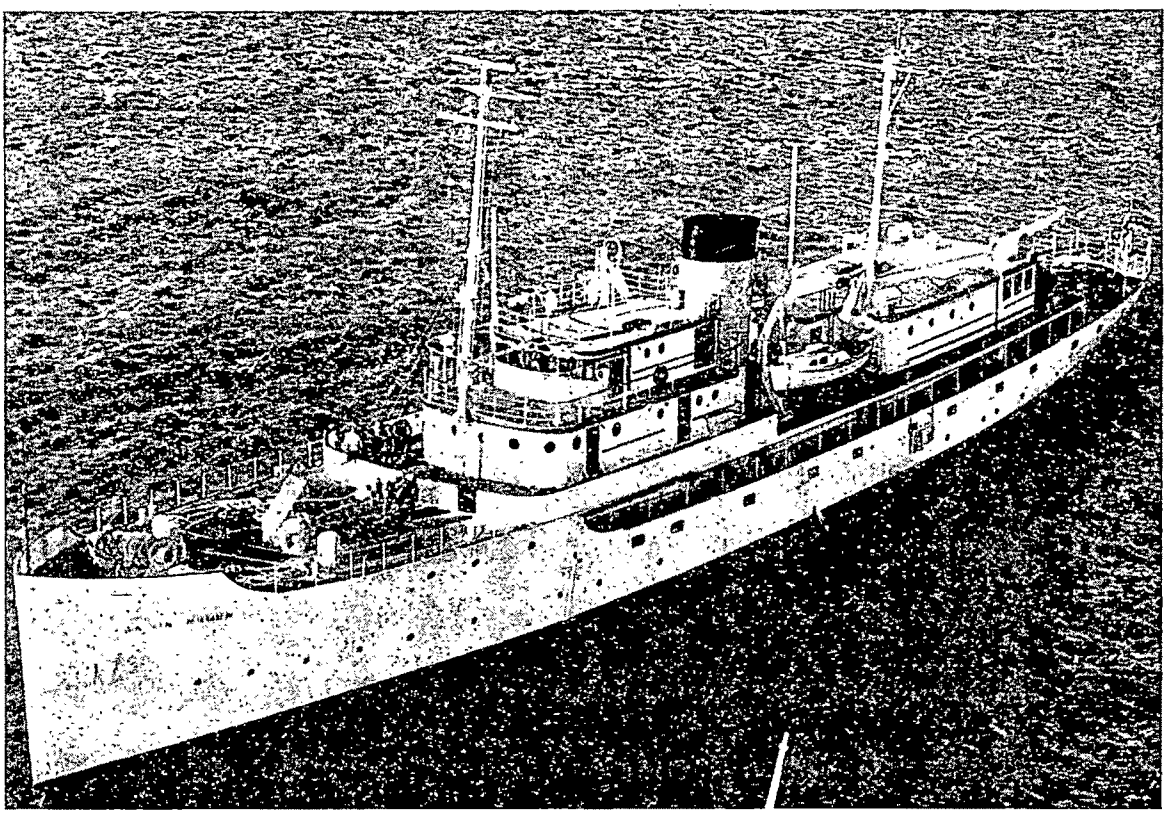


Fig. 1. National Science Foundation vessel *Anton Bruun*

collections that come to the Smithsonian Institution. The present policy of providing space and facilities for outside specialists and graduate students to work in the Museum of Natural History is being continued, and reciprocal arrangements for Museum personnel to work in other laboratories are encouraged.

Recognizing that a scientist often may do much valuable research on personally collected materials, the programme of the Smithsonian Institution provides for staff participation in marine expeditions. Eleven marine scientists from the Smithsonian Institution will participate in the International Indian Ocean Expedition and three are participating in the Tropical Atlantic Expedition.

In order to give staff scientists more time for research and exploratory field work, the Institution hopes to provide for an increase in the number of technical assistants, in supporting services, and in funds for travel. In addition to participating in research cruises, staff scientists are expected frequently to examine reference materials in other museums, thus achieving greater completeness of coverage and accuracy of data.

Adequate study of some materials collected by the oceanographic programme will require study of related materials from freshwater and land; thus it is anticipated that all divisions will continue to process fresh-water and terrestrial specimens, in addition to the handling of marine organisms.

The internal organization of the Museum of Natural History permits oceanographic work to be conducted in several divisions within three departments. Studies of recent invertebrates are at present carried on in two divisions of the Department of Zoology—the Divisions of Marine Invertebrates and of Molluscs. Staff members of these divisions have collected in the Atlantic, Pacific and Indian Ocean areas during the past year. Because of the complexity of the taxonomic groups under consideration, additional administrative units in the area of marine invertebrates may prove desirable.

In reference to marine vertebrates, personnel from the Divisions of Fishes, Reptiles and Amphibians, Birds, and Mammals (all within the Department of Zoology) contribute to the oceanography programme. Vertebrate specialists from the National Museum have actively participated in the International Whaling Commission and on Navy shark panels for many years.

One of the areas particularly in need of strengthening at the Smithsonian, as in other organizations, is that dealing with marine plants. At present, one staff member of the Division of Cryptogams (in the Department of Botany) is participating in the International Indian Ocean Expedition, conducting a study of nannoplankton dinoflagellates with the *Anton Bruun* (Fig. 1) as his base.

Within the Department of Geology, the present Division of Invertebrate Paleontology and Paleobotany curates collections of minute fossil and recent organisms including Foraminifera, Radiolaria and coccoliths, which, in association with bottom sediments, have important implications for oceanography. The prodigious numbers of specimens that will be collected during the oceanographic cruises should provide unprecedented opportunities to obtain information on the paleoclimatology of marine environments as well as on the evolution and biogeography of both fossil and recent organisms.

The Smithsonian Institution is the principal repository of geological collections made with the use of Federal funds; similarly it serves as a repository for cores, dredged-sediment samples, and rocks from the ocean bottom. In anticipation of a large increase in the receipt of oceanic sediments, research and curating are planned in such fields as petrology, sedimentary petrology, clay mineralogy, carbonate sedimentology, and geochemistry.

Manpower recruitment. The National Academy of Sciences Committee on Oceanography noted in its 1959 report (Brown, Harrison *et al.*, *Oceanography* 1960–1970) that "many lines of marine research depend upon precise definition of species". It recommended that museums

"be given financial support so that this kind of work can be carried forward". The shortage of systematists was recognized and a modest appropriation was suggested to keep this field of biology from "dying out".

Recently the Interagency Committee on Oceanography of the Federal Council for Science and Technology asked its manpower and training panel to investigate the manpower requirements for oceanography. The preliminary report of this Panel concluded that in view of the tremendous quantities of marine specimens being collected by oceanic survey and research cruises, an inadequate number of taxonomic scientists is available to process the collections promptly and adequately. Since taxonomy is basic to effective research in most biological fields, the shortage of taxonomists has very serious implications for the marine sciences, particularly during the present exploratory phases.

Although hoping to recruit new staff scientists, the Smithsonian Institution recognizes that specialists are not available for employment in each of the more than 60 major groups of marine specimens which will be collected. Co-operative arrangements and contracts with other public and private organizations will continue to encourage the use of specialists wherever they are located in the United States and abroad.

Smithsonian Oceanographic Sorting Center

One of the principal delays in the processing of marine materials has been the separation of bulk collections into units that can be handled effectively by specialists. Such collections include heterogeneous groups of plants and animals, often mixed with sediments. Many hours must be spent in carefully picking over the collections to separate them into the major components for identification and population counts. For example, the sorting of one pint of tropical marine plankton into biological groups may take the full time of one person for as much as twenty days.

In order to increase the efficiency of processing of samples and thus to permit more rapid publication of data, the Smithsonian Institution has established in Washington, D. C., the Smithsonian Oceanographic Sorting Center. The Center has employed personnel to follow the progress of marine biological samples from collection to publication and to assist oceanographers through all phases of the handling of specimens.

Although the Sorting Center is temporarily located in leased space, plans are being developed to move it to another localty in Washington where it will be adjacent to some of the oceanic activities of other agencies. It is anticipated that competence will be available in the Center to handle all types of collections of marine fauna, flora and sediments.

Separation of collections.—The initial separation into major groups will be performed by a staff of technicians under the supervision of experienced personnel who are aware of the various difficulties that may be encountered. It is hoped that many of the marine scientists who make collections will visit the Sorting Center to assist the supervisors in the training of technicians in the proper separation of the organisms within their interest.

Identification of specimens. It is expected that arrangements will be made as necessary on either a research or a service basis for the identification by specialists on each group of specimens which is sent to the Sorting Center. Lists of specialists on the various marine taxa will be obtained and maintained in the sorting centre. The opinion of advisory committees will be used to assure that specimens are sent to the best qualified and willing specialists. Specimens will be made available for research by qualified scientists without regard to institutional affiliation.

When collected materials are of interest primarily for distributional studies, it is anticipated that service identifications will be required. In such cases, when publication is not anticipated by the persons making the taxonomic determination, the Sorting Center hopes to defray expenses of such identification.

Provision of data. The Sorting Center will provide data concerning the composition of collections, including algae and sediments, by field station. It should be possible to relate such information to physical and chemical data from the same station on file in the National Oceanographic Data Center for ecological and other studies.

Progress reports on the processing of biological data from major expeditions will be provided to scientists on request. This service will assist biologists in co-ordinating their studies with the multi-agency or multi-national programme under which the data were collected. To avoid the overloading of individual scientists and to expedite the processing of collections, a current list of identification work in progress by various scientists also will be maintained.

In so far as is feasible, a programme for the recording and retrieval of data will be undertaken with the co-operation of National Oceanographic Data Center, Science Information Exchange, the U.S. Bureau of Commercial Fisheries, and other groups, as well as the Food and Agriculture Organization of the United Nations and other international groups. As appropriate, the Center will investigate the presentation of marine biological data through biogeographic maps and other devices that illustrate distribution and abundance of marine organisms.

Since the Sorting Center exists as a service organization, rather than as one dedicated to research, it is expected that encouragement will be given to the preparation and publication of processed data outside the Center. Normally, only when an obvious need exists for more rapid data processing, or when the data might not be available otherwise, would the staff of the Sorting Center publish the data on its own volition.

Co-ordination function for expeditions. While the Sorting Center, as outlined here, should meet the most direct needs for specimen processing, it is hoped that several inadequacies in the present mechanism for treatment of marine biological materials can be remedied. It is planned that the Center staff, as requested, assist with all aspects of the processing of marine collections taken during multi-agency and multi-national expeditions, such as the International Indian Ocean Expedition.

During the planning of expeditions, the Center will serve as a source of information on such items as methods of preservation and the storage, labelling and shipping of collections. The Center will maintain lists of biologists desiring to participate in marine expeditions to specific areas and the staff will provide information to them on the availability of research space and on alternative cruise plans.

Through the preparation of summaries of existing data the needs for biological information from areas of the world's oceans will be brought to the attention of scientists. For example, the Smithsonian Institution has prepared and distributed an illustrated field guide to marine and insular birds of the Indian Ocean for the use of participants in the International Indian Ocean Expedition. The guide summarizes published information on the biology of birds and suggests the sorts of additional fundamental observations which are needed. A guide to the scombroid fishes of the Indian Ocean has also been prepared, and additional aids for field identification and data gathering will be distributed as they are available.

Availability of space for research and training. It is not expected that the Sorting Center in Washington, D.C., will process all biological collections, for some scientists will continue to feel that the only satisfactory sorting of special collections is that performed under their immediate supervision. In such cases, the Sorting Center can provide two kinds of services. Individual scientists, having limited

space at their own institutions, may use the facilities of the Center and request the assistance of the staff in separating marine materials of special interest. Preservatives, containers and other necessary support can be made available on reasonable notice and after appropriate arrangements.

A second function of the Sorting Center could be the training of technicians for sorting, packaging, shipping, preserving and other scientific handling of specimens. Under appropriate sponsorship persons desiring to work in the Sorting Center may participate, under supervision, in all phases of the operation.

Distribution of Specimens

Although last in this discussion, it is obviously of first importance to scientists that specimens be made available in a number of places for future study. The Sorting Center will use advisory committees of Government and non-Government scientists to plan the equitable distribution of the specimens to appropriate well-curated museums for permanent storage. On the advice of such committees it is anticipated that the U.S. National Museum and other museums, on expression of their willingness to accept specimens, will receive materials for their collections. The specimens not otherwise claimed or distributed will be placed in the U.S. National Museum, to be available for study under the usual procedures.

In summary, the Smithsonian Oceanographic Sorting Center is visualized as a service organization for the benefit of scientists and scientific organizations both within and without the Federal Government. Collections submitted for processing will not automatically become the property of the U.S. National Museum. Indeed, it is the desire and the intention of the Smithsonian Institution to aid the interested scientific community to make an equitable distribution of all such materials, placing them in institutions throughout the country where they will most effectively advance the biological and geological aspects of the national oceanographic efforts.

DIRECTORATE OF OVERSEAS SURVEYS

BRIGADIER MARTIN HOTINE, director since 1946 and adviser on surveys successively to the Secretary of State for the Colonies and to the Secretary for Technical Co-operation, will retire on October 10 after forty-six years service to the Crown, and after making a very substantial contribution both to the scientific and technical basis of surveying and to its practical application. Brigadier Hotine was commissioned in the Royal Engineers in 1917; after service in Persia, Iraq and India he was appointed research officer to the Air Survey Committee during 1925–28, and was responsible for the development of the Arundel method of triangulation from air photographs. This, and other practical methods of air survey not involving the use of the more elaborate plotting instruments developed on the Continent, were described in his book, *Surveying from Air Photographs*, which was published in 1931; and many techniques still in use are based on them. In many respects this is still the basic text-book on the subject.

After a period of service at the War Office he spent two years in Central Africa on observations for the 30th arc of meridian, during which he made important contributions to the procedures for observing geodetic triangulation and to the precise measurement of first order bases with invar tapes, where accuracies better than one part in a million were required over distances of several miles. In particular, his paper on "The General Theory of Tape Suspension on Base Measurement", published in the *Empire Survey Review*, started from fundamental principles and led to important changes in the handling and in the techniques of measurement using invar tapes, which were at that time the only way of achieving these accuracies over such distances.

Another important result of his experience in Africa was his realization of the survey requirements of underdeveloped countries and of the need for a central agency to carry out the major tasks of geodetic control, aerial photography and basic topographical mapping, which later bore fruit in the Directorate of Overseas Surveys. On being posted to the Ordnance Survey in 1933 he was immediately concerned with an appraisal of the state of the control framework of Great Britain, which had been established in the nineteenth century; and his work led to the decision to re-triangulate the country. The work began in 1935 under Hotine's energetic direction, and the methods and procedures he then devised were generally followed until the end of the triangulation in 1962.

With the outbreak of the Second World War, he was posted to General Headquarters, British Expeditionary Force, and his technical instructions did much to inform junior survey officers about the French triangulation system. After Dunkirk he served in East Africa and Greece, returning to the War Office to become, for the rest of the War, the director of Military Survey and chief of the Geographical Section, General Staff, in 1941. There he was responsible through the Military Survey Service for meeting the needs of the defence services for maps, aeronautical charts and survey data for all their military operations throughout the world. In particular, he created a close working arrangement with the mapping services of the United States. He left the Army in 1946 so that his services could be made available to set up and direct the organization which has now grown into the Directorate of Overseas Surveys. The Directorate started with an establishment of about 180 in temporary accommodation at Bushy Park, and was transferred to its present offices at Tolworth in 1951, where its staff amounts to about 450. During the seventeen years of its existence, the Directorate has produced original topographical mapping, mostly at 1/50,000 scale, of well over one million square miles, most of it in Commonwealth countries (both dependent and independent) in Africa. The Directorate continues to give its services to overseas countries as part of the contribution made by Her Majesty's Government through the Department of Technical Co-operation to development overseas, and it is at present operating in more than thirty overseas countries.

This kind of wholesale survey and mapping of underdeveloped countries posed a number of fundamental questions to which Brigadier Hotine and his staff had to find answers. The smaller scales originally envisaged were found to be inadequate for development planning, and the scale of 1/50,000 was generally found to be the most suitable. From the start very high standards were set in the accuracy and in the beaconing of the triangulation framework so that this would be permanent and adequate for the future amplification of control for larger scale mapping of particular projects or areas. In the same way, the maps issued by the Directorate were designed on regular sheet lines and to a specification rigorous enough for their forming part of the national map system of the country concerned, though the actual areas were covered in the order of priority dictated by its development plans.

These higher standards meant a slower output, but they had the advantage that the maps and survey framework were of permanent value, and available for many uses, instead of being restricted in value to a specific

project. There were, however, cases where the urgent requirements of particular projects prompted the production of maps on little or even no control, showing detail only, without contours. This saved time which would have been spent on the extra ground work required for plotting the contours, and it was possible to add the contours in subsequent editions when control had been fixed.

At the beginning and until 1950 the Directorate relied entirely on photographs taken by the Royal Air Force; but in later years considerably more use has been made of air photographs taken on contract by civilian firms. When precise distance measurement on the ground by radio became a practical proposition with the development of the tellurometer in 1956, the Directorate was the first extensive user of this equipment and was able to increase its rate of establishing control frameworks by two or three times and to carry out some tasks hitherto regarded as impracticable. Before the establishment of the Department of Technical Co-operation there was frequently a risk that work of technical assistance started in the Colonies under arrangements for colonial development and welfare would be interrupted when these countries achieved independence. The Directorate of Overseas Surveys had a part to play in averting this, and Hotine's personal visits to overseas countries have played a great part in preserving continuity in survey work.

The use of satellites and the possibilities of space travel have made it specially necessary to consider geodesy as a truly three-dimensional subject instead of regarding it as principally two-dimensional with corrections and modifications to allow for the third dimension, as had been the fashion previously. Hotine has been one of the pioneers in this field and has taken a leading part in the discussions held by geodesists and mathematicians at Venice in 1959, Cortina in May 1962, and Munich in October 1962; indeed, his papers on 'non-classical' geodesy are well known for the lead they have given on this subject.

Brigadier Hotine was president of the Commonwealth Survey Officers' conference in 1955 and in 1959 and again this year. This conference, which is sponsored by the Department of Technical Co-operation, is organized by the Directorate of Overseas Surveys under the direction of the heads of the four Government Survey departments. It succeeded the original conferences of Empire Survey Officers held in 1928, 1931 and 1935 at which the idea of a central survey organization was first suggested. The conference now comprises 200–300 members and has achieved a high standard of discussion, consultation and record due to its careful planning, informal and friendly nature, and to his firm but tactful presidency.

Brigadier Hotine was awarded the C.B.E. in 1945, the C.M.G. in 1949, the Founder's Medal of the Royal Geographical Society in 1947, and the President's Medal of the Photogrammetric Society in 1955. He is an Officer of the Legon of Merit (U.S.A.).

Brigadier Hotine will be succeeded as director and adviser by Mr. G. J. Humphries. From the University of Reading, Mr. Humphries went to Nigeria in 1928 as a surveyor and served in the Army from 1939 until 1946, ending with the rank of lieutenant-colonel. From 1944 he was on Brigadier Hotine's staff at the War Office and was responsible for a large part of the planning and setting up of the Directorate of Colonial Surveys. He was in charge of the organization and running of the field parties as well as acting as deputy to the present director. He was awarded the O.B.E. in 1954.

GAMMA-GLOBULIN VARIABILITY: A GENETIC HYPOTHESIS

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THE large number of antibodies an individual can form is difficult to reconcile with the presumed constancy, at least in the germ line, of the genes controlling γ-globulin structure. A hypothesis is described in this article suggesting a mechanism whereby a few specialized genes could control the synthesis of a large number of different proteins. The hypothesis is consistent with established genetic principles and with present concepts of protein synthesis.

Nature of γ-globulin variability. The three main classes of human γ-globulin, $7S\gamma$-, γ_{1A}, (or β_{2A}-), and γ_{1M}- (or β_{2M}-) globulins, appear to have in common a polypeptide chain with specific antigenic properties determined by the autosomal locus *Inv*[1–4]. The $7S\gamma$-globulins have in addition a second characteristic polypeptide chain with specific antigenic properties determined by the locus *Gm*[5,6]. The γ_{1A}- and γ_{1M}-globulins also have specific antigenic determinants not shared by the other classes, suggesting that two additional genetic loci control the synthesis of a second polypeptide chain in these γ-globulins[4], although no polymorphisms corresponding to these presumed loci have yet been described.

Variations in the electrophoretic behaviour of the polypeptides of different human serum myeloma globulins have been demonstrated by Poulik and Edelman[7] using urea and thiol dissociated proteins. The conditions of the electrophoresis (in starch gels containing 8 M urea) make it very probable that these variations reflected differences in the amino-acid sequences of the constituent polypeptides of the several myeloma globulins. Similar variations have also been observed[8] in the properties of the low-molecular-weight polypeptides of guinea pig antibodies directed against specific haptens, so that variation in the amino-acid sequences of γ-globulin polypeptides seems to be a normal phenomenon. In order to determine the nature and extent of this variability it would be desirable to isolate and study the structure of polypeptides from mono-clonal antibodies. This has not yet been done. However, recent work on the nature of Bence Jones proteins[9] and on the occurrence of proteins with similar properties in the urine of normal individuals[10,11] suggests that Bence Jones proteins provide equivalent material by an 'accident of nature'. Comparisons of the structures of different Bence Jones proteins, which are known to be highly variable, should therefore reveal the extent and types of variation which must be explained by any hypothesis concerning the variability of γ-globulins.

Bence Jones proteins may differ in molecular weight, electrophoretic mobility, iso-electric point, amino-acid composition, *N*-terminal amino-acid, and antigenic character[12]. They can all, however, be classified immunologically[13] as type *A* or *B* (equivalent to types II and I of myeloma globulins[14,15]), and Putnam and Miyake[16] have shown that this classification is correlated with their *N*-terminal amino-acids. All type *B* Bence Jones proteins have the same *N*-terminal amino-acid (aspartic acid or its amide), but the *N*-terminal amino-acids of the type *A* Bence Jones proteins are variable. For example, some type *A* proteins had no *N*-terminal amino-acid residue detectable, while *N*-terminal threonine, leucine, tyrosine

or valine was found in others. Many Bence Jones proteins have been tested for their reactions in the *Gm*- and *Inv*-detecting systems[17,18]. All failed to react in the *Gm*-system, and some also failed to react in the *Inv*-system, but many were *Inv* (a+b−) or *Inv* (a− b+). No unequivocal examples of *Inv* (a+ b+) reactions were found. Usually, but not invariably, type *B* Bence Jones proteins proved to be either *Inv* (a+) or *Inv* (b+); all type *A* were *Inv* (a− b−). Tryptic peptide 'finger prints' have been described by Putnam[19,20] for three type *B* and five type *A* Bence Jones proteins. No two were identical, but those of type *B* shared some 8 peptides out of a total of about 20, and the type *A* Bence Jones proteins also shared some 7–9 peptides. However, Putnam reported that probably no peptides were identical in the tryptic digests of the two types. One of the peptides common to all three type *B* proteins lacked a basic amino-acid suggesting that they share a common *C*-terminal amino-acid sequence, in addition to having aspartic acid in the *N*-terminal position.

Any hypothesis concerning the variability in expression of the γ-globulin loci must therefore be able to explain the presence of different amino-acid sequences in polypeptide chains having identical *N*-terminal amino-acids and some common amino-acid sequences (including the *C*-terminal sequence), which also have enough structure in common to establish an antigenic character (*B*) and the ability to react with the *Inv*-detecting systems. The hypothesis must also account for the existence of an antigenically distinct type (*A*) of polypeptides which have variable structures (including their *N*-terminal residues) and some common amino-acid sequences, but which fail to react with the *Inv*-detecting systems and have overall amino-acid sequences sufficiently different from type *B* to lead to an essentially completely different set of tryptic peptides.

Antibody specificity. Before considering possible mechanisms for producing variable polypeptides from a given set of structural genes, the question must be asked whether variations in the amino-acid sequences of γ-globulin polypeptides could account for antibody specificity. In several cases (for example, ribonuclease[21], alkaline phosphatase[22], human haptoglobins[23]) the secondary and higher orders of structure have been shown to be completely determined by the primary amino-acid sequence of the corresponding polypeptides, as judged by the recovery, without the addition of complex organizing factors, of the native configuration of the proteins after unfolding with urea and thiols. A change in the amino-acid sequence of a γ-globulin polypeptide could therefore give rise to different antibodies directly as a consequence of the changed sequence and/or indirectly by causing a change in the folding of the molecule. However, as pointed out by Edelman and Benacerraf[2], the number of different polypeptides necessary to account for the diversity of antibodies would, on this simple hypothesis, have to be very large. On the other hand, as indicated here, most antibodies probably contain two types of polypeptide chains, both of which appear able to vary[7]. The required variation in primary structure would consequently be much reduced (cf. Edelman and Benacerraf[2]) if the final configuration of the molecule is not fully determined prior to the juxtaposition of both chains, for, in these circumstances, a change in the amino-acid sequence of either polypeptide chain could affect the final configuration of the combining site (even if this site directly involves only one of the chains). For example, if two different γ-globulin polypeptides could have p and q different sequences, then a total of pq different configurations of antibody molecules might be obtained by their mutually determined folding. This suggestion implies that the three dimensional structure of γ-globulin is sensitive to changes in the primary amino-acid sequences of both its constituent polypeptides. Suitable reconstitution experiments with separated polypeptides from different mono-clonal antibody preparations could be used to test this.

The complete determination of antibody configuration by the primary amino-acid sequences of the corresponding polypeptides is probably a necessity in cells which can form antibody in the absence of antigen. However, it is conceivable that the 19*S* antibodies, which are produced only in the presence of antigen by cells with no 'immunological memory'[24], may have configurations in part determined by the antigen. Even if this proves to be the case, the problem still remains of the mechanism whereby polypeptides with many different amino-acid sequences can be synthesized under the direction of a limited number of loci by cells which can multiply extensively and still continue to produce these polypeptides.

Possible genetic mechanisms for the variability. Two of the mechanisms which have been discussed in the past for changes in protein structure via genetic events appear able to produce large changes by single events. One of these involves chromosomal rearrangements, and specific examples of its involvement are known in the case of the human haptoglobins[25], Lepore hæmoglobins[26], and possibly also in the case of thalassæmias in general[27]. The other mechanism involves the change in amino-acid sequences which is presumed to result from addition or deletion of base pairs from protein structural genes with a consequent shift in the coding frame of reference; the evidence for this type of change is, at present, inferential; but the arguments are extremely persuasive[28,29]. It is suggested here that chromosomal rearrangements involving the γ-globulin structural genes (conceivably also sometimes resulting in a change in amino-acid sequences as a consequence of a shift in the reading frame) are responsible for the variation in the products of the γ-globulin loci in different antibody producing cells. These chromosomal rearrangements are presumed to occur during the somatic cell divisions leading to the mature immune system. Since chromosome pairing and inter-chromosomal crossing over are thought to be rare events in mitosis, the rearrangements are suggested as occurring usually within a single chromatid, or between sister chromatids. (There are ample precedents for somatic events involving intra-chromatid or sister strand crossing over in *Vicia*[30], *Bellevalia*[31] and maize[32,33].) However, it should be noted that the expression of only a single allele in myeloma globulins and Bence Jones proteins could be interpreted as indicating the occurrence of inter-chromosomal crossing over.

A model γ-globulin gene. In order to illustrate the hypothesis more extensively a specific model will be developed, although it is recognized that others can be conceived. The present model, while perhaps not likely to be proved correct in its details, can nevertheless serve as a basis for the design of experiments to test the overall hypothesis that γ-globulin variability is the consequence of somatic chromosomal rearrangements involving the γ-globulin structural genes. Consideration of the effects of different types of chromosomal rearrangement suggests that the observed variations in γ-globulin structure could be accounted for by rearrangements involving inversions.

Messenger-RNA active in protein synthesis appears to be synthesized in relation to only one strand of the DNA double helix of a protein structural gene[34,35]. This conclusion, based on experimental observations, is supported by theoretical difficulties which make it virtually impossible to reconcile the results of coding experiments[36,37] with the structure of known proteins and the effects of 'point' mutations on proteins if both strands of DNA were copied. If part of the DNA of a protein structural gene is inverted by a chromosomal rearrangement, then, to preserve the sense of direction of the strands, part of the previously uncopied strand will become continuous with the copied strand (Fig. 1). The messenger-RNA copied from the inverted section of the gene will consequently have a changed base sequence (being that

complementary to, and in the reverse order of, the original message), and will instruct the synthesis of a very different sequence of amino-acids from that coded by the same region of DNA before it was inverted. (Cytologically and genetically detected inversions are frequent in many organisms which change the order, but not the expression of the genes in the inverted regions. In these relatively large-scale inversions the relationship between a structural gene in the inverted region and the corresponding indicator (whatever it may be) which determines the active strand of DNA is presumably unchanged. The small-scale inversions considered in this article are assumed to involve regions of DNA for which the active strand is already defined by an indicator outside the inverted regions. It must be stressed, however, that the hypothetical mechanisms for variation in the γ-globulin polypeptides discussed here would not be invalidated if both strands of DNA prove able to dictate the synthesis of messenger-RNA since inversions would change the base sequences in both messages.)

In order for the γ-globulin genes to participate in somatic chromosomal rearrangements of the type illustrated in Fig. 1, without requiring any crossing-over to occur other than in homologous regions of DNA, the genes would have to contain local inverted duplications of base sequences over enough length to permit intragenic synapsis and crossing-over. Since a sufficiently large number of rearrangement products must be possible to generate enough different amino-acid sequences to explain antibody specificity, several pairs of sites where inversion can occur must be present within the gene. This suggests the presence of duplications (without inversion) in the genes. However, some or all of the several pairs of inversion sites could be different.

The number of inverted duplications in a locus needs not be large to obtain sufficient variability. Fig. 2 shows schematically some of the possible rearrangements with only two inverted duplications and two duplications in the original gene. The products of any given rearrangement are available for further rearrangements during subsequent cell divisions, so that the full range of variability could be produced in many different cell lineages.

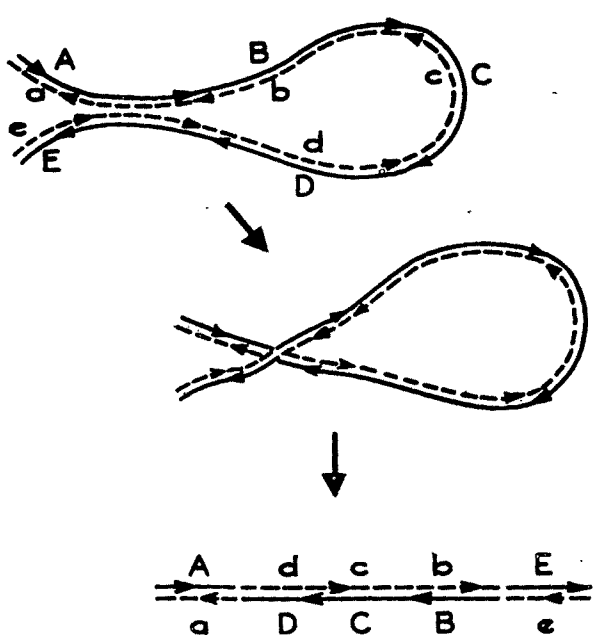
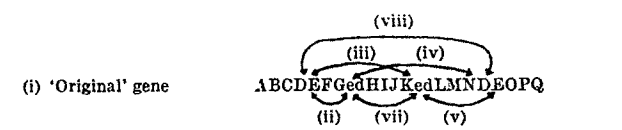


Fig. 1. A diagram representing the consequences of an intra-chromatid inversion. The complementary strands of DNA are represented by solid and dotted lines; their directional senses are shown by arrows. The inversion causes a portion of the 'dotted' strand to become continuous with the 'solid' strand. The capital and small letters are used to represent complementary sequences of bases



(ii) Inversion	ABCDEgfedHIJKedLMNDEOPQ
(iii) Inversion	ABCDEkjihDEgfedLMNDEOPQ
(iv) Inversion	ABCDEFGednmlDEkjihDEOPQ
(v) Inversion	ABCDEFGedHIJKednmlDEOPQ
(vi) Unequal crossing over with sister strand	ABCDEFGedHIJKedHIJKedLMNDEOPQ
(vii) Loss of a small acentric ring	ABCDEFGedLMNDEOPQ
(viii) Loss of a large acentric ring	ABCDEOPQ

Fig. 2. A schematic representation of some of the rearrangement products which are possible in a region of DNA with two duplications and two inverted duplications. Only one strand is illustrated and capital letters and small letters are used to imply base sequences related in the reverse complementary sense (for example, M could be ATGCAA and m would then be TTGCAT)

The observations made on the variation of structure of the type *B* Bence Jones proteins can be accounted for by rearrangements similar to those illustrated in Fig. 2 if the 'original' gene is regarded as a simplified model of an allele at the *Inv* locus. Thus all the rearrangement products would dictate the synthesis of polypeptides with several amino-acid sequences in common (including the *N*- and *C*-terminal sequences) which could account for the immunological characteristics shared by all type *B* Bence Jones proteins. Yet the 'finger prints' of these polypeptides would not be identical.

The observations made on the type *A* Bence Jones proteins are more difficult to reconcile with the control of the structure of these proteins by a single locus. Since no type *A* proteins have been reported as reacting in the *Inv*-detecting systems, they may well be the products of some locus (not yet described) other than the *Inv* locus. This could account for the complete dissimilarity of 'finger-prints' of the *A* and *B* types, but would not solve the problem of the mechanism permitting variation of the *N*-terminal residues of these proteins. Unfortunately no experimental evidence has been published relative to the nature of the signal in naturally occurring messenger-RNA which indicates the commencement of polypeptide synthesis (in synthetic messenger-RNA it is, presumably, the end of the RNA molecule). However, Nirenberg[38] has raised the possibility that the beginning or end of the message, in terms of protein synthesis, might be the result of a region of messenger-RNA which can form a hairpin-like helical structure (since double helical RNA will not stimulate *in vitro* amino-acid incorporation). Such regions would necessarily be coded in DNA by closely linked inverted duplications, and could permit the type of rearrangements (inversions over the *N*-terminal signal) required to account for the variable *N*-terminal residues in the type *A* Bence Jones proteins. Examples of the events envisaged are presented in the next section.

An examination of the reasonableness of the model. The reasonableness of any model involving inversions can in part be assessed by inspection of the base triplets which code for specific amino-acids. Lists of these triplets are increasing so rapidly at the time of writing that I have used a list presented in a lecture by Dr. Severo Ochoa in Madison during February. This tentative list (of 41 coding triplets) was based on the work of the groups of Nirenberg and Ochoa, and on an analysis by Jukes[39] of data derived from amino-acid substitutions observed in mutant proteins. Eighteen of the twenty amino-acids can be coded by triplets which in their reversed complementary form also code for amino-acids (even with the present incomplete list). It is perhaps not coincidental that two amino-acids (glutamic acid and serine) which each constitute more than 10 per cent of many γ-globulins can be coded by their reversed complements (AAG and CUU respectively). Suitable hydrogen-bonding sites in the final γ-globulin polypeptides could thus be maintained, even after the occurrence of inversions, if these two hydro-

Regions of duplication or inverted duplication		I	II	III	IV	V
Original gene	DNA	←GAA GAG TTC→←GAA CTC TTC GTT→←CAA AAT GAA CTC TTC→←GTT TTA TTG ACA→←AAG GAA GAG TTC→←TGT CTT ACT CAC AAC→←GAC TGT GAA GAG TTC→ →CTT CTC AAG←→CTT GAG AAG CAA←→GTT TTA CTT GAG AAG←→CAA AAT AAC TGT←→TTC CTT CTC AAG←→ACA GAA TGA GTG TTG←→CTG ACA CTT CTC AAG←				
No rearrangement	M-RNA	GAA CUC UUC GUU→←CAA AAU GAA CUC UUC→←GUU UUA UUG ACA→←AAG GAA GAG UUC→←UGU CUU ACU CAC AAC→←GAC UGU GAA GAG UUC→← CUU GAG AAG				
	Protein	(NH_2) Val — Gln. Asn. Glu. Pro. Ser — Val. Tyr. Cys. Thr — Arg. Glu. Gly. Ser — Leu. Leu. Thr. Pro. Asn — Ala. Leu. Glu. Gly. Ser —				
III–IV Inversion within chromatid (see Fig. 4)	M-RNA	GAA CUC UUC GUU→←CAA AAU GAA *CUC UUC CUU→←UGU CAA UAA AAC→←GAA GAG* UUC→←UGU CUU ACA CAC AAC→←GAC UGU GAA GAG UUC→← CUU GAG AAG				
	Protein	(NH_2)Val — Gln. Asn. Glu. *Pro. Ser. Leu — Leu. Gln. Ilu. Asn — Glu. Gly.* Ser — Leu. Leu. Thr. Pro. Asn — Ala. Leu. Glu. Gly. Ser —				
III–V Inversion within chromatid	M-RNA	GAA CUC UUC GUU→←CAA AAU GAA *CUC UUC ACA GUC→←GUU GUG AGU AAG ACA→←GAA CUC UUC CUU→←UGU CAA UAA AAC→←GAA GAG* UUC→← CUU GAG AAG				
	Protein	(NH_2)Val — Gln. Asn. Glu. *Pro. Ser. Thr. Ala — Val. Gly. Met. Arg. Thr — Glu. Pro. Ser. Leu — Leu. Gln. Ilu. Asn — Glu. Gly.* Ser —				
II–IV Inversion within chromatid involving *N*-terminal region	M-RNA	GAA *CUC UUC CUU→←UGU CAA UAA AAC→←GAA GAG UUC AUU UUG→←AAC GAA GAG* UUC→←UGU CUU ACU CAC AAC→←GAC UGU GAA GAG UUC→← CUU GAG AAG				
	Protein	*(NH_2) Leu — Leu. Gln. Ilu. Asn — Glu. Gly. Ser. Ilu. Cys — Asn. Glu. Gly.* Ser — Leu. Leu. Thr. Pro. Asn — Ala. Leu. Glu. Gly. Ser —				
II–V Inversion within chromatid involving *N*-terminal region	M-RNA	GAA *CUC UUC ACA GUC→←GUU GUG AGU AAG ACA→←GAA CUC UUC CUU→←UGU CAA UAA AAC→←GAA GAG UUC AUU UUG→←AAC GAA GAG* UUC→← CUU GAG AAG				
	Protein	*(NH_2) Thr. Ala — Val. Gly. Met. Arg. Thr — Glu. Pro. Ser. Leu — Leu. Gln. Ilu. Asn — Glu. Gly. Ser. Ilu. Cys — Asn. Glu. Gly.* Ser —				
IV × V′ Partial duplication by crossing over with sister strand	M-RNA	GAA CUC UUC GUU→←CAA AAU GAA CUC UUC→←GUU UUA UUG ACA→←AAG GAA G(*AG UUC→←UGU CUU ACU CAC AAC→←GAC UGU GAA G*)$_3$AG UUC→← CUU GAG AAG				
	Protein	(NH_2) Val — Gln. Asn. Glu. Pro. Ser — Val. Tyr. Cys. Thr — Arg. Glu (*Gly. Ser — Leu. Leu. Thr. Pro. Asn — Ala. Leu. Glu*)$_2$ Gly. Ser —				

Fig. 3. A hypothetical model for part of a structural gene for a γ-globulin polypeptide to illustrate some of the consequences of intra-chromatid inversions and sister strand crossing over. The DNA of the original gene is shown at the top, and below are the messenger-RNA sequences copied from the active strand before and after different re-arrangements of the structural gene. The corresponding amino-acid sequences are shown below each type of messenger-RNA. The regions involved in the rearrangements are shown in italics. The sense of direction of the nucleic acid strands is indicated by arrows which also represent segments of the molecules with sequences of bases not written *in extenso*. Details of the intra-chromatid crossing-over event leading to the inversion III–IV are shown in Fig. 4

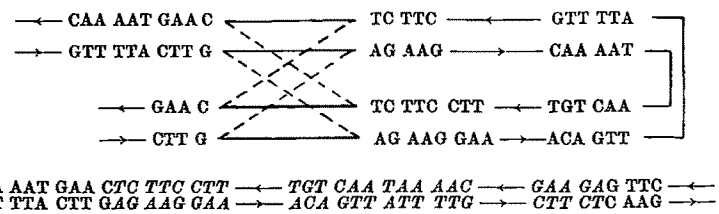


Fig. 4. Details of a typical intra-chromatid crossing-over event leading to an inversion (the III–IV inversion of Fig. 3). Homologous regions of DNA are assumed to be necessary for the occurrence of synapsis and crossing-over. The dotted lines indicate the continuity of the strands after the crossing-over. The inverted region is italicized

gen-bonding amino-acids were coded by the triplets indicated.

There is convincing independent evidence for the existence of local inverted duplications in DNA of the type here suggested as involved in the γ-globulin loci. Thus the recent work of Spencer *et al.*[40] on the helical hairpin-like nature of soluble-RNA, and the observations of Goodman and Rich[41] that soluble-RNA is synthesized in relation to specific regions of DNA leave little doubt of the occurrence of regions of DNA with quite small-scale inverted duplications of about 40 base pairs length.

As a final enquiry into the reasonableness of the model involving inversions, an attempt has been made to present in detail in Figs. 3 and 4 a hypothetical structural gene for a γ-globulin polypeptide of the kind involved in the Bence Jones proteins of type *A* with examples of the effects of different rearrangements on the final amino-acid sequences (including the *N*-terminal residue). It must be stressed that inversions over the *N*-terminal signal leading to a change in the *N*-terminal residue would not be excluded if some specific sequence of bases codes for the *N*-terminus. The necessary crossing-over could still be achieved if the reversed complement of the whole or part of the sequence of bases corresponding to the 'beginning of message' sign occurred in the active strand of the amino-acid coding region of the gene. On the other hand, if the reversed complement of part or all of the 'beginning of message' sign (whatever its nature) does not occur in the amino-acid coding region of the gene, then inversion over this sign is not likely to be possible. The *Inv* genes may be of this type since the *N*-terminal residue of type *B* Bence Jones proteins shows no variation. Rabbit γ-globulins also have one polypeptide with a fixed *N*-terminal sequence[42], (NH_2)Ala.Leu.Val.Asp.Glu—, and so probably inversions cannot occur over the *N*-terminal end of the gene controlling this polypeptide. In more general terms, it is clear that for a part (or the whole) of a gene to be susceptible to inversion it must be flanked by inverted duplications.

Considerations of this type suggest that some of the unusual properties of the alleles at the *Gm* locus can be interpreted in terms of the location of inversions within these genes. Thus in heterozygotes (Gm^a/Gm^b) the Gm^b allele is expressed in myeloma globulins with only about one-fifth the frequency of the Gm^a allele, and in six negroes (Gm^{ab}/Gm^{ab}) all the myeloma globulins were ($Gm(a+\ b-)$ (ref. 18). If that portion of the genes Gm^b and Gm^{ab} which controls the $Gm(b+)$ phenotype contains an inverted duplication of some other part of the gene (or is between two inverted duplications) then its expression would be susceptible to suppression by inversion. If the $Gm(a+)$ phenotype were determined by part of the gene outside any inverted duplications it would not be suppressed by the rearrangements.

In the preceding discussion the tacit assumption has been made that the duplications in the γ-globulin structural genes involve integral numbers of amino-acid coding units (triplets). If they do not, then some rearrangements will cause a change in the sequence of the resultant polypeptide of the type discussed by Crick *et al.*[28]. This raises the interesting possibility that type *A* as well as type *B* Bence Jones proteins could in fact be determined by the *Inv* locus, but that in the *Inv* genes the inverted duplication suggested as coding for the 'beginning of message' sign involves a non-integral number of coding units. Absence of inversion over the *N*-terminal sign would lead to the fixed *N*-terminal amino-acid (aspartic acid) of type *B* Bence Jones proteins and be accompanied by reading of the message with one frame of reference, but an inversion over this region would change the *N*-terminal amino-acid and be accompanied by reading of the message with a different frame of reference (corresponding to type *A* Bence Jones proteins). Ability to react in the *Inv*-detecting systems and with antibodies against type *B* proteins would be lost as a consequence of the completely changed amino-acid sequence dictated by the new frame of reference.

Experimental tests of the hypothesis. The most direct and realizable method for testing the hypothesis would seem to be the determination of amino-acid sequences in a number of Bence Jones proteins. The relationships between the sequences obtained from a given structural gene before and after different rearrangements have occurred are illustrated in Fig. 3, and permit some predictions to be made. A prerequisite of the occurrence of many rearrangement products is the presence of several inverted duplications within the gene, but until the genetic code is completely known these will be difficult to detect by amino-acid sequence studies. However, there may also be duplications within the gene, which would be detected in the Bence Jones proteins as repeated amino-acid sequences (for example, Glu.Gly.Ser is repeated in Fig. 3) unless the duplications involve a non-integral number of coding units. After an inversion, whole sequences will be changed *en bloc* but will still be between unchanged sequences when integral numbers of coding units are involved (for example, Glu.Pro.Ser—Val.Tyr.Cys.Thr—Arg.Glu.Gly.Ser becomes Glu.Pro.Ser.Leu—Leu.Gln.Ilu.Asn—Glu.Gly.Ser). The position in the whole polypeptide of these new sequences will be variable depending on the particular inversion (cf. the positions of Leu.Gln.Ilu.Asn after the four inversions illustrated). Inversions over the *N*-terminal signal of different lengths are presumed necessary to give type *A* Bence Jones proteins with different *N*-terminal residues. The distance from the *N*-terminus to the position where common sequences occur should consequently differ in such proteins (cf. the position of the common sequence Leu.Gln.Ilu.Asn in the two inversions II–IV and II–V), as should the lengths of the common sequences. These predictions could be tested by sequence determinations, particularly of the *N*-terminal regions, on type *A* Bence Jones proteins with different *N*-terminal residues.

General observations. If the present hypothesis of somatic rearrangements in the structural genes for the γ-globulins has any validity, it is possible that this type of change is involved in other sorts of differentiation. Certainly the types of mechanism here discussed, which permit an initially fixed genotype to be changed by somatic events occurring during development (cf. Nance[27]), should be considered in relation to differentiation in general.

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OBITUARY

Prof. B. B. Bevan-Baker

Bevan-Baker, who died on July 1 at the age of seventy-three, was a man of rich and varied talents whose early promise of the highest scientific and mathematical achivement was tragically frustrated by the ill-health that beset him from middle life. But until illness enforced an early retirement, he gave distinguished service to the University of London and Royal Holloway College.

Bevan Braithwaite Baker was born in 1890; in ultimately adopting the surname Bevan-Baker he was merely acquiescing in a usage of many of his friends. He entered University College, London, in 1906 and graduated in physics at the age of nineteen. He then did research on crystal structure in metals in London and in Munich; in some of this work he was associated with his contemporary, E. N. da C. Andrade (now Professor), to whom he evidently owed his introduction to the subject. Baker's interests then changed and he took London degrees in mathematics in 1912 and 1914. During the First World War he served with the Friends' Ambulance Unit in Italy, this being in accordance with his Quaker upbringing. On his return, he was from 1918 until 1920 assistant in pure mathematics in University College. From 1920 until 1924, Baker was lecturer in mathematics in Edinburgh under E. T. Whittaker. This was the time of his fruitful mathematical activity. His main work was a series of papers on periodic orbits, but he wrote also on such varied topics as the hypergeometric function, the path of an electron in the neighbourhood of an atom, and an extension of Heaviside's operational calculus. Baker was elected a Fellow of the Royal Society of Edinburgh in 1921; he gained a D.Sc. at Edinburgh in 1923 for a much extended account of his dynamical work. He was secretary of the Edinburgh Mathematical Society from 1921 until 1924.

From 1924 until 1944, Baker was professor of mathematics in the University of London and head of the Department of Mathematics in Royal Holloway College. He played a big part in the general affairs of the College in peace and in war. He was called on to serve for two consecutive three-year terms as a Staff Governor, a most exceptional procedure. During his headship, the undergraduate school of mathematics flourished greatly and its students gained more than a proportionate share of high honours in University of London examinations. A few of these women mathematicians began postgraduate work at Royal Holloway College before going on to research elsewhere, and they now hold distinguished positions in universities in Britain and overseas. Although Baker was disappointed at not doing more to develop a postgraduate school, this was actually the beginning of what has now become a well-known research centre (now taking both men and women students). But no account of Baker's time at Royal Holloway College should omit mention of Miss Marion Pick, a graduate of the College and a lecturer throughout Baker's term of office, who supplied the immediate stimulus to the students who were to make a career in mathematics and who also kept the Department in full operation during Baker's illnesses.

Baker was the most unselfish of men and bestowed innumerable kindnesses on colleagues and students. At one time he was an impressive figure of immense girth. This was because of thyroid trouble and after this was cured he became over-slim. During the rest of his working life he had a series of illnesses until his doctors ordered his retirement at the early age of fifty-four. Baker was denied the stamina to do much research work after joining Royal Holloway College. His name appears as joint author with E. T. Copson of *The Mathematical Theory of Huygens' Principle* (Oxford, 1939), but again his health caused his contribution to be far less than he wished.

Music was Baker's ruling passion; he was a pianist of the highest class for a non-professional, and he played various other instruments and took part in operatic and other singing. Family opinion in his youth had dissuaded Baker from following music as a profession, so it was a wonderful consolation for him for many years to share in and to enrich the musical life of Royal Holloway College. In 1918 Baker married Margaret Barbour of Edinburgh, sister of the distinguished geologist, G. B. Barbour of Cincinnati. She sustained him in every way until her death in 1961. Until then he had enjoyed his retirement in improved health but his health declined again after that. Baker is survived by four of his five children.

W. H. McCrea

NEWS and VIEWS

C.S.I.R.O. Division of Soils: Mr. J. K. Taylor

MR. J. K. TAYLOR will retire in December 1963 as chief of the Division of Soils, Commonwealth Scientific and Industrial Research Organization, Australia. Mr. Taylor graduated in agriculture at the University of Sydney in 1920 and became demonstrator in agricultural chemistry at that University. In 1921 he was awarded a Walter and Eliza Hall research fellowship for postgraduate research in soils and plant nutrition at the Universities of Sydney and California. He obtained his M.Sc. degree at the latter University in 1923, his B.A. degree at the University of Sydney in 1924, and was appointed lecturer in agricultural and dairy bacteriology at Hawkesbury Agricultural College, New South Wales. In 1927, Mr. Taylor was appointed to the Division of Soils Research of the Council for Scientific and Industrial Research, the forerunner of C.S.I.R.O., where he undertook the first soil surveys conducted by the Council. Mr. Taylor was closely associated with his chief, Prof. J. A. Prescott, in building up the Division and expanding its soil survey activities. He also played an important part in the establishment of regional laboratories in various parts of Australia so that the work of the Division could be carried out more effectively. Mr. Taylor was appointed deputy chief of the Division in 1944 and chief in 1947. Since then, the scope of the Division's research has been greatly increased and there are now strong research groups concerned with the chemistry, physics, and microbiology of soils, and with clay mineralogy, pedology and soil survey. Mr. Taylor has been closely associated with many aspects of the development of soil science in Australia. He played a leading part in the formation of the Australian Society of Soil Science and was its president during 1958–60.

Prof. E. G. Hallsworth

PROF. E. G. HALLSWORTH, professor of agricultural chemistry at the University of Nottingham, has been appointed to succeed Mr. Taylor. Prof. Hallsworth graduated at the University of Leeds in 1936 and became assistant lecturer in agricultural chemistry at that University. He obtained his Ph.D. from Leeds in 1939 and in 1940 went to Australia as lecturer in agricultural chemistry in the University of Sydney. While at Sydney he developed investigations on pedogenesis and fertility of soils and published a series of papers with his students, dealing with particular soils of New South Wales ranging from the alpine humus soils to the gilgais. He built up a substantial knowledge of these soils during this period and was able to make a critical review of shortcomings in the great soil group system of classification when applied to Australian soils. In 1950 he returned to England to become professor of agricultural chemistry in the University of Nottingham, where he took a leading part in building up one of the most flourishing university departments of agriculture in Britain. At Nottingham, Prof. Hallsworth undertook research on the micro-element nutrition of legumes. He and his colleagues demonstrated recently that cobalt has a part to play in nitrogen fixation by nodulated clover. During 1960–61 Prof. Hallsworth revisited Australia as visiting professor of soil science in the University of Western Australia.

Biochemistry at Ghana: Prof. C. B. Coulson

DR. C. B. COULSON, at present head of the Department of Biochemistry and Biophysics of the Arthur D. Little Research Institute, near Edinburgh, has been appointed to the recently instituted chair of biochemistry, nutrition and food science in the University of Ghana at Legon. Dr. Coulson, born in Nottinghamshire and educated at the Riley High School, Hull, and High Pavement School, Nottingham, graduated with honours in chemistry in Prof. R. D. Haworth's Department in the University of Sheffield in 1949. His research on plant proteins and amino-acids in Prague gained him the Doctor of Natural Sciences of the Charles University in 1952. Work in the same field gave him an M.Sc. degree (University of Sheffield), while his work on biochemical aspects of triterpenoidal saponins led to the award of a Ph.D. of the University of Wales. In 1952, as a junior research fellow of the then Institute of Seaweed Research, he examined the proteins and amino-acids of marine algae. In 1955, after military service, he joined the research staff of the Department of Agricultural Chemistry (now Department of Biochemistry and Soil Science) under Prof. W. Charles Evans at the University College of North Wales, Bangor. He continued work there as an Imperial Chemical Industries Research Fellow and as a lecturer in biochemistry and soil organic matter. His research interests extended to farm animal biochemistry and soil biochemistry as well as triterpenoidal saponins. In 1960 Dr. Coulson took up his present post and has been concerned with research on wheat gluten, anti-tubercular glycopeptides of milk whey (with Prof. T. Fujiwara, of the University of Osaka) and the industrial utilization of peat humic acids, both at the Institute and for O.E.C.D,

The Department of Biochemistry, Nutrition and Food Science, in the Faculty of Agriculture of the University of Ghana, is to be associated with the Food Technology and Marketing Investigation Unit (F.A.O.), which is to be set up with a grant of nearly two million dollars spread over five years from the United Nations Special Fund and the Government of Ghana. The main task of the Department will be to develop the output of specialists in the fields covered by the Department and to extend research in biochemistry, nutrition and food science within the context of West Africa.

The National Council for Technological Awards

THE sixth report of the National Council for Technological Awards covers the year ended March 31, 1963, in which 927 Diplomas in Technology were conferred, making a total of 2,019 since September 1958 (Pp. 59. London: The National Council for Technological Awards, 1963). At the end of the year, 7,310 students were following courses leading to the Diploma and the number of first-year students had risen to 2,715. Some 350 industrial organizations provided industrial training for students on whom the Diploma was conferred during the year. Over the past three years the proportion of college-based first-year students has increased from 17 per cent to 31 per cent in engineering courses, and from 53 per cent to 71 per cent in other technologies. Three awards were made of Membership of the College of Technologists. Among appendixes to the report is a list of 117 courses, at 28 colleges, which are now recognized by the Council as leading to the Diploma in Technology.

Effective Use of Outside Research Facilities

A BROCHURE, issued by the Battelle Memorial Institute, outlines the procedures which are being used successfully by some corporations to establish a close working relationship with outside research laboratories such as the Battelle Memorial Institute (*A Practical Plan for the Effective Use of Outside Research Facilities*. Pp. 8. Columbus, Ohio: Battelle Memorial Institute, 1963). The benefits both to the corporation and the research institute resulting from such a relationship are outlined and the

booklet indicates concisely the characteristics and value of contract research for industry. Emphasis is laid on the importance of determining the areas in which outside research facilities promise to be most useful and also the facilities, experience and talent which the research institute can apply in these areas. The way in which these two are related may vary considerably. A further booklet, *Group Research*, also issued by the Battelle Memorial Institute, describes the nature of the group research projects conducted by the Institute during more than thirty years, their conduct and administration and the general advantages of such research, as well as the contribution which a research institute can offer to this type of co-operative research. Stress is laid on the value of the objective approach which such an institute can bring, and it is claimed that outside research organization with experience of group research can define and analyse problems effectively, nominate potential research approaches and formulate programmes which take account of technical, economic and social aspects.

Training Guide, 1963

The *Training Guide, 1963*, published by the Royal Institute of Chemistry, gives details of qualifications and courses, grants and scholarships, and firms sponsoring sandwich courses for students in chemistry (Pp. ii+36. London: Royal Institute of Chemistry, 1963. 2*s*. 6*d*.). Lists of universities and colleges offering full-time degree courses in chemistry as well as of technical colleges offering full-time, sandwich or part-time courses leading to qualifications in chemistry or applied chemistry are also included.

Index to Current Periodicals received in the Library of the Royal Anthropological Institute

The library of the Royal Anthropological Institute receives more than 470 current periodicals connected with every branch of anthropology. Now an index to these periodicals is being prepared under various headings—physical anthropology, archæology, cultural anthropology, ethnography and linguistics (1, Part I; January–March, 1963. Subscription 40*s*. per annum, four parts). Copies can be obtained from the Librarian of the Royal Anthropological Institute, 21 Bedford Square, London. The production of periodical literature has so increased in recent years as to make it almost impossible to keep track of what has appeared. The *Indexes* of the Royal Anthropological Institute will prove of real use to students in the various branches of anthropology and will meet a very pressing need.

Black Soils of India

The *regur* soils (meaning sticky black earth) of India, also called black cotton soils, have excited a good deal of interest among soil scientists, because of their extent, economic importance and characteristic properties. A recent publication summarizes the information available (66 references) and is illustrated by several maps and photographs showing the location, vegetation and profiles of these soils (*Black Soils of India*. By S. P. Raychaudhuri, B. B. Roy, S. P. Gupta and M. L. Dewan. Pp. iv+47. New Delhi: National Institute of Sciences of India, 1963). They cover an area of about 800,000 sq. km. of the Deccan peninsula. The climate is monsoon, the average annual temperatures being 20° C in December and 30°–35° C in May; the average rainfall is between 500 and 1,000 mm, mostly from June until September. The natural vegetation is tropical dry deciduous and tropical thorn forest, the dominant species being *Anogeissus latifolia*, *Terminalia tomentosa* and *Acacia catechu*. The parent material of the soils is mainly Deccan trap, alluding to the step-like aspect of basaltic rocks, of the Cretaceous period; the black soils are found not only on the volcanic plateau but also on transported material derived from the trap and on other formations of basic character. The typical black soil on flat or gently undulating ground may be several metres deep without zoning; it is shallower and not so dark in colour on slopes. It is slightly alkaline, low in organic matter—the percentage of carbon being about 0·5—but contains, on the average, 50 per cent clay and has a high cation exchange capacity saturated mainly with calcium and magnesium. The chief clay mineral is montmorillonite. It shows great expansion and shrinking on wetting and drying; the aggregates are stable, the permeability is good. Cotton, jowar, ground-nut and wheat are the important crops, and also sugar cane if irrigation is possible. These soils are placed in the world group of black soils of the tropics and subtropics ('grumusols').

Announcements

Sir Edward Playfair, chairman of International Computers and Tabulators Ltd., has been appointed president of the British Computer Society in succession to Mr. R. L. Michaelson. Sir Edward is a governor of the Imperial College of Science and Technology, a member of the boards of Westminster Bank Ltd., Westminster Foreign Bank Ltd., and Glaxo Laboratories Ltd., and is also a member of the Royal Fine Arts Commission. He has held various Government positions.

The "Polarmeeting 1963" of the German Society of Polar Research will be held in Karlsruhe during October 6–9. Further information can be obtained from Karl-Heinz Tiedemann, 23 Kiel, Eckernforder Strasse 1.

A one-day symposium on "Food Supply and Nature Conservation" will be held at the Cambridgeshire College of Arts and Technology on November 2. Further information can be obtained from Dr. R. I. B. Cooper, Cambridgeshire College of Arts and Technology, Collier Road, Cambridge.

A meeting of the Photoelectric Spectrometry Group on "Reflectance Spectroscopy" will be held at the Imperial College of Science and Technology, London, on October 25. Further information can be obtained from the Honorary Secretary, the Photoelectric Spectrometry Group, Unicam Instruments Ltd., York Street, Cambridge.

The Technicon Instruments Company's third annual symposium, entitled "Automated Chemical Analysis and Automated Analytical Techniques", will be held during November 5–6 at the Royal College of Surgeons, Lincoln's Inn Fields, London. Further information can be obtained from the Technicon Instruments Co., Ltd., Hanworth Lane, Chertsey, Surrey.

The Institute of Physics and the Physical Society has announced that its 1964 Exhibition of Scientific Instruments and Apparatus will be held in the Halls of the Royal Horticultural Society, Vincent Square, London, S.W.1, during January 6–9, 1964. The 1965 Exhibition will be held in the first week of April in the Manchester College of Science and Technology.

An international Solar Symposium will be held at the University of Sydney, under the auspices of the Australian Commonwealth Scientific and Industrial Research Organization and the University of Sydney, during November 12–15. Its purpose is to review present-day solar research, to discuss plans for the forthcoming solar cycle and to consider the problems of postgraduate training in astrophysics. Further information can be obtained from the Organizing Committee, Solar Symposium, Radiophysics Division, C.S.I.R.O., University Ground, City Road, Chippendale, Sydney, N.S.W., Australia.

Erratum. In the seventh paragraph of the article entitled "Decomposition of Alkali Metal Nitrates by Light Ions of Kilovolt Energy", by Dr. S. R. Logan, which appeared on p. 369 of the July 27 issue of *Nature*, "34 eV/amp" and "10 eV/amp" should read 34 eV/Å and 10 eV/Å respectively.

LIQUID METALS

A CONFERENCE was held at the Fulmer Research Institute, Stoke Poges, to bring together as many as possible of the workers concerned with the experimental study of liquid metals. The meeting was convened with the help and encouragement of Dr. B. R. T. Frost, of the Metallurgy Division of the Atomic Energy Research Establishment, Harwell. Prof. J. D. Bernal (Birkbeck College, University of London) acted as chairman.

Dr. J. E. Enderby (University of Sheffield) directed attention to the considerable variation found in the absolute value of the resistivity (ρ) (at constant volume) and the way in which it varies with temperature. The resistivities of sodium and potassium are strictly proportional to the absolute temperature; in contrast, divalent metals, zinc, cadmium and mercury, have a negative temperature coefficient. Values of the resistivity ratio ($\rho_{liquid}/\rho_{solid}$) vary between 1·45 and 2·2; a trend exists through the alkali metals and the noble metals which is related to the energy-gap across the Brillouin zone boundary. The change in thermoelectric power across the melting-point has the value 0 for sodium, silver and gold, but for copper it is as high as 9·5. Hall coefficient measurements give the free electron value. Thus for metals such as zinc and cadmium the coefficient changes sign and becomes negative on melting.

Mr. B. W. Mott (Harwell) dealt with a modification of the Hildebrand rule which can be used to predict immiscibility in alloy systems. A parameter is calculated, the Mott number, which depends on the atomic volume, heat of evaporation and electronegativity of each of the components, together with the melting-point of the more refractory metal. There will be immiscibility if the Mott number is lower than the smallest Pauling valency. The main difficulty involves the selection of electronegativity values, those given by the Gordy relationship predict immiscibility in 84 per cent of the 886 systems examined.

Simple X-ray diffraction patterns, obtained from pure molten metals such as sodium and copper, can be contrasted with those from metals where structure can be shown to exist, gallium, bismuth and tin. In the case of liquid tin Mr. B. R. Orton (Fulmer Research Institute, now at Brunel College of Advanced Technology) was able to show that, by careful calculation of the radial distribution function, two atomic species are present in the melt. One can be identified with covalent grey tin, the other forms the random matrix of metallic white tin. For other metals which occupy the *B* groups of the periodic table it is possible to show that the participation of the covalent bond increases with valency. Diffraction patterns from liquid alloys also show structure. In the gold–tin alloy system a dominant structure is found which corresponds in composition to the solid-state electron compound, Au_3Sn. Other alloy systems show structure (copper–indium, copper–tin, silver–indium, gold–gallium and gold–indium). At the same valency electron concentration, different alloys of the above group show similar diffraction patterns.

Dr. H. N. V. Temperley (Atomic Weapons Research Establishment, Aldermaston) proposed that previous machine calculations, based on the rigid sphere model of a liquid, should help in the understanding of solidification. There is, however, very poor agreement with approximate theories in the high-density region. In addition to more detailed calculations, a new field is opened in Bernal's statistical geometry. The two-dimensional case is being investigated first. Two models of the lattice type are considered, rigid square and rigid rod. The first has the restriction that the presence of a molecule at one point on a square lattice prevents the occupation of the four neighbouring points. This model shows a transition between a completely disordered arrangement at low density and an alternating structure at high density. Transitions from one to the other are found to be first-order transitions in the Ehrenfest sense. This is in qualitative agreement with the results for rigid spheres. In the second model the molecules are identified with pairs of neighbouring points, no lattice point being occupied more than once. This model is more satisfactory for mathematical study, though only very general results are obtained.

Dr. P. A. Egelstaff (Harwell) described how cold neutrons can be utilized in the study of diffusion in liquids. Most of the existing theories consider the diffusion process to be either gas-like or solid-like. If these models are applied to the viscosity and diffusion data on liquids it is concluded that liquid diffusion is unlike either of the two starting points. A new model is formulated that involves the co-operative action of a number of atoms, termed 'globules'. Predictions are made, using this model, which are confirmed by experimental results, thus giving direct evidence of co-operative units in liquid metals.

Another model was proposed, by Dr. J. Wilson (University of Birmingham), in which the liquid is visualized as consisting of atomic clusters having a definite structure in a matrix of randomly arranged atoms. A state of dynamic equilibrium exists between the clusters and the matrix. The structure of the clusters need not be the same as that of the corresponding solid. The lifetime of the clusters, composed of 50–150 atoms, may be one or two orders greater than the relaxation time of the liquid. This model provides an explanation of the minimum value found in the plot of viscosity against composition for certain eutectic alloys. The minimum represents a transition point between the liquid alloy being dominated by one or other of the pure components. Other published data confirm this model

Describing the inadequate state of the theories of thermodynamics when applied to the explanation of the nature of the bonds in alloy formation, Dr. S. Ll. Leach (Royal School of Mines, University of London) suggested an alternative procedure. The thermodynamic properties must be considered with other physical properties (resistivity and X-ray diffraction measurements) to give a complete picture. He quoted, as an example, the gold–tin alloy system, where all this information is available. From the X-ray measurements it is deduced that there are two types of bond, the short-range covalent and long-range metallic bond. The published resistivity measurements support this view. A maximum, which is independent of temperature, is found at the composition Au_3Sn. It seems possible that as the temperature of the liquid is raised the covalent bonds break down and the electrons released just compensate for the increase in thermal scattering. The success of this approach has led the Royal School of Mines to embark on a programme of experimental measurements of a number of physical properties on one or two selected alloy systems.

The chairman, Prof. J. D. Bernal, concluded the meeting with some general comments on the nature of pure liquids and alloys. The transition from solid to liquid could, he said, be associated with higher vibration-levels, changes in geometrical configuration, greater disorder, lower co-ordination number and lower mean lattice energy. For ideal liquids, machine calculations have emphasized the importance of the short-range forces. If these forces only are taken into account then a discontinuity is found which corresponds to the solid to liquid transition. To understand the liquid to gas transition the

behaviour of argon above the critical point is important. There is no single fluid phase but two distinct states of matter even though there is no visible discontinuity.

Commenting on the models of Dr. Egelstaff and Dr. Wilson, Prof. Bernal suggested that the presence of globules could be detected by the use of small-angle scatter and an estimate of their size obtained from viscosity measurements.

Turning next to alloys, Prof. Bernal put forward the idea that they could be considered as emulsions and cited as evidence the presence of the γ-phase in alloys of copper, silver or gold with zinc. In this phase the atoms are distributed at random over the lattice sites. However, the electron compound found in the gold–tin alloy system could be the cause of the structure observed in the X-ray diffraction patterns.

B. R. Orton

RADIOACTIVE CHEMICALS, LABELLED COMPOUNDS AND RADIATION SOURCES

FOR most purposes, particularly in engineering and radiotherapy, where the radiological properties of radioisotopes are of paramount importance, it is generally sufficient to refer to the tabulated physical data given in Part 1 of *The Radiochemical Manual* for a suitable choice of radioactive material. However, in the case of radioactive tracers for use in the biological sciences, chemical technology and related fields, the purity, chemical stability and the characteristic behaviour of the isotopes as radioactive chemicals are often the decisive factors, and for these Part 2 of the *Manual**, which has recently been published and which deals with the chemical aspects of radioactive materials, must be consulted.

The range of radioactive chemicals is very extensive, and Part 2 has therefore been limited to a number of articles on selected topics likely to be useful to users of radioactive tracers. The examples referred to are drawn largely from the practical experience of the scientific staff at the Radiochemical Centre at Amersham. The contents of Part 2 consist of three sections, dealing with the sources of radioactive chemicals, some characteristic features of the chemicals, and notes on labelled compounds of the elements carbon, hydrogen, sulphur, phosphorus, iodine and chlorine.

The first section contains four chapters in which the distinctive features which affect the manufacture of radioactive reagents are outlined. Radiological protection and remote handling frequently limit the types of chemical manipulation which can be carried out. The half-life of the isotope is the important factor in the organization of supply and distribution, and the chemical scale of manufacture which rarely exceeds one gram is a unique feature. The distinction between primary isotopes and labelled compounds is clearly drawn and the various methods of preparation are explained. Isotopic labelling and its wider implications, the synthesis of labelled compounds by chemical and biological methods and some of the unresolved problems such as the isotopic labelling of natural products by biosynthesis, are also discussed.

Chapters 5–7, which form the second section, are concerned with the concepts of purity and its determination, instability, and the behaviour of minute quantities and dilute solutions of radioactive chemicals. The final six chapters in the third section refer individually to compounds of each of the six elements which are most commonly concerned in tracer experiments. Carbon-14, which emits only β-particles, is produced by means of a reactor in adequate quantities and at high specific activity, and, since it has a half-life of 5,760 years, it is a most favourable tracer isotope. It has been used for the intensive study of biochemical processes and has now virtually displaced the stable isotope carbon-13 and the short-lived (20 min) radioactive isotope carbon-11. Tritium-labelled compounds are now second in importance to carbon-14 compounds in biochemical tracer work, but tritium is not used as a hydrogen tracer but as an accessory tracer for a carbon atom or a molecule or a molecular fragment. Tritium is available in quantity and high specific activity (up to 29 c./m.amp) and its half-life of 12·3 years is conveniently long.

Sulphur-35 is the only practicable label for sulphur atoms. Its half-life is 87·2 days, and although it can be produced by direct neutron irradiation, it is better extracted as sulphate at high specific activity from neutron-irradiated potassium chloride. The main groups of chemical in which sulphur-35-labelled compounds are of interest are listed in the *Manual*. Phosphorus-32 is relatively easy to prepare. Its half-life is 14·2 days and its main applications are in inorganic fertilizers, nerve gases, systemic insecticides, and plasticizers. Iodine-131 is in abundant supply from irradiated tellurium or from fission products, but its short half-life prevents stockholding of iodine-labelled compounds. Other isotopes of iodine, iodine-132, iodine-125 and iodine-124, are also available. Chlorine-36 has a long half-life of $3{\cdot}03 \times 10^5$ years and is a pure β-emitter of medium energy. Its production is expensive and its specific activity is low, less than 4 mc./m.amp. Chlorine, however, is an important industrial chemical and therefore chlorine-36 has valuable applications. It is also in many ways an ideal radio-isotope for use in the teaching of practical chemistry.

Part 2 of the *Manual* concludes with thirteen tables of synthetic routes to labelled compounds, a glossary of terms used, and numerous references to published work relating to the topics discussed and the tables. For details of the products, radioactive chemicals and radioactive sources, and services provided by the Radiochemical Centre the reader is referred to the catalogue. *Radioactive Products*†, available on request from the Centre.

S. Weintroub

* *The Radiochemical Manual.* Part 2: *Radioactive Chemicals.* Pp. v+78. (Amersham: The Radiochemical Centre, 1963.) 25*s*.

† *Radioactive Products: Radioisotopes, Labelled Compounds, and Radiation Sources.* Pp. 119. (Amersham: The Radiochemical Centre, 1962.)

RESEARCH INTO TECHNICAL EDUCATION

THE report of a working party, with Prof. A. V. Judges as chairman, set up at the second session of a conference on June 6, 1961, presided over by Dr. Dunsheath, and attended by representatives of the University of London, the Regional Advisory Council for Technological Education (London and Home Counties), local authorities, technical colleges and research institutions, has now been published under the title *Research into Technical Education**. The working party was charged, *inter alia*, with considering priorities in research in this field and, apart from the specific proposals for research which are detailed, the report gives in its introduction an admirable survey of the present position and of the

* *Research into Technical Education: Report of a Working Party.* Pp. 34. (London: The Regional Advisory Council for Technological Education. London and Home Counties, 1963.) 5*s*.

several authorities and institutions that are already active. Appended to the report, moreover, are notes on relevant activities of the National Foundation for Educational Research and the British Association of Commercial and Industrial Education, details of projects already in progress in the London colleges, of the two educational research projects in the Department of Mathematics at the Hatfield College of Technology and of the Nuffield research projects at the Birmingham College of Advanced Technology and at Brunel College.

Although the working party does not lose sight of its main concern—the technical colleges—this general survey is of considerably wider interest and the comments made are probably valid over a fairly wide field of secondary and of higher education. More research is obviously being carried out into technical education than is generally realized. However, the research position is scrappy and disjointed, and the working party believes that the present investment rate is ahead of intelligent application. In particular, besides greater availability of statistics, in the institutional field the clearing services need overhauling to facilitate awareness of what is happening in cognate fields. The working party found great difficulty in discovering the annual investment in the development of research into technical education, but if the planning of research into specialized training within industry is to be absorbed into the prime costs of industry and marketing, it suggests that assistance is needed in four ways.

First, established posts are necessary in the centres of higher education in the faculties of arts, education or applied science. Secondly, research units need to be established in universities, colleges of advanced technology, and other institutions, with an independent budget, and headed by specialists who may or may not be members of the academic staff. Thirdly, there is room for specific enquiries in any establishment, industrial or academic, with the use of a research studentship or *ad hoc* grant. Fourthly, development might be promoted within the National Foundation for Educational Research of the existing division concerned with research and information on technical education.

All this needs manpower and suitable recruitment, and the spread of incentives are at the heart of the whole problem of launching large-scale projects into new territory. The working party particularly stresses the need for more specialized workers in educational psychology, statistical methods, and the design of educational experiments, economic analysis, and sociology. It lists the topics for research in five broad groups, but makes no attempt to indicate a desirable priority. The groups cover entry to technical education and training, methods of teaching and training; relation between technical education and industrial and commercial training; problems of a broad scope, such as forecasting needs for technical personnel, aspects of public examinations, economics of the technical education; and comparative studies of technical education.

Even the titles of the broad groups of projects listed in this report indicate the implication of such research in other fields of education. The working party considers the question whether the developing situation calls for national supervision by some body after the style of the Medical Research Council. It is firmly of the opinion that, whether or not such surveillance is desirable, existing organizations must accept the need for close co-operation and the pooling of information and must agree on some form of clearing-house facilities. The Ministry of Education and the Scottish Education Department, the Ministry of Labour, the National Foundation for Educational Research, the Scottish Council for Research in Education, the National Advisory Council on Education for Industry and Commerce, the British Association for Commercial and Industrial Education are all concerned in one way or another, and the universities, local education authorities and organizations of teachers also have interests to develop. All these in coming together should attempt some more formal and structural form of co-ordination.

The working party considers that the initiative in securing closer co-operation should be taken by the Government, and points out that the case for creating a national council or grants committee, with powers to handle funds, to consider the direction of policy and to report to the Privy Council through a Minister, would be stronger if considerable sums from the national exchequer became available for disbursal among institutions engaged with research projects. Such a step would not, however, remove the need for much closer co-operation generally; it reiterates that the lead in developing new work must be taken by the National Foundation, the universities, the technical teacher training colleges, industrial firms and associations, local education authorities, and associations of principals and teachers in technical colleges, regional advisory councils, and the technical colleges and teachers themselves. It sees hopeful signs in the creation of the intelligence and research organization of the Ministry of Education, and the increased support given to the National Foundation for Educational Research. Furthermore, it suggests that the universities through their Departments of Education may be able, in the next quinquennium, to direct more of their resources into problems of technical education and to encourage postgraduate research in this field. This is important in view of the great need for research workers, and the working party suggests that conditions for the award of academic qualifications as a result of educational research should be broadened to give postgraduate workers in this field an incentive comparable with that of postgraduate workers in scientific and technical research.

MODIFICATION OF THE WEATHER

THE great economic potential of successful weather control led the United States Congress in 1958 to ask the National Science Foundation to launch a full-scale investigation of this exciting field of modern meteorology. Within the Foundation, a Weather Modification Research Programme has been developed as an integral part of the more inclusive Programme on Atmospheric Sciences, which required funds amounting to 6,615,000 dollars during the fiscal year ending June, 1962. Of this sum, 1,340,000 dollars were set aside to support weather modification research under a system of grants and contracts from the Foundation to research groups at university, Government and other laboratories.

The one major technique in use for weather modification is only fifteen years old. This is 'cloud seeding', that is, the introduction, into a cloud, of material calculated to have an effect on the constituent cloud particles. Certain clouds and types of fog can be modified; and, under certain conditions, rainfall can be somewhat augmented and hail discouraged. The possibilities merit further careful investigation.

A summary of progress made in the United States National Weather Modification Programme up to 1962 appears in a report[1] issued by L. J. Haworth, director of the National Science Foundation. Work has been concentrated on basic studies into the nature of cloud phenomena, on the study of known cloud-seeding methods, and on the investigation of other methods of local modification of clouds. The last-mentioned investigation includes methods of changing ascending or descending air currents, or of

changing the electrical field of the cloud by, for example, hanging miles of electrified wire across canyons in North Mexico. The Foundation also sponsors investigations in atmospheric dynamics and the general circulation, and these may make significant contributions to the investigation of weather modification over large regions; and on the large scale may eventually determine what kinds of man-made influences can affect large weather patterns, and climate. Large-scale weather modification is, by nature, international in scope, and co-operation and co-ordination is essential between some twenty nations at present making research efforts in weather modification.

The report also contains the results of a Rand Corporation study group[2], briefed by the Foundation to analyse past and present attempts at modifying weather and to consider suggestions for achieving weather control; the Rand group emphasized the importance of co-ordination of theory with mathematical models of the atmosphere for the electronic computer, with laboratory models and other laboratory studies, as well as with field observations. Modification may be possible through an understanding of the physical processes involved within a cloud; or of the air currents associated with it; or of the major synoptic weather systems and the general circulation. Various associated investigations should be encouraged, for example, palæoclimatology may be helpful in revealing causes of the great climatic changes of the past. The various effects of man-made atmospheric pollution should be studied, for example, carbon dioxide, and the pollution of the upper atmosphere by space vehicles and rockets.

Theoretical studies are aided by large electronic computers for solving the equations of motion of simplified models of the atmosphere. The use of such models permits the research worker to test the effect of suggested methods of weather modification, for example, the effect of damming the Bering Strait; or of removing ice from the Arctic Sea; of controlling evaporation over the ocean by covering the surface with a chemical film; or of changing the radiation balance over snow surface by a coating of carbon black; or of finding the most favourable circumstances in which a small modification might act as a 'trigger mechanism' to set off a larger modification. Calculations may also be made on small-scale diurnal phenomena, for example, the behaviour of fog and stratus cloud at a coast line. Even smaller scale atmospheric motions within clouds may be studied, for example, to show collision frequencies of cloud droplets and possible changes due to modified electric fields.

Besides including further computer studies, the future plans of the Foundation again involve such projects as cloud-seeding research and the effects of 'seeding' on hurricanes, research on cloud electrification and research on the control of the hydrological cycle through control of evaporation.

Space technology and radiation studies may even lead to control of the radiation balance by altering the composition of the upper levels of the atmosphere, or by introducing artificial cloud layers in suitable places. The report concludes that the time for rapid progress appears to be at hand with a variety of new theoretical and practical tools, provided that the project continues to attract well-trained and talented man-power. W. Garriock

[1] National Science Foundation, Washington. *Weather Modification:* Fourth Annual Report for Fiscal Year ended June 30, 1962. Pp. v+84+7 plates. (Washington, D.C.: Government Printing Office, 1963.) 50 cents.
[2] Greenfield, S. M., *et al.*, *A Rationale for Weather Control Research, Trans. Amer. Geophys. Union*, **43**, No. 4, 469 (December 1962).

A WORKING MODEL OF THE MECHANICAL ESCAPEMENT IN SU SUNG'S ASTRONOMICAL CLOCK TOWER

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In 1960, Drs. Needham, Wang Ling and Price published a detailed description of the mechanism in the Chinese astronomical clock tower built for the Emperor by Su Sung in A.D. 1092 (ref. 1), in which the mechanical power for driving the mechanism was provided by a large wheel eleven feet in diameter mounted with its axis horizontal, the wheel carrying thirty-six scoops equally spaced around its circumference. The wheel moved intermittently as the result of water flowing into the scoops in turn, the weight of water in a full scoop being sufficient to tilt a weighted lever obstructing the movement of the wheel, and the water poured out of each scoop after it had passed the weighted lever. The wheel was brought to rest after the passage of each scoop by stops worked by latches while the wheel was in motion. The authors claimed that the apparatus was of great importance to the history of mechanical clockwork since it provided a 'missing link' between the water clocks and clepsydra of antiquity and the first mechanical escapements developed in the West during the Middle Ages. They suggested: "that the whole design is reminiscent of the anchor escapement of the late seventeenth century, since the driving wheel is a scape wheel and the pallets are inserted alternately at two points on its circumference separated by 90° or less . . ." In a footnote on the same page they observe, "the more subtle aspects of Su Sung's mechanism will not reveal themselves until a working model has been constructed. We may hope that such a task will be undertaken . . ."

The present authors took up this challenge and have now produced a working model (their Mark III model: the first two models, though they operated successfully, did not meet all the requirements specified in *Heavenly Clockwork*) which appears to meet the requirements specified in the text and, furthermore, incorporates a component essential to the operation of the mechanism and described by the translators simply as "some kind of star-shaped gadget", the function of which they were unable to guess. We have named it "an interrupter cam".

Our own reading of the text of *Heavenly Clockwork* together with experiments we have made with two earlier models lead us to believe that Su Sung's escapement was composed of eight distinct elements each one of which was essential for its operation.

They are shown diagrammatically in Fig. 1 and were: (*A*) The 'scape' wheel carrying thirty-six scoops equally spaced around the periphery. As the wheel turned it was arrested as each scoop reached the horizontal diameter where it was filled with water until the wheel moved on for the next scoop to be filled after tilting the weighted lever (*B*). Each scoop carried a projecting pin at one side for operating the escape latch (*C*). (*B*) A weighted lever hinged near the centre provided with a counter poise at one end and a forked end for stopping the scoops at the other. A pin between the fork of this lever operated the interrupter cam (*G*) just before the wheel was stopped by the buffer and caused to recoil. (*C*) An escape latch connected by the chain (*D*) to the overhead stopping lever (*E*) which carried a buffer stop at one end. This buffer stop normally rested between two adjacent pins at the

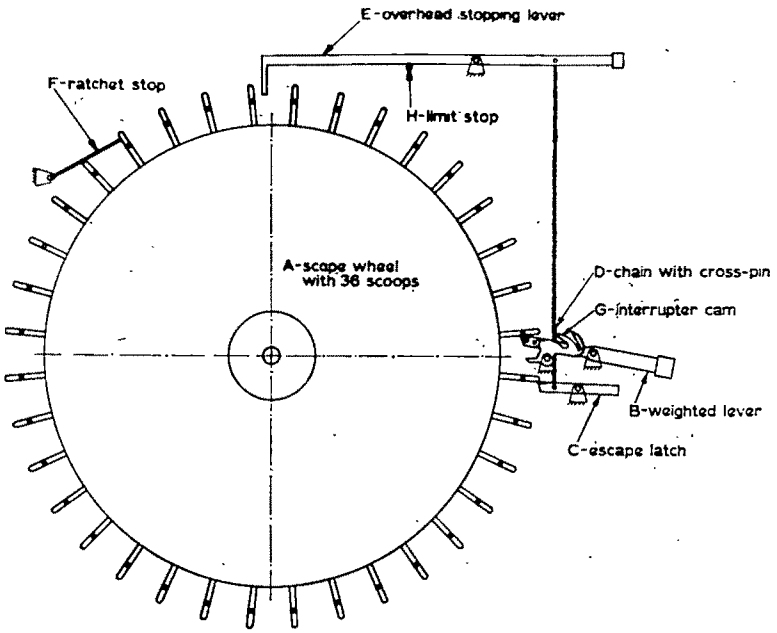


Fig. 1

top of the wheel. When the escape latch operated, the buffer stop was raised and lifted over the next pin to rest again in the space between the next two pins. (*D*) A chain connecting the escape latch (*C*) to the overhead stopping lever (*E*) so that during the first part of the motion of the wheel the buffer stop was lifted whereas when the next pin approached it did not operate, thus allowing the upper stop (*E*) to function and bring the wheel to rest. The model (and probably the original) was provided with a cross-pin fixed to the chain and, as the weighted lever tilted, its movement inserted the interrupter cam below the pin so that the lower part of the chain was lifted, causing the escape latch to miss the next pin on the wheel just before the recoil, though the same pin was able to operate the escape latch again after the wheel had been stopped, recoiled, and come to rest (Fig. 2). (*E*) The overhead stopping lever carrying a buffer at one end and weighted so that this buffer remained in position with the lever resting on the limit stop (*H*) except when the chain was pulled down by the escape latch to allow the next pin to pass the buffer. (*F*) A ratchet stop near the top of the wheel to prevent the wheel from recoiling too far when its forward motion was arrested by the buffer. The parts of the mechanism were disposed so that one of the pins rested on this ratchet as each scoop was being filled. (*G*) An interrupter cam to lift the lower end of the chain and thus the escape latch during the later part of the wheel's motion, so that during this time the escape latch could not function and therefore the buffer stop remained in position and was able to operate as a stop. The interrupter cam was probably turned by a pin projecting from the weighted lever as in the model and as it turned the lower part of the chain was lifted by the cross-pin on the chain on to the cam, thus raising the escape latch so that it missed making contact with its pin during the later part of the motion of the wheel. (*H*) A limit stop on which the overhead stopping lever could rest at all times except during the short periods when the chain was pulled down by the escape latch causing the buffer to rise over the pins one at a time.

We have not previously come across this combination of motions in any mechanism nor have we seen any reference to it in historical or horological publications. It is surprisingly sophisticated for its period, but it most probably represents the system used on the original celestial globe of Su Sung for the following reasons:

(1) All the eight components of the escapement are depicted on the original Chinese woodcuts in Su Sung's book.

(2) All the eight components of the escapement are necessary for its functioning. If any are removed it will not work.

(3) All but the last two components—the cam and the stop are described in the translation of the original Chinese script of Su Sung.

(4) The similarity of the "star-shaped gadget" (Fig. 3*a*) shown on the Chinese illustration, and the interrupter cam (Fig. 3*b*) the shape of which was determined by the geometrical requirements of the escapement supports the belief that the "star-shaped gadget" was in fact an interrupter cam made to perform this function.

(5) The position where the cam is shown on the illustration is the place where it must be located since it must lift the chain and be operated by the weighted lever.

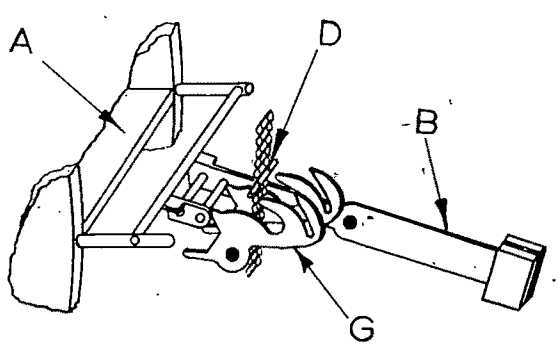


Fig. 2

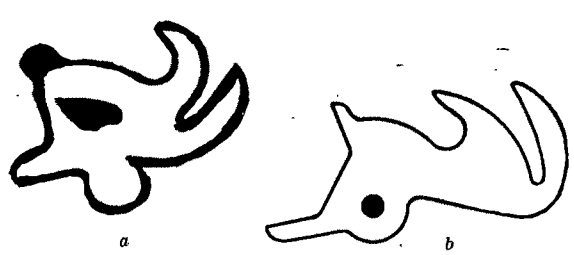


Fig. 3

Until we had discovered the function of the interrupter cam and added it to the assembly the most baffling feature of the mechanism (as described in *Heavenly Clockwork*) was that the upper stop carrying the buffer could never stop the wheel, since it was lifted clear of the wheel every time the wheel was due to be stopped, that is, the buffer could not 'buff'.

[1] *Heavenly Clockwork. The Great Astronomical Clocks of Medieval China.* (Camb. Univ. Press.) A preliminary notice appeared in *Nature*, **177** 600 (1956).

SLOW EXPANSION OF A PLASMA COLUMN WITHOUT THE APPLICATION OF A MAGNETIC FIELD

By Dr. P. AVIVI, N. BEN-YOSEF, F. DEUTSCH and Dr. A. S. KAUFMAN*
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THE device *Maya* was built with the view of creating and investigating a plasma originating in the positive column of an arc discharge, during and subsequent to the rapid ejection of the discharge-tube walls. A suitable magnetic field would be applied to confine the exposed plasma and the neutral gas streaming from the positive column into the surrounding vacuum region would be absorbed by a titanium getter. *Maya* may be regarded as the complement of confinement devices using injection of plasma into a magnetic field.

During the testing of *Maya* without an applied magnetic field, it was observed that electrostatic probes, situated outside the initial boundary of the positive column in hydrogen, responded to the arrival of electrons only after a delay of about one to two milliseconds from the commencement of ejection. In addition, if two probes were set at the same radial distance from the discharge tube, then the one farther from the exposed column responded to the arrival of electrons a perceptible fraction of a millisecond later than the probe situated more closely. Similar effects were observed for positive-ion collection.

This article is a preliminary account of these observations. If it is assumed that the probes do not perturb the plasma motion, the results indicate that the expansion rate of the plasma column into vacuum is very slow—about 10^4 cm sec^{-1}.

The device *Maya* comprised three main sections—the plasma chamber, the ballistic section and the buffer (Fig. 1).

The plasma chamber was a stainless steel cylinder of length 40 cm and inner diameter 16 cm. Concentrically disposed was a cylindrical discharge tube formed from either duralumin or stainless steel, with an insulating lining of inner diameter ranging from 2·7 cm to 3·1 cm according to circumstances. Before ejection, the discharge tube was seated in the cup, which was lined with 'Teflon'. In this position, the discharge tube and plasma chamber were both electrically insulated and hermetically sealed from each other. The two vessels were evacuated separately and the pressure in the space between them was between 2×10^{-4} torr and 1×10^{-3} torr. No attempt was made to attain a still lower pressure.

Arc discharges were produced in a continuous stream of hydrogen at a pressure of 3×10^{-2} torr and the gas flow was maintained during ejection. No undue care was taken as to the purity of the hydrogen. A hot cathode was used and the anode consisted of a disk of molybdenum attached to an exposed stem of stainless steel. The cathode and plasma chamber were fixed at earth potential and the potential difference between the electrodes during the running of the arc was in the range from 80 V to 150 V for most of the runs. The discharge current prior to ejection ranged from 0·25 to 0·77 amp. It was maintained during ejection and, in general, increased at a steady rate during ejection to a value which was about 30 per cent greater than before ejection commenced.

* In former publications, A. S. Kaufman was known as S. Kaufman.

After the arc was struck, axial ejection of the discharge tube followed. The ejection was arranged by firing a projectile in the ballistic section so that the momentum would be transferred to the discharge tube via the neoprene membrane. The mechanical buffer arrested the motion of the discharge tube after it had left the plasma chamber. The speed of ejection was measured by timing the passage of the discharge tube between two phototubes prior to the retarding action of the buffer. The average speed of ejection was in the range from $1{\cdot}7 \times 10^3$ cm sec^{-1} to $2{\cdot}5 \times 10^3$ cm sec^{-1}. The signal from the first phototube also acted as a trigger for the oscillographs.

Electrostatic plane probes were situated at different levels and radii in the space between the vessels so as to determine the location of the plasma during ejection. The probes, formed from tungsten rods of diameter 1 mm, were sheathed in glass to the extremity. Each probe was connected directly to a separate dry-cell battery and thence to an oscillograph, with a resistor of 10 kΩ in parallel to earth. One of the probe mounts on the first level could be used for a second timing arrangement in which a photomultiplier detected the light from the arc

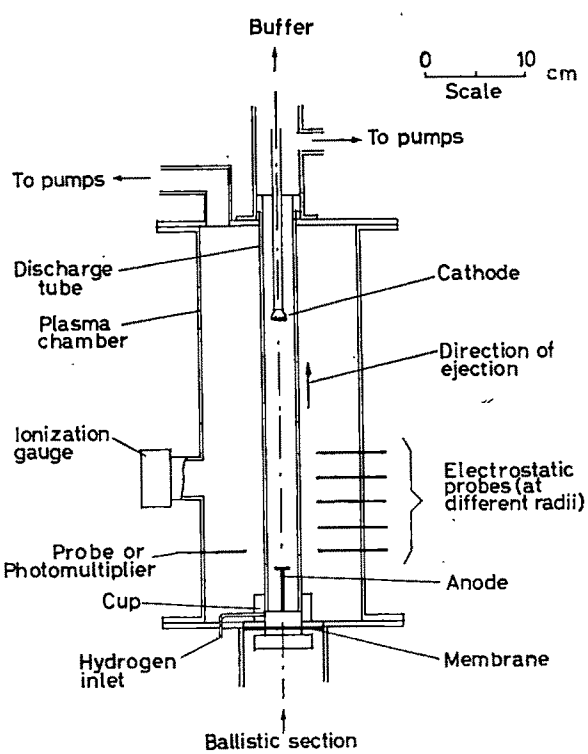


Fig. 1. Longitudinal section (schematic) of *Maya*

when the end of the discharge tube reached this level. Rapid changes of gas pressure in the plasma chamber were recorded by an ionization gauge situated at the height of the fourth level of probes and at a distance of 7 cm outside the wall of the plasma chamber. The changes in ion current were fed into a fast-rise amplifier and thence into an oscillograph.

In a short account, the observations can best be presented by selecting a typical run. Fig. 2 depicts the probe signals where probes 1, 3 and 4 were set at a positive potential of 145 V and probe 6 at a negative potential of 40 V, all with respect to the cathode. The location of the probes is shown in Table 1. The first three probe traces show that a time in excess of 1 msec elapsed before any overall collection of electrons took place. A period of several milliseconds then ensued before these signals reached some steady value (not shown in Fig. 2). In addition, there was a distinct delay in response of 0·3 msec between probes 3 and 4 for the run in question. The result of many runs was that when the probes were set either at the same level or at the same radial distance, the more distant probes responded to the arrival of electron current later than those situated closer to the exposed column, even when the probe positions and potentials were altered. In many cases, but not apparently in all, a pip made its appearance just prior to, or with, the main signal (see the trace of probe 4). The time of current collection was reckoned from the appearance of this pip or from the clear bend in the trace when the pip was absent.

Table 1. Computed Speeds of Plasma Expansion (Run 34)

Probe	z (cm)	r (cm)	Method A $10^{-4}\ V_p$ (cm sec^{-1})	Method B $10^{-4}\ V_p$ (cm sec^{-1})
4	7·4	1·4	0·9 (probes 1,4)	0·6
1	9·9	3·8		0·8
3	12·4	1·5	1·3 (probes 3,4)	0·9
6	7·4	5·5	not calculated	

z, Vertical distance from probe level to the bottom of the plasma chamber.
r, Radial distance from collecting surface of probe to the external wall of the discharge tube.
V_p, Speed of plasma expansion.

A number of runs were also performed with positive-ion collection. The initial delay in response and the delay between probes were evident as for the collection of electrons. It is significant that the positive ions reached one probe earlier than the electrons reached a second probe placed on the same level and at the same radial distance—in one particular run, by as much as 0·3 msec. The collection of positive ions by probe 6 is shown in Fig. 2, but the amplifier sensitivity in this case was too low to indicate exactly the start of current collection.

The ionization gauge was used to measure the response time for the neutral gas. In a typical case, it responded to the fastest neutral particles 1·4 msec prior to the start of the electron collection on a probe at the first level.

When the photomultiplier was connected, it was possible to determine the time delay in the trigger signal with respect to the commencement of ejection. For the run in question, this time was 0·4 msec with a maximum possible error of $\binom{+0\cdot2}{-0\cdot0}$ msec. Thus, in Fig. 2, the recorded initial delays in probe response were shorter than the real delays by this time. It was also possible to locate the position of the discharge tube at the time of the response of the probes. For the same run, the length of exposed column between the cup and the discharge tube was 1·2 cm when the nearest probe (No. 4) began to respond.

All the results quoted here were obtained in the absence of an externally applied magnetic field. On the application of a steady longitudinal field of several hundred œrsteds, the delay between probes was increased—in one case, to 0·8 msec.

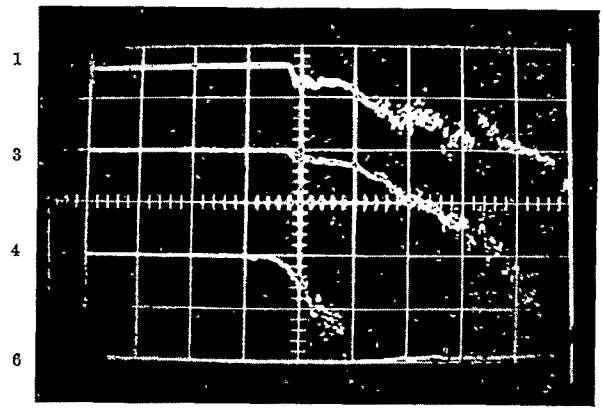


Fig. 2. Probe signals as a function of time (run 34); abscissa, each main division is 0·5 msec; ordinate, for probes 1, 3, 4, each main division is 2 V, for probe 6, each main division is 0·5 V; positive current is in the positive direction; discharge current (before ejection), 0·50 amp; arc voltage, 150; average velocity of ejection, $2{\cdot}0 \times 10^3$ cm sec^{-1}

The principal features of these observations will now be interpreted. The electrons and ions detected by the probes must have originated in the arc discharge. The possibility of a glow discharge between the anode and the plasma chamber can be excluded because the potential difference between them was less than the normal cathode fall required to maintain a glow discharge. It is thus evident that during ejection the plasma column expanded from the boundary of the discharge tube into the space between the two vessels.

The speed of plasma expansion will now be computed by two different methods. Basic to both are the following assumptions—the probes do not influence the rate of plasma expansion, there is no accelerated motion of the plasma, expansion takes place along a straight line, and that it displays circular symmetry.

In method A we also assume that the speed of expansion is independent of the direction of expansion. Then from the delay in response between a pair of probes it is possible to compute speeds of expansion as given in Table 1. It is not essential to know exactly when the plasma started to expand. The speeds are not substantially altered whether the plasma began to expand as the tube left the cup or even when the tube reached a level 2 cm from the cup. The fact that there is no single-valued speed of expansion from different pairs of probes, and this is clearly confirmed by many other runs, may be due to the incorrectness of the assumptions. For example, the speed of expansion is indeed a function of direction, or the plasma possesses accelerated motion in the longitudinal direction. The results examined are compatible with the latter possibility and also indicate that the speed of expansion in the radial direction is less than that in the longitudinal direction.

Method B is based on the time that elapsed before each individual probe responded. At this stage, it is not possible to determine exactly the moment when the plasma was capable of streaming from the discharge tube into the plasma chamber. We arbitrarily assume that the plasma began to move when the tube cleared the cup. In addition, it is supposed that there was no delay between the arrival of electrons at a probe and the recording of the probe signal. This method yields speeds of a similar order of magnitude to method A but somewhat lower (Table 1). The acceleration of the plasma in the longitudinal direction is also demonstrated.

When the probe traces were recorded with high amplification, a small fluctuating signal was discernible during the delay until electron collection at the knee of the trace occurred. If we assume that this signal was genuinely due to the arrival of electrons of the most probable velocity, then their concentration was about 10^7 cm^{-3} as compared with an estimated concentration of at least 10^9 cm^{-3}

at the wall of the positive column before ejection. A concentration of 10^7 cm^{-3} must be regarded as an upper limit.

In conclusion, from an examination of some twenty runs, the initial delay in electron collection and the delay between probes both indicate the same result, namely, a speed of plasma expansion of about 10^4 cm sec^{-1}. Further support is derived from the observation that a time of several milliseconds elapsed from the start of electron collection until a steady signal was attained. The computed results are consistent with a model in which the plasma, after leaving the discharge tube, is accelerated in the longitudinal direction. However, in view of the preliminary nature of the results, some other model of expansion cannot be excluded.

Little[1] investigated the rate of expansion of a highly ionized plasma in mercury vapour into a tenuous and feebly ionized region. A measured velocity of about 10^5 cm sec^{-1} agreed with the calculated wave velocity for the propagation of electro-acoustic waves in the plasma. In the case of hydrogen, the calculated wave velocity would be about 10^6 cm sec^{-1}, which is much greater than the measured speeds of expansion in the present work.

The evidence for the slow expansion of the plasma column rests solely on the probe signals. Such slow motion in the absence of an external magnetic field has been unknown hitherto, so it is natural to suspect the probes. If a probe were to introduce some delay in collection, then a case could be made against method *B* but not against method *A*, since identical probes were used. Furthermore, in one run during which the probes responded at different times as usual, there was afterwards a break in the discharge current which was not maintained. The collection of current by all the probes then ceased simultaneously.

At this stage it would be premature to offer an explanation of the slow expansion: However, it is certain that it is not an aerodynamic effect related to the ejection; the neutral particles reached the ionization gauge much more quickly than the charged particles reached the probes. It is possible that the effect is associated with the stability of the plasma sheath and that some form of electrostatic confinement retards the motion of the plasma.

Measurements with electrostatic probes show that the plasma originating in the positive column of an arc discharge expands into vacuum during the removal of the discharge-tube walls at a speed of about 10^4 cm sec^{-1}. The results may be of importance for work related to the confinement of plasmas from material walls. The account is only preliminary and in future work it will be important to confirm the slow rate of expansion by an independent method of measurement (for example, microwaves) and to determine its dependence on such parameters as the discharge current, the gas and its pressure, and the velocity of ejection.

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CROSS-STRATIFICATION AND PALÆOGEOGRAPHY OF THE LOWER GREENSAND OF SOUTH-EAST ENGLAND AND BAS-BOULONNAIS, FRANCE

By J. NARAYAN

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THE Lower Greensand, in which the Hythe and Folkestone Beds are the principal members, is a shallow marine deposit dating from the Lower Aptian to Lower Albian. The principal British outcrops lie in the Isle of Wight, southern Dorset, Wiltshire, the Weald, East Anglia. The Weald outcrop continues into the Bas-Boulonnais.

Kirkaldy[1] described the deposit as 'shallow marine' with the vertical and lateral variations in lithology characteristic of the 'belt of variables'. According to Casey[2] the Lower Greensand was deposited in two main basins, one to the north and one to the south of the London ridge; but present work on the cross-stratification suggests that this may not have been the case in Folkestone Beds times.

Cross-stratification is present throughout the Lower Greensand, but in places has been partly destroyed by organisms and growth of ferruginous concretions. Two main types of cross-stratification are encountered in the Hythe and the Folkestone Beds: (1) planar, (2) trough, as defined by McKee and Weir[3]. In thickness the units vary considerably from a few centimetres to about three metres. This variation may be due to unevenness of the floor. In the Folkestone Beds the trough cross-stratification is associated chiefly with the finer sands, and the planar type with the coarser sands. Deformed cross-stratification[4] is present locally. Other sedimenta-

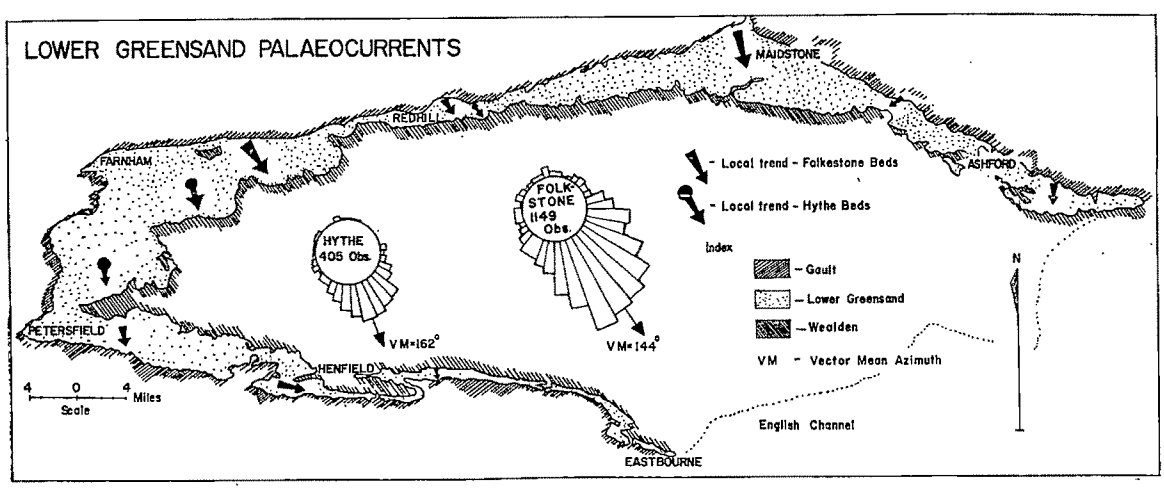


Fig. 1. General current pattern in the Hythe and the Folkestone Beds. Each arrow shows the general trend of the current in that locality. The size of the arrow, however, is irrelevant. The rose diagrams show the general distribution for the whole area with the vector means

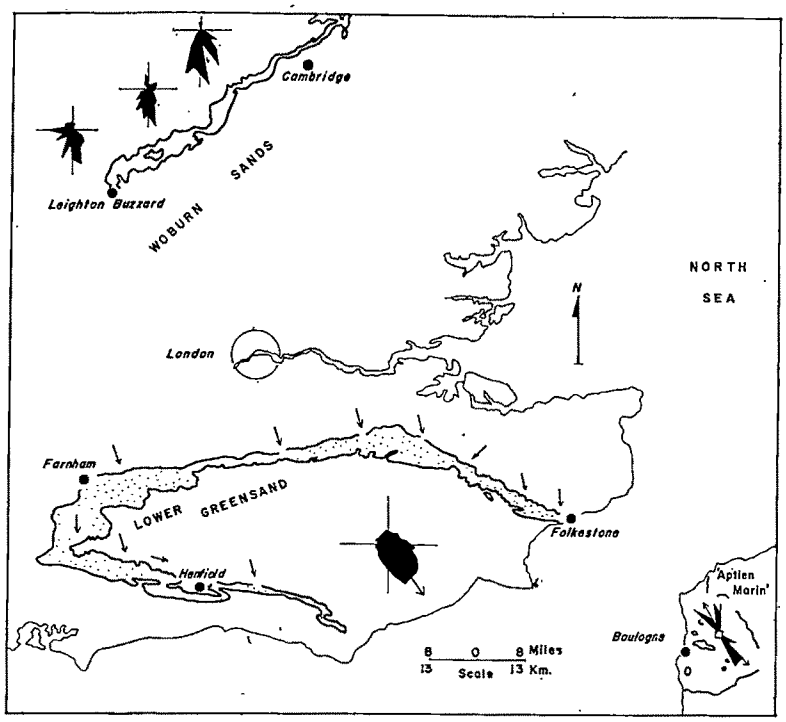


Fig. 2. Comparative current pattern of the Folkestone Beds, the Woburn Sands (after ref. 10) and 'Aptien marin' of Bas-Boulonnais, France. Arrows indicate vector means

tion structures include channelling, burrowing and small-scale ripple marks. There are two main types of channels: (1) those which were eroded and filled simultaneously, and (2) those which were filled later. Burrowing as described by Middlemiss[5] is very common. The calcareous sandstones of the Hythe Beds show parallel laminations defined by the variations in glauconite content. These laminations are almost horizontal, indicating a slacker water environment. A slower rate of sedimentation is supported by the churned nature of the sediment as pointed out by Middlemiss[5]. The equivalent horizon in the Isle of Wight is Group VI (ref. 6) which is also churned up. Hence quieter sedimentation appears to have set in over a wide region during this particular period.

Cross-stratification dip directions in the Hythe and the Folkestone Beds were measured in a statistically randomized manner. Four hundred and five observations were made in the Hythe Beds. The vector mean azimuth[7] is 162°. Fig. 1 gives the general distribution of the current pattern. The vector magnitude (L) is 73·4 per cent, which is well below 10^{-20} probability level (Rayleigh test). The maximum dip attained by the foresets is 38°. In general, however, their inclination is less than 20°, and almost horizontal near Petworth.

Eleven hundred and forty-nine cross-stratification dip directions were measured in the Folkestone Beds. The vector mean azimuth was calculated for the whole area (144°) and for each locality (Fig. 1). The vector magnitude is 65·1 per cent, which is well below 10^{-20} probability-level (Rayleigh test). The maximum cross-bed thickness observed was about 3 m and the maximum foreset dip 38°.

In the Bas-Boulonnais 94 observations were made in the 'Aptien marin'. Two main current directions prevail (Fig. 2). The vector mean azimuths are respectively 133° and 330°.

Analysis of the cross-stratification data (Figs. 1 and 2) shows that the palæocurrents were essentially constant from the north-west throughout the period. The cross-bedded units are associated with the silt bands tending to thicken towards the bases of the units. When traced upstream, they appear to split into several bands and then disappear within the units. This indicates a rhythmically pulsating current, perhaps tide-generated as indicated by Allen[8]. Study of Recent sand bodies in the North Sea by Stride[9] has revealed a definite pattern in their distribution resulting from currents always running parallel to the coastline. Applying this to the Lower Greensand of the Weald and Bas-Boulonnais suggests a north-west–south-east coastline. Schwarzacher[10], studying the Woburn Sands in East Anglia, found almost the same current direction (Fig. 2). It may well be that the London ridge did not exist during the deposition of the Folkestone Beds and that the southern and northern sedimentary basins were directly linked. Perhaps, the so-called London ridge carried only a thin veneer of sediment, later stripped off evenly.

Wood[11] has studied the heavy detrital minerals of the western part of the Weald and found an increase in kyanite from the Hythe to the Folkestone Beds. Whereas he cites Armorica as a possible source, the present work shows that the sediments last drifted from the north.

Further study of the detritals and the sedimentary structures of the Lower Greensand should reveal more precisely the regional environment.

I thank Prof. P. Allen, Mr. J. R. L. Allen and Dr. R. Casey for their advice.

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ANALYSIS OF PHOTOSYNTHETIC REACTIONS BY THE USE OF MONOCHROMATIC LIGHT

By Dr. KUNIO TAGAWA, HARRY Y. TSUJIMOTO and Prof. DANIEL I. ARNON
Department of Cell Physiology, University of California, Berkeley

EXTENSIVE work with algal cells in several laboratories, reviewed recently by Smith and French[1] and by Witt *et al.*[2], has led to a now widely held view that photosynthesis involves the co-operation of at least two pigment systems, chlorophyll *a* and an 'accessory' pigment system such as chlorophyll *b*, each pigment activating a separate photochemical reaction which is essential to the overall process. However, these investigations with intact cells were concerned mainly with enhancement of quantum yields and with spectral changes in cellular constituents following activation of specific pigments, and have yielded little biochemical evidence correlating component reactions

of photosynthesis with specific pigment systems. To quote Smith and French[1], "such evidence comes primarily from action spectra for various measurable effects", but because of the experimental difficulties involved "the complete accounting, on a quantitative basis, for the detailed shape of action spectra in terms of the absorption spectra and of the diverse functions of the several pigments involved remains a hope for the future".

The aim of this investigation was to correlate, experimentally, pigment function with those partial reactions of photosynthesis which have been isolated at the sub-cellular level (in chloroplasts) and have thus been freed from the structural and functional complexity of whole cells. Sub-cellular photosynthesis was sub-divided into its component reactions by fractionating chloroplasts[3] and by altering their biochemical environment[4]. Preliminary experiments in this laboratory[5,6] attempting to correlate this biochemical analysis with effects of monochromatic light were encouraging. This article reports on the photochemical activity of isolated chloroplasts that were illuminated by monochromatic light at a wave-length (663 mμ) which is absorbed by both chlorophylls *a* and *b*, and at a wave-length (708 mμ) which is absorbed by the chlorophyll *a* pigment system but not by chlorophyll *b*.

Biochemical Analysis of Photosynthesis by Chloroplasts. Before reporting on the photochemical activity of chloroplasts in monochromatic light, it will be useful to summarize their photochemical reactions in white light. Apart from oxygen evolution[7] (Hill reaction) and total synthesis of carbohydrates[8,9], the photosynthetic capacity of isolated chloroplasts is now known to include photosynthetic phosphorylation[8] (photophosphorylation), that is, a light-induced synthesis of adenosine triphosphate. Once the discovery of photophosphorylation in spinach chloroplasts had been extended to photosynthetic bacteria[10], algae[11] and various higher plants[12], it became clear that this conversion of radiant energy into the chemical bond energy of adenosine triphosphate is an essential component of all photosyntheses in Nature[13–15].

At first, adenosine triphosphate was the sole product of photophosphorylation, but later a second type of photophosphorylation was discovered, again initially in spinach chloroplasts[16] and afterwards in photosynthetic bacteria[17] (compare also refs. 18 and 19), in which the photoproduction of adenosine triphosphate was coupled with the photoreduction of pyridine nucleotide. The new reaction, named non-cyclic photophosphorylation (which in chloroplasts is also coupled with oxygen evolution), and the older type, renamed cyclic photophosphorylation[20], jointly constitute the photochemical phase of photosynthesis that generates the two components of assimilatory power (adenosine triphosphate and reduced pyridine nucleotide) which together drive the dark, enzymatic conversion by chloroplasts of carbon dioxide to carbohydrate[21,22].

This biochemical partition of sub-cellular photosynthesis into a light and dark phase was corroborated by a separation, in a physical sense, of the photosynthetic apparatus in chloroplasts. Chloroplasts were fractionated into a water-insoluble green portion (grana) which contained the photosynthetic pigments that are concerned with the photochemical phase and a non-green, water-soluble fraction which contained the enzymes involved in the dark conversion of carbon dioxide to carbohydrate[23]. Photosynthesis by chloroplasts was next carried out in two successive steps[3]: first, the photochemical phase which, apart from oxygen, yielded the assimilatory power, and second, the chemosynthetic phase in which the assimilatory power was used in the dark to convert carbon dioxide into carbohydrates.

Our recent investigations of the photochemical phase of photosynthesis in chloroplasts have centred on ferredoxin[24–27]. The water-soluble extract of chloroplasts contains not only the enzymes of carbon assimilation[3,23] but also a protein, ferredoxin, which has been identified as a key intermediate in photophosphorylation. (Chloroplast ferredoxin has also been described as the methemoglobin-reducing factor[28], the TPN-reducing factor[29], photosynthetic pyridine nucleotide reductase[30] (PPNR) and the red enzyme[31]. The ferredoxin nomenclature[24,32] used here is based on the recently found chemical and functional similarities in what appears to be a family of ferredoxins in bacteria[33] and in photosynthetic cells[24].)

Ferredoxin is the most electro-negative constituent isolated so far from the photosynthetic apparatus of green plants[24]. When ferredoxin is photoreduced[25] by grana, its subsequent fate determines whether cyclic or non-cyclic photophosphorylation takes place. Non-cyclic photophosphorylation results if reduced ferredoxin is reoxidized by triphosphopyridine nucleotide (a dark reaction mediated by a flavoprotein enzyme[24,31,32], ferredoxin-triphosphopyridine nucleotide reductase[34,35]) and cyclic photophosphorylation results if reduced ferredoxin is reoxidized not by triphosphopyridine nucleotide but by a bound chloroplast component of the cyclic photophosphorylation system[26].

The photoreduction of ferredoxin and the resultant cyclic or non-cyclic photophosphorylation were found to be basically independent of photoproduction of oxygen by chloroplasts[27]. To demonstrate this independence it was necessary to use special experimental devices such as inhibitors of oxygen evolution, anaerobic conditions and, most recently, far-red monochromatic light[27]. Cyclic photophosphorylation catalysed by ferredoxin was found to occur at 708 mμ—a wave-length of light which is absorbed by the chlorophyll *a* pigment system but not by chlorophyll *b* (ref. 36) and at which photoproduction of oxygen by chloroplasts is greatly diminished[27,37]. These results are consistent with the view that photoproduction of oxygen by chloroplasts is an accessory photochemical

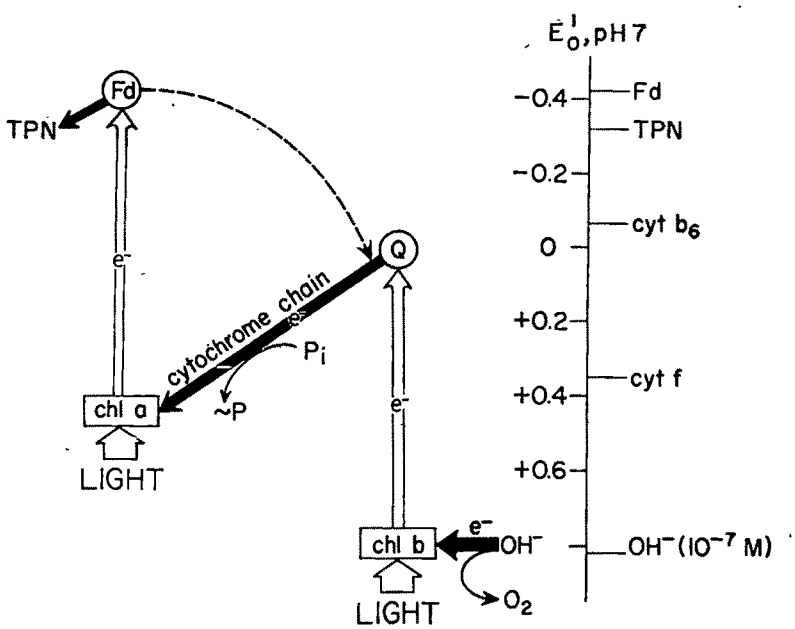


Fig 1. Scheme for two photoreactions and their relation to ferredoxin-dependent cyclic and non-cyclic photophosphorylation in chloroplasts. Details in the text

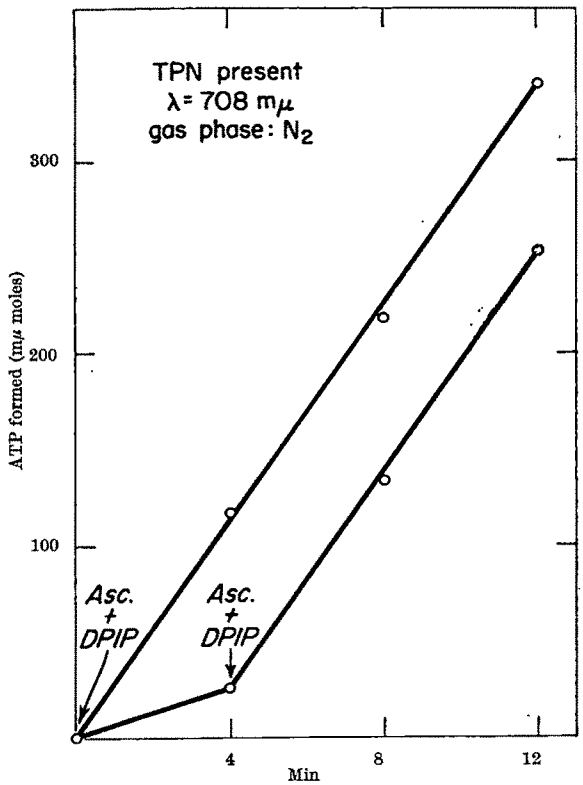


Fig. 2. Non-cyclic photophosphorylation at 708 mμ. The reaction mixture contained in a final volume of 3·0 ml., broken chloroplasts equivalent to 400 μg of chlorophyll, 500 μg of spinach ferredoxin and the following in μmoles: *tris* buffer, pH 8·3, 100; $MgCl_2$, 5; ADP, 2; $K_2HP^{32}O_4$, 2; and triphosphopyridine nucleotide (TPN), 2. Sodium ascorbate (20 μmoles) and dichlorophenol indophenol (DPIP) (0·2 μmoles) were added at the times indicated. The reaction was carried out in Warburg vessels under nitrogen at 15° C. Light intensity was 5·0 μeinsteins per min. The reaction vessels were capped with rubber serum bottle stoppers, through which, at the indicated times, 0·5 ml. aliquots of the reaction mixture were withdrawn (with a syringe) for ATP determination. The ATP formed is plotted as mμmoles ATP formed per 1·5 ml. of reaction mixture

reaction dependent mainly on chlorophyll *b*, which in chloroplasts serves as an 'accessory' pigment system, analogous to accessory pigment systems such as the phycobilins, found only in oxygen-evolving photosynthetic cells.

To recapitulate, the biochemical analysis of the photochemical activity of isolated chloroplasts has led to a distinction between two main component photochemical reactions (Fig. 1): (1) the photoproduction of oxygen, a reaction for which the accessory chlorophyll *b* pigment system is essential; (2) the photoreduction of ferredoxin (leading to triphosphopyridine nucleotide reduction and photophosphorylation), a reaction which is basically independent of chlorophyll *b* in that it can be experimentally driven by the chlorophyll *a* pigment system alone. We will present new evidence that supports this interpretation and also clarifies further the relationship between cyclic and non-cyclic photophosphorylation in chloroplasts.

Cyclic and Non-cyclic Electron Flow at 708 *mμ*. In the scheme, represented by Fig. 1 (based on Fig. 3 in ref. 4) we envisage that non-cyclic photophosphorylation by chloroplasts is coupled with an 'uphill' flow of electrons from water (OH^-) to triphosphopyridine nucleotide—an electron flow which is driven by two photochemical reactions working in series[4,38]. The first photoreaction lifts electrons from the redox potential (at *p*H 7) of water-oxygen ($E_0' = 0{\cdot}82$ V) to that of plastoquinone[39] ($E_0' \sim 0$ V) and is driven by chlorophyll *b* (henceforth referred to as photoreaction *B*). The second photoreaction lifts electrons from the level of cytochrome *f* (ref. 40) ($E_0' = 0{\cdot}365$ V) to that of ferredoxin[24] ($E_0' = -0{\cdot}43$ V) and is driven by the chlorophyll *a* pigment system (henceforth referred to as photoreaction *A*). A 'primary' phosphorylation site, common to both the cyclic and non-cyclic photophosphorylation pathways, is considered to be coupled with a 'downhill', dark electron transfer—one in accordance with the thermodynamic gradient—which joins the two photochemical reactions and probably involves a transfer of electrons from plastoquinone (*Q*) to the chloroplast cytochromes and thence to chlorophyll *a* (long black arrow in Fig. 1). In addition, the ferredoxin-catalysed cyclic photophosphorylation is envisaged as having at least one more phosphorylation site, coupled with the electron transport sector that is marked in Fig. 1 by a broken line.

It can be predicted from the scheme in Fig. 1 that at 708 mμ, non-cyclic photophosphorylation, coupled with triphosphopyridine nucleotide reduction, should occur when the ascorbate-dichlorophenol indophenol (ascorbate-DPIP) couple and not water is the electron donor system. The transfer of electrons from water to triphosphopyridine nucleotide would require the participation of both photoreactions *B* and *A*, whereas the transfer of electrons from the ascorbate-DPIP couple to triphosphopyridine nucleotide would require the participation of only photoreaction *A*.

These predictions were based on the following observations: (1) The ascorbate-DPIP couple photoreduces triphosphopyridine nucleotide in the presence of inhibitors of oxygen evolution[41]. (2) The ascorbate-DPIP couple serves as the electron donor system for a triphosphopyridine nucleotide-coupled non-cyclic photophosphorylation when the flow of electrons from water is blocked[4]. (3) The photoreduction of triphosphopyridine nucleotide by the ascorbate-DPIP couple, as contrasted with the photoreduction by water, is not affected by an extraction of plastoquinone (*Q* in Fig. 1) from chloroplasts[39,42].

Fig. 2 shows how the predictions from the scheme have been experimentally verified. At 708 mμ, a wave-length at which photoreaction *B* could not occur (because chlorophyll *b* does not absorb at this wave-length), little non-cyclic photophosphorylation took place unless ascorbate-DPIP was supplied as the electron donor system

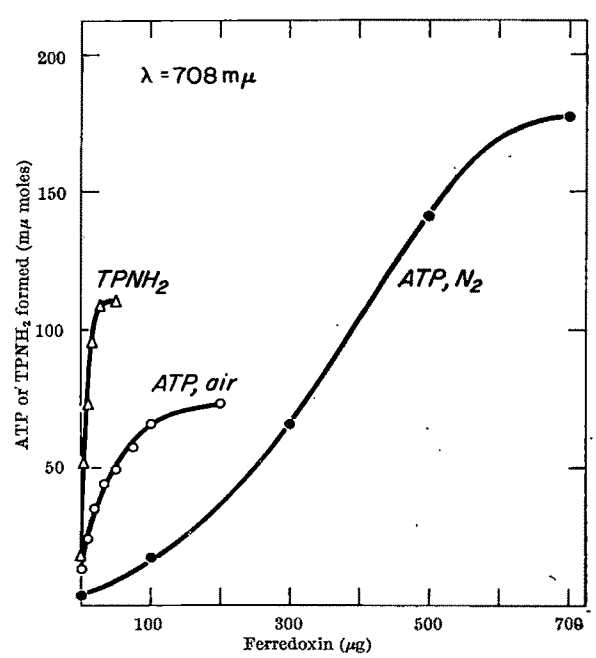


Fig. 3. Photochemical reactions of isolated chloroplasts at 708 mμ as a function of ferredoxin concentration. Left curve: photoreduction of triphosphopyridine nucleotide (TPN) by the ascorbate-DPIP couple under nitrogen (see legend for Fig. 2). Middle curve: cyclic photophosphorylation under air. Right curve: cyclic photophosphorylation under nitrogen. No co-factor of photophosphorylation other than ferredoxin was added to the reaction mixtures

for the reduction of triphosphopyridine nucleotide. The low rate of non-cyclic photophosphorylation which was observed prior to the addition of the ascorbate-DPIP couple (lower curve, Fig. 2) was probably due to chromatic impurities remaining in the 708 mμ light beam.

Fig. 3 shows cyclic photophosphorylation at 708 mμ as a function of ferredoxin concentration under aerobic and anaerobic conditions. Lower concentrations of ferredoxin were more effective under aerobic than under anaerobic conditions in catalysing cyclic photophosphorylation, but a higher maximal rate of photophosphorylation was observed under anaerobic conditions. Fig. 3 also shows that, in the presence of ascorbate-DPIP as the electron donor system, ferredoxin has a greater affinity for the reduction of triphosphopyridine nucleotide than for the cyclic electron flow pathway. The reduction of triphosphopyridine nucleotide proceeded equally well under aerobic and anaerobic conditions.

The apparent Michaelis constants have been computed from data represented by Fig. 3 in order to compare the affinity of ferredoxin for the cyclic and non-cyclic electron flow pathways in chloroplasts. The values for non-cyclic, aerobic cyclic and anaerobic cyclic photophosphorylation are, respectively: $3{\cdot}3 \times 10^{-7}$ M, $2{\cdot}3 \times 10^{-6}$ M and $1{\cdot}9 \times 10^{-5}$ M.

The high affinity of ferredoxin for the non-cyclic electron flow is in agreement with the previously observed dominance in chloroplasts of non-cyclic photophosphorylation over cyclic photophosphorylation, so long as triphosphopyridine nucleotide is available as the electron acceptor[26]. This high affinity of ferredoxin for the non-cyclic electron flow to triphosphopyridine nucleotide also explains the inhibitory effect of triphosphopyridine nucleotide on the ferredoxin-catalysed cyclic photophosphorylation at 708 mμ, which is shown in Fig. 4. These results are also consistent with the scheme in Fig. 1.

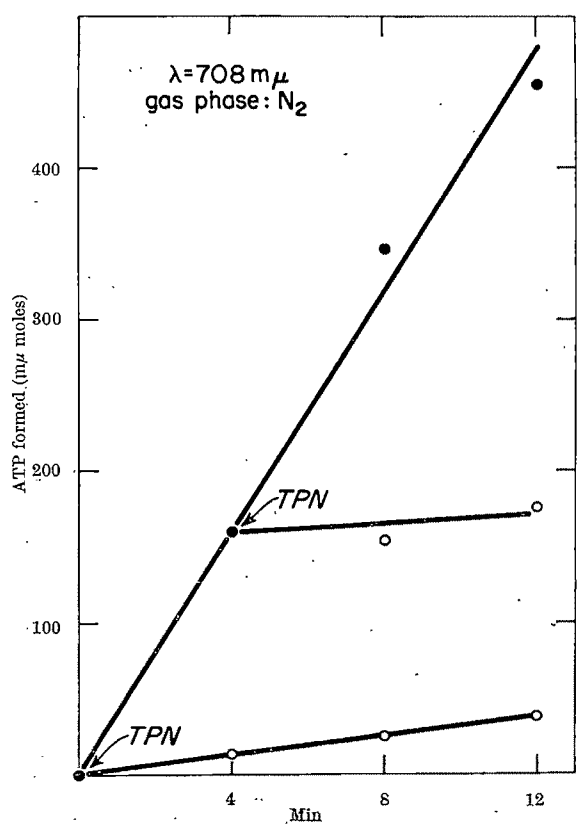


Fig. 4. Effect of triphosphopyridine nucleotide on ferredoxin-dependent cyclic photophosphorylation under anaerobic conditions at 708 mμ. The experimental conditions were as given for Fig. 2, except that ascorbate and DPIP were omitted. TPN (2·5 μmoles) was added only as indicated

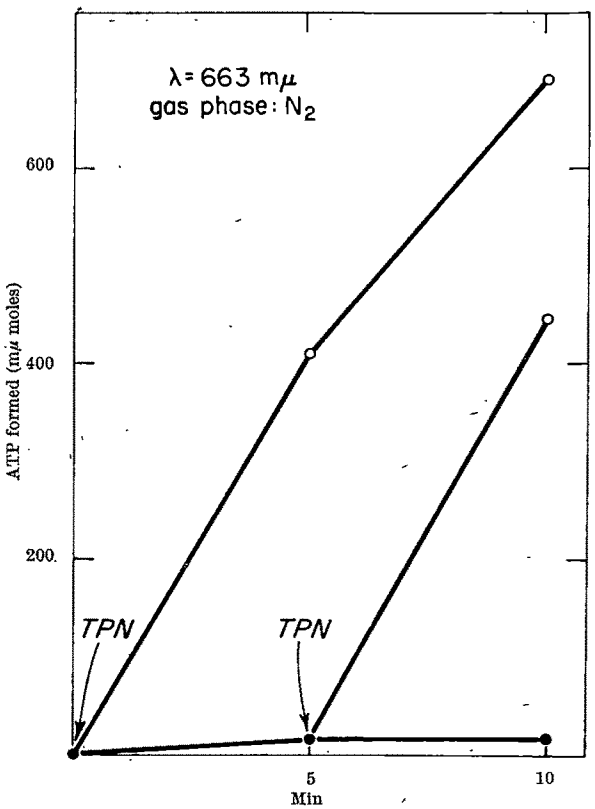


Fig. 5. Effect of triphosphopyridine nucleotide on ferredoxin-dependent cyclic photophosphorylation under anaerobic conditions at 663 mμ. Experimental conditions were as given for Fig. 4, except that the reaction mixtures were pre-illuminated for 5 min at 663 mμ, under nitrogen. Light intensity was 6·0 μeinsteins per min throughout

At 708 mμ photoreaction *B* is inoperative; therefore, water cannot serve as the electron donor and the added triphosphopyridine nucleotide now acts as a 'trap' which drains off electrons (via ferredoxin) and thus prevents their cyclic flow. Without an alternative electron donor system (ascorbate-DPIP couple) that would be effective at 708 mμ (cf. Fig. 2), ferredoxin would remain in the oxidized state and, as a result, all photophosphorylation, cyclic and non-cyclic, would of necessity cease. It is noteworthy that although reduced ferredoxin is auto-oxidizable, molecular oxygen did not, like triphosphopyridine nucleotide, act as an electron trap to prevent the cyclic electron flow.

Photophosphorylation at 663 mμ. Fig. 5 shows that at 663 mμ, under anaerobic conditions, cyclic photophosphorylation did not occur (bottom curve). However, the addition of triphosphopyridine nucleotide readily established non-cyclic photophosphorylation (middle and upper curves). At 663 mμ both photoreactions *A* and *B* were possible and hence, in accordance with the scheme in Fig. 1, the transfer of electrons from water to triphosphopyridine nucleotide and the accompanying phosphorylation would be assured.

The inability of monochromatic light at 663 mμ to support cyclic photophosphorylation under anaerobic conditions (bottom curve in Fig. 5) was observed after pre-illuminating the chloroplast system at this wave-length

Table 1. Effect of Pre-illumination at 663 mμ and Aeration on Ferredoxin-dependent Cyclic Photophosphorylation at 708 mμ

Treatment	mμmoles ATP formed/4 min
1. Control	127
2. 5 min pre-illumination in N_2	16
3. 5 min pre-illumination in air	105
4. Treatment 2 followed by 5 min aeration in the dark	126

The reaction mixture was as given for Fig. 4. The light intensity was 2·5 and 4·9 μeinsteins per min at 663 mμ and 708 mμ, respectively. Pre-illumination at 663 mμ was for 5 min. The reaction time was 4 min.

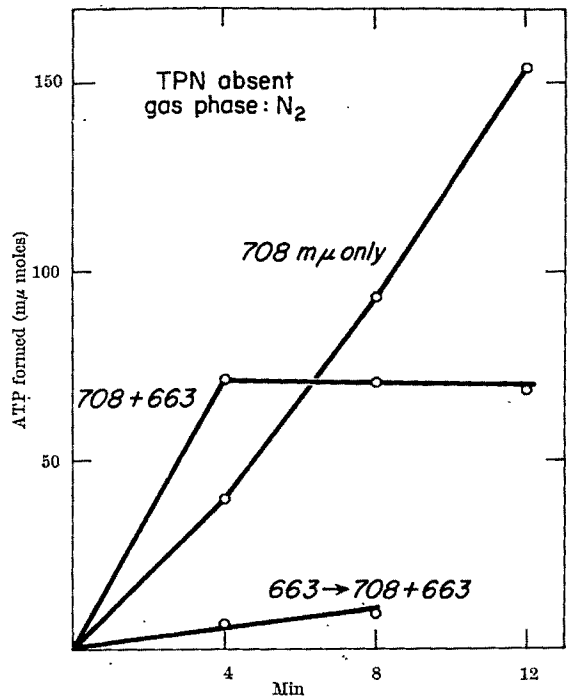


Fig. 6. Effect of additional illumination at 663 mμ on ferredoxin-dependent cyclic photophosphorylation at 708 mμ, under anaerobic conditions. Bottom curve: 4 min pre-illumination at 663 mμ under nitrogen, followed by joint illumination with the two monochromatic beams (663 mμ and 708 mμ). Middle curve: illumination was by the 708 mμ band only without pre-illumination. Left curve: joint illumination with the two beams (663 mμ and 708 mμ) without pre-illumination. Reaction mixture was as given for Fig. 4. Light intensity was 2·6 and 3·1 μeinsteins per min at 663 mμ and 708 mμ, respectively

for 5 min, under nitrogen. It was essential to maintain anaerobic conditions during the pre-illumination period to observe the subsequent suppression of anaerobic cyclic photophosphorylation at 663 mμ. Cyclic photophosphorylation was not suppressed when the pre-illumination was carried out in air or when anaerobic pre-illumination was followed by 5 min of aeration in the dark, prior to exposure to the actinic light beam at 663 mμ (Table 1).

The results shown in Fig. 5 and Table 1 are explained by the following hypothesis. As already stated, non-cyclic electron flow in chloroplasts is a unidirectional electron transfer from water to triphosphopyridine nucleotide and is driven by photoreactions *B* and *A*. This unidirectional electron flow keeps the intermediates in the electron transport chain in a partly reduced state. The problem of 'over-reduction' does not arise so long as the electron acceptor, triphosphopyridine nucleotide (or ultimately carbon dioxide) is available. A different situation, however, arises in the case of cyclic photophosphorylation. To maintain a cyclic electron flow from reduced ferredoxin back to the electron transport chain, the intermediates in the chain must be at least partly oxidized. If they are kept in a reduced form they cannot accept electrons from reduced ferredoxin.

Our hypothesis proposes that molecular oxygen normally regulates the redox balance for the electron transport chain involved in cyclic photophosphorylation by chloroplasts. In the presence of oxygen, the electron transport system of chloroplasts does not become over-reduced by the flow of electrons from water. But under anaerobic conditions, the flow of electrons from photoreaction *B* over-reduces the components of the electron transport chain in chloroplasts and this over-reduction cannot be counterbalanced by the regulatory oxidizing action of oxygen.

In accordance with previous interpretations[13,5], the present hypothesis excludes a proposed electron acceptor role for oxygen in cyclic photophosphorylation (chloroplasts do not contain cytochrome oxidase and do not respire[43,44]), but envisages for oxygen an important role as a redox regulator for cyclic photophosphorylation. This hypothesis explains why in white light, or in shorter wave-lengths of monochromatic light which permit photoreaction *B* to occur, aerobic conditions are necessary for the ferredoxin-dependent cyclic photophosphorylation. Oxygen is needed to prevent photoreaction *B* from over-reducing the system, thereby bringing cyclic photophosphorylation to a halt. Under these conditions of illumination, ferredoxin-dependent cyclic photophosphorylation can be made to proceed without oxygen only if photoreaction *B* is blocked by the use of specific inhibitors, for example, *p*-chlorophenyldimethyl urea[26]. Alternatively, cyclic photophosphorylation can be made to proceed under anaerobic conditions by using far-red monochromatic light which excludes photoreaction *B* (ref. 27 and Fig. 2).

Photophosphorylation with Two Beams of Light. The hypothesis just presented is also supported by experiments with two beams of light, as illustrated in Figs. 6 and 7. Fig. 6 shows that, under anaerobic conditions, cyclic photophosphorylation at 708 mμ is inhibited by the addition of a second monochromatic beam of light at 663 mμ. This chromatic inhibition, which we attribute to over-reduction by the 663 mμ beam, occurred immediately when illumination by the combined 708 and 663 mμ beams was preceded by pre-illumination at 663 mμ (bottom curve, Fig. 6). Without a pre-illumination treatment, the inhibitory effect of the 663 mμ beam, added to the 708 mμ beam, was observed only after 4 min. Evidently an interval of time was needed to bring about a sufficient state of over-reduction by the 663 mμ beam.

Unlike cyclic photophosphorylation, non-cyclic photophosphorylation with triphosphopyridine nucleotide was favoured by the combination of the two beams of monochromatic light (Fig. 7). No phosphorylation coupled with photoreduction of triphosphopyridine nucleotide by water occurred at 708 mμ alone (bottom curve, Fig. 7). Supplementary illumination at 663 mμ,

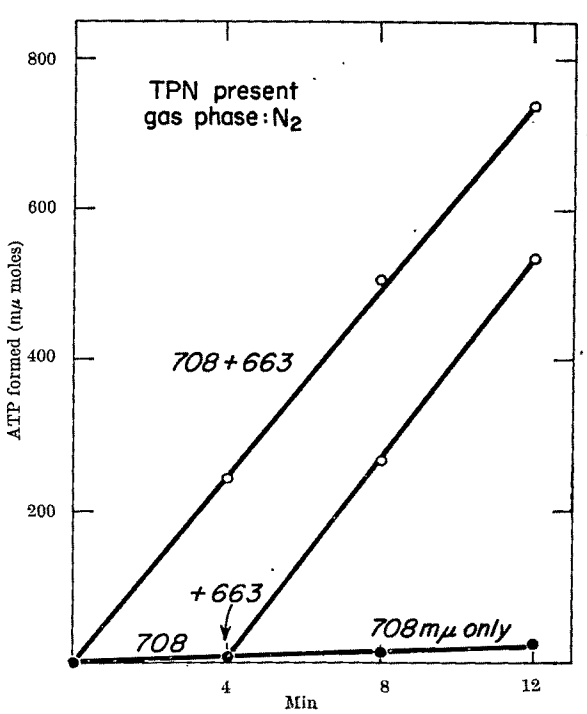


Fig. 7. Effect of additional illumination at 663 mμ on non-cyclic photophosphorylation at 708 mμ, under anaerobic conditions. Illumination at 708 mμ was given in all cases, but additional illumination at 663 mμ was supplied at the start of the experiment (top curve) or after 4 min (middle curve). No pre-illumination was given. Light intensity was as given for Fig. 6. Reaction mixture was as given for Fig. 4 except that 2·5 μmoles TPN was added in all cases

when given at the start, made photoreaction *B* and the resultant non-cyclic photophosphorylation possible from the beginning of the illumination period (Fig. 7). Alternatively, the non-cyclic photophosphorylation could be delayed by delaying the addition of the 663 mμ beam (middle curve in Fig. 7).

Concluding remarks. The findings reported here support the idea that in illuminated chloroplasts the conversion of radiant energy into chemical energy is accomplished by two main photoreactions: photoproduction of oxygen, dependent on light absorbed by the chlorophyll *b* pigment system, and photoreduction of ferredoxin, dependent on light absorbed by the chlorophyll *a* pigment system. The experiments with monochromatic light reported here were not designed to give any information about the association of other pigments, such as carotenoids or variants of chlorophyll *a* (ref. 1), with the two main pigment systems in chloroplasts. This question must be left for future experimentation.

Our results indicate that at selected far-red wavelengths of light, which are not absorbed by chlorophyll *b* and give no oxygen evolution, the chlorophyll *a* pigment system can drive the photoreduction of ferredoxin and the ensuing cyclic and non-cyclic photophosphorylation (the latter only when a suitable electron donor is supplied). The photoactivity of chlorophyll *a* cannot replace the photoactivity of chlorophyll *b*.

The two photochemical reactions shown in Fig. 1 are envisaged as being joined by a 'thermochemical bridge', connecting reduced plastoquinone with cytochrome. Such an interpretation is consistent with the observations of Myers and French[45] on whole cells, which argue against a physical transfer of energy from one photosynthetic pigment to another. In their experiments, enhancement of quantum yields by two beams of light could be seen when the two beams were not supplied simultaneously but were alternated in periods of several seconds—an interval of time too long for a direct energy transfer mechanism. However, we consider it still premature to go beyond tentative formulations on the possible energy transfer mechanisms in chloroplasts.

The idea of redox balance as a condition for cyclic photophosphorylation in chloroplasts has its counterpart in photosynthetic bacteria[18,19,46]. However, since photosynthesis in bacteria, unlike chloroplasts, produces no oxygen, oxygen cannot be invoked as a redox regulator for bacterial cyclic photophosphorylation under physiological conditions. In chloroplasts, according to our hypothesis, oxygen fulfils an important physiological function as a redox regulator in cyclic photophosphorylation. Thus, chloroplasts which lack the enzymatic equipment to use oxygen for respiration are considered to be dependent on oxygen for regulation of photochemical activity.

The methods used in this investigation are described elsewhere[27]. One of us (D. I. A.) thanks Prof. Otto Warburg for the hospitality of his Institute where, as a guest investigator in 1955–56, he learned the optical and photometric techniques used in this investigation. We thank Prof. J. E. Gullberg for advice and Lars O. Björn for assistance in the initial assembly and testing of the optical apparatus. Richard K. Chain, Berah D. McSwain and William Ufert have given excellent technical assistance with the present experiments. This work was supported by grants from the U.S. National Institutes of Health and the Office of Naval Research.

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FLAVOUR OF BEEF AND WHALE MEAT

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As a result of a series of investigations of beef, pork and lamb[1–3], we concluded that an identical basic meaty aroma is associated with the lean portion of these red meats and that species flavour differences reside in the fat. To check the validity of the conclusion, that lean meats contribute an identical meaty aroma, we have now compared the volatiles, both chemically and organoleptically, of lean whale and lean beef. If differences in

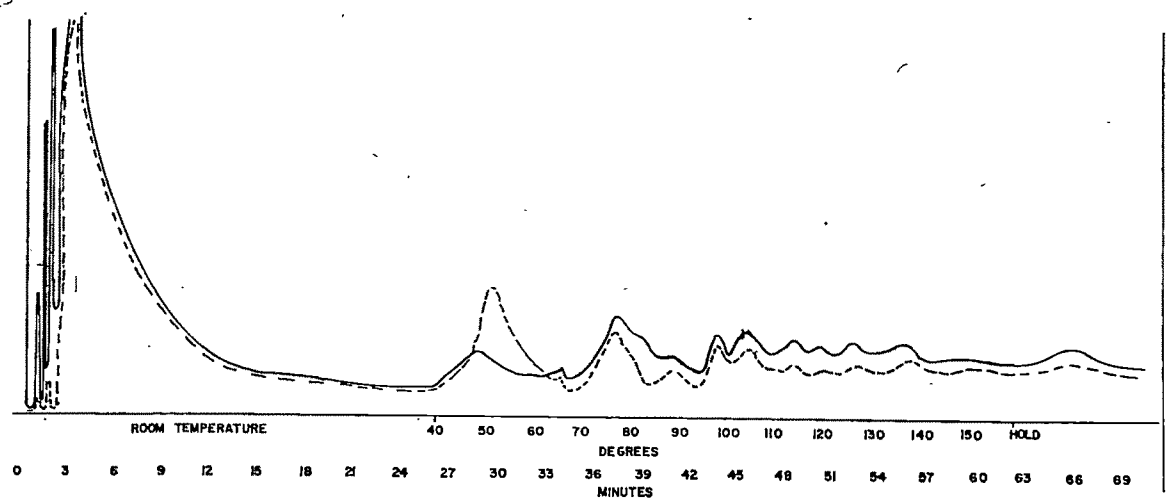


Fig. 1. Comparison of the chromatographic patterns obtained from lean beef and lean whale volatiles. The third peak on whale pattern is absent in beef and represents trimethylamine. Thick line, whale volatiles; dashed line, beef volatiles

the basic meaty flavour derived from lean meats of different species of animals do exist, one might expect to find them in a mammal that lives in an environment and subsists on a diet so completely alien to that of its terrestrial relatives.

Approximately 20 lb. of lean whale meat were obtained from a 49-ft. sei whale, *Balaenoptera borealis*[4], less than 20 h after it was killed. The sample was frozen, packed in dry ice and flown to the laboratory where it was kept at dry-ice temperature until needed.

For flavour evaluations, steaks 1 in. thick were cut from the sample, heated to an internal temperature of 150° F and tasted by the laboratory personnel. The results were uniformly unfavourable; all tasters said the steaks were 'fishy', and descriptive terms such as 'metallic', 'oily', and 'rancid' were also used by the panel. The procedures used to concentrate the flavour precursors in lean meats[1–3] were applied to the lean whale meat. The following operations were carried out at 0°–4° C. Whale meat (1 kg) was ground and homogenized with 1,500 ml. of water. The slurry was centrifuged, filtered and the extract lyophilized to yield 50 g (5 per cent of starting weight) of a water-soluble red-brown powder. 100 ml. of a 5 per cent solution of the powder (*p*H 5·2) were refluxed. This solution gave a strong 'fishy' aroma that soon disappeared and was replaced by a typical meaty aroma indistinguishable from the cooked meat aroma obtained from similar solutions prepared from beef, pork or lamb powders. The whale 'broth' by odour evaluation was rated excellent and described as 'meaty' but without any species identity being attached to the 'meaty' aroma.

Chemical comparisons of lean beef and whale volatiles were made on freeze-dried extracts of the lean meats; 30 g of powder were vacuum pyrolysed at 100° C and 10^{-5} mm mercury. Total volatiles were condensed in a trap surrounded by liquid nitrogen. The total volatiles were in turn fractionated at 10^{-6} mm mercury by allowing the trap containing the total volatiles to come to room temperature and collecting fractions at liquid nitrogen temperature and at dry ice–acetone temperature. A small amount of residue remained in the total condensate trap. The techniques and apparatus were previously described[1,2]. The aroma of the fractions from whale were identical to their counterpart fractions from beef. Of particular interest was the residue in the total condensate trap, which in both whale and beef consisted of a very small amount of a colourless, viscous, water-soluble liquid of a pleasant, fruity odour that, on exposure to air, slowly darkened and assumed a 'meaty' aroma. The volatiles were analysed for acidic and basic compounds by conversion to their salts and their subsequent analyses[1]; for carbonyl compounds, by conversion to 2,4-dinitrophenylhydrazones[1], and by separation of these derivatives on paper[5,6]; for hydrogen sulphide, by conversion to methylene blue by a modified procedure of Marbach and Doty[7]; and for lactic acid, by the colorimetric method of Hullin and Noble[8].

Compounds positively identified in the volatiles from whale, other than water and carbon dioxide, included ammonia, methylamine and trimethylamine, hydrogen sulphide and methyl mercaptan, formaldehyde, acetone and acetaldehyde, and lactic acid and its ammonium salt. Of these compounds, only trimethylamine was absent in the beef volatiles.

In a separate set of experiments, 5 g of whale powder were heated for 45 min at 100° C with nitrogen flowing over the sample, at a rate of approximately 30 ml./min. The volatiles were trapped on a collection coil[9] and separated by gas chromatography. The column was an 8 ft. × ⅛ in. outer diameter, stainless steel coil; the liquid phase was 20 per cent castorwax on acid-washed 'Chromosorb-*P*'. The column was temperature programmed, and a flame ionization detector was used[10]. 5 g of beef powder were treated in identical fashion. The gas chromatographic patterns are shown in Fig. 1. The qualitative agreement between the two traces is excellent. Only one peak is not common to both, and that is the trimethylamine peak present in whale. The gas stream was split after emergence from the column, part going to the flame detector and the rest to the nostrils of an observer. The path-lengths, after emergence from the column, were so adjusted that the odour could be noted as the peak was being recorded. No one compound could be assigned the designation of 'meaty'; however, the collective fractions above 70° C were much more characteristic of meat than were the collective fractions below 70° C. These chromatograms were obtained at high sensitivity, and the peaks at the higher temperatures correspond to microgram amounts of material.

Studies of whale depot fat have shown this to be highly unsaturated[11–13]. If the intramuscular lipid fraction was similarly unsaturated, oxidation could account for the observed off-flavour of the whale steaks. The fatty composition of the lipid fractions extractable from lean whale and lean beef were therefore compared. 100 g of diced meat were extracted according to the procedure of Folch *et al.*[14] with 2 : 1 chloroform–methanol. The lipid fraction, after clean-up and solvent removal, was dissolved in a minimum of 20 : 1 chloroform–methanol and chromatographed on silicic acid. Chloroform containing increasing amounts of methanol was used to successively elute two triglyceride fractions, a cephalin fraction,

Table 1. Fatty Acid Composition of Lipid Fractions (% of Total Fatty Acids) obtained from Lean Beef and Whale. Superscripts refer to Number of Double Bonds in Acid. The Dash signifies that the Acid was absent

Acid	Triglycerides		Cephalins		Lecithins and Sphingomyelins	
	Beef	Whale	Beef	Whale	Beef	Whale
C_{14}^{0}	5·15	7·96	2·28	9·60	4·25	9·91
C_{14}^{1}=	1·73	1·60	1·17	4·95	1·63	7·29
C_{14}^{2}=	1·73	—	1·52	—	1·08	—
C_{16}^{0}	33·15	13·62	6·63	11·57	18·25	23·80
C_{16}^{1}=	5·10	11·69	2·06	6·17	3·38	10·56
C_{18}^{0}	16·43	5·65	23·91	25·95	7·01	6·54
C_{18}^{1}=	31·09	16·35	13·47	20·41	27·57	17·50
C_{18}^{2}=	4·31	6·19	11·96	4·68	23·05	—
C_{18}^{3}=	1·31	—	2·92	—	1·36	—
C_{20}^{0}	—	3·18	—	2·55	—	0·93
C_{20}^{1}=	—	2·52	—	—	—	—
C_{20}^{3}=	—	—	4·11	—	2·71	—
C_{20}^{4}=	—	—	22·72	6·84	6·07	4·62
$C_{20}^{5\text{ and }6}$=	—	14·50	1·81	7·28	0·96	16·64
C_{22}^{0}	—	3·47	—	—	0·28	—
C_{22}^{3}=	—	—	1·43	—	0·92	—
C_{22}^{4}=	—	3·91	3·68	—	2·07	—
$C_{22}^{5\text{ and }6}$=	—	9·36	0·33	—	—	2·21

Table 2. Fatty Acid Composition of Lipid Fractions (% of Total Fatty Acids) of Lean Beef and Whale in Terms of Unsaturation

Acid type	Triglycerides		Cephalins		Lecithins and sphingomyelins	
	Beef	Whale	Beef	Whale	Beef	Whale
Saturated acids	54·73	33·88	32·82	49·67	29·79	41·18
Mono unsaturated acids	37·92	32·16	16·70	31·53	32·58	35·35
Dienoic acids	6·04	6·19	13·48	4·68	24·13	—
Trienoic acids	1·31	—	8·46	—	4·99	—
Tetraenoic acids and greater unsaturation	—	27·77	28·54	14·12	9·10	23·42
Wt. of fraction (g)	4·438	3·767	0·432	0·312	0·443	0·390

and a mixed lecithin–sphingomyelin fraction[15]. The fatty acid composition of the combined triglyceride fraction and each of the other fractions was determined by hydrolysis of an appropriate aliquot, followed by conversion of the fatty acids to their methyl esters and their subsequent separation by gas chromatography[15,16].

Retention volume data were obtained on both a non-polar 'Apiezon' substrate and on a polar diethyleneglycol succinate ('DEGS') substrate. Packed columns, 20 per cent substrate on acid-washed 'Chromosorb *P*', 7 ft. × ⅛ in. outer diameter stainless steel, were used. Columns were operated isothermally at 202° C with the 'Apiezon' column and at 192° C with the 'DEGS' column[10]. Identification of the acids was in the main based on both a comparison of the retention volumes of the methyl esters with knowns and on plots of log of retention volume versus chain length for both liquid phases[16]. In Table 1, the fatty acid composition is given for the combined triglyceride fractions, the cephalin fraction, and the combined lecithin–sphingomyelin fraction in whale and beef. The profound difference in the fatty acid composition of the triglyceride fractions is particularly striking. Beef triglycerides contain no fatty acids above C_{18}; whale triglycerides contain approximately 37 per cent C_{20} and C_{22} fatty a... and about 75 per cent of these contain four or more doub... bonds. The differences in terms of unsaturation in the fatty acid composition of whale and beef liquids are summarized in Table 2.

In a comparison of the phospholipids in lean pork and beef, we noted that the isolated, highly unsaturated, phospholipid fractions quickly assumed fishy, unpleasant off-flavours, while in the total lipid extract, which contained large amounts of the relatively saturated triglycerides, this development of off-flavour was retarded[15]. The whale triglyceride fraction, on the other hand, is actually more unsaturated than the phospholipid fraction and, rather than retarding the development of oxidative off-flavours, probably enhances their development. This high degree of unsaturation of the lipid fraction triglycerides, in combination with the trimethylamine presumably formed by the reduction of the trimethylamine oxide present in the whale's food supply, could well account for the poor flavour of the whale meat. The organoleptic and chemical evaluation of the water-extractable portion of the lean whale meat confirms the supposition that a basic meat flavour originates in the lean portions of meats. A corollary can be added; the lean meat, which is in essence an aqueous phase, can act as a reservoir for extraneous, water-soluble materials, in this case, for trimethylamine oxide, that may also affect flavour.

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MICROBIOLOGY OF ANTARCTIC SEA-ICE

Microalgae and Antarctic Sea-ice

THOSE who have travelled through far southern antarctic waters will be familiar with the brown coloration commonly visible as bands in sea-ice, especially in the lowermost layers. This colour is caused by diatoms growing on or trapped within the ice. In spite of their widespread occurrence and prolific growth, they do not appear to have attracted closer study in their normal habitat, although a good deal of material from these populations has, without doubt, been recovered in phytoplankton hauls, for example, Heiden and Kolbe[1], Hart[2].

One reason for our lack of knowledge of this flora centres round the fact that most investigations in the past have been carried out from ships rather than from fixed stations associated with bases on the antarctic mainland. Even working through holes in the ice, it is clear that very little or no information concerning the attached flora would be obtained unless special techniques were adopted.

One of us[3] first became interested in these organisms at the Australian Antarctic Station, Mawson, during 1956, when they were found well developed on the undersurface of sea-ice some 1·4 m thick at a time when the amount of light penetrating to their habitat must have been very limited. More recently, during the 1961–62 summer season at McMurdo Sound, diatoms were found in large quantities associated with sea-ice 2–3 m thick. At that time, it was not possible to undertake a study in any detail[4]. However, the opportunity was taken during the 1962–63 austral summer to collect a series of samples in this area to determine the species present. It is the purpose of this communication to direct attention to the main findings.

The flora was sampled on seven occasions between December 18, 1962, and January 7, 1963, at an ice-hole station over 25 m of water near Cape Armitage on Ross

Island. Aqualung equipment was used to obtain the material directly from the lower layers of the ice. During this period, the water below the ice was almost devoid of phytoplankton.

A total of 32 species of microalgae, all diatoms, were recovered from the samples. Numerical dominance was shared by 11 species, all of which were present in at least 6 of the 7 samples. These organisms were *Amphiprora kjellmanii, A. oestrupii, Biddulphia weissflogii, Coscinodiscus subtilis, Eucampia balaustium, Fragilaria linearis, Nitzschia martiana, N. seriata, Pleurosigma antarcticum, Rhizosolenia alata* and *R. rostrata*. It should be noted that, of the 11 species, 6 have been recorded previously by Heiden and Kolbe[1] among pack ice or close to the mainland of Antarctica, and 5 included by Hart[2] in his group of typically neritic and ice-edge forms.

Here, it should be mentioned that we are not satisfied with the terminology which has been applied to these organisms, for example, by Hart. A clear distinction should be drawn between species especially adapted for life in the sea-ice habitat and those which are permanently planktonic. There are, in effect, two plant communities; the epontic community and the planktonic community. The epontic community in this enviroment consists, as it does in the estuaries of lower latitudes, of attached species of *Pleurosigma, Nitzschia, Amphiprora, Fragilaria*, etc., as well as of non-attached species living with them as part of that community, that is, species of *Biddulphia, Coscinodiscus, Asteromphalus*, etc.

With the deterioration of the sea-ice during the summer, the identity of this community is inevitably lost. Although the members of the epontic community must, at these times, be recovered with the plankton, it should be remembered that their development has taken place under conditions which, in several respects, are dissimilar to those to which the typical plankton is exposed. The application of the term 'meroplankton' to such organisms seems to us misleading and inappropriate in that it gives no indication of their origin. To describe the members of this community as 'ice-edge' forms is clearly not acceptable.

There are several reasons which make the recognition of separate epontic and planktonic communities important. Apart from the intrinsic biological interest of microalgae in the sea-ice habitat, it is clear that, in the presence of an ice-associated flora, assessment of the primary productivity of this region cannot be based solely on measurements of photosynthetic activity in the water column below the ice. Further, it seems probable that the development of the epontic community, by further reducing levels of submarine illumination, may exert a significant limiting influence on the activities of phytoplankton present beneath the sea-ice. In this connexion, it is noted that a sudden increase in under-ice illumination, particularly during the 1961–62 summer, appeared to be related, at least in part, to the loss of the epontic community coincident with deterioration in the lower layers of the sea-ice.

While present results (see following communication) indicate that at least low levels of illumination are necessary for the growth of diatoms associated with sea-ice, it is by no means certain that heterotrophic growth does not occur. Here, it may be relevant to note that potentially photosynthetic micro-organisms have been recorded at depths from 500 to 4,000 m in the open sea by Bernard[5], by a group at Miami and by Wood (unpublished). These workers have found chlorophyll-containing coccolithophores, dinoflagellates (*Exuvialla* and *Gymnodinium*), blue-green algae (*Nostoc* and *Oscillatoria*) and chrysomonads (*Isochrysis* spp.) at such depths, in some cases in samples taken under aseptic conditions, and have cultivated them or shown the presence of chlorophyll by fluorescence. Oppenheimer and Wood[6] found chlorophyll-containing organisms in sediments to a depth of 11 cm, and Claus[7] has found up to 90 species of chlorophyllous algae in caves recently opened in Hungary. Kriss and Rukina[8] found photosynthetic sulphur bacteria at 2,500 m in the Black Sea. The presence of potentially photosynthetic organisms as apparently normal inhabitants of regions of subliminal or zero illumination seems worth comment, especially as they occur in such widely different habitats.

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Diatoms of Antarctic Sea-ice as Agents of Primary Production

In the preceding communication, Bunt and Wood have given a brief description of the epontic community of diatoms associated with the lower layers of the sea-ice in McMurdo Sound. I should like, now, to describe the ice habitat and to present information relating to the concentrations of organisms, which this habitat supports and to their activities as agents of primary production.

There were several problems of a practical nature peculiar to this investigation. During the 1961–62 austral summer, the possibility of obtaining estimates of the concentrations of organisms in the ice flora by taking samples from ice upturned in the path of ice-breakers was abandoned when it was observed that an appreciable proportion of the population was dislodged into the water below the ice and could not be recovered quantitatively. Further, the material remaining attached to the ice rapidly became frozen on exposure to the air so that it was unsuitable for laboratory measurements of photosynthetic activity. It became apparent that sampling would have to be undertaken directly from the lower layers of undisturbed sea-ice and that such a procedure would require diving equipment. Very little diving has been undertaken in polar waters, particularly in areas entirely covered by sea-ice. Nevertheless, having had brief experience with such activities in ice-free water at Mawson in 1956 and having received some advice from Peckham (personal communication) for conditions in McMurdo Sound, it was decided that such an approach might be practicable. The equipment used was similar to that described by Fane[1].

Access to the water was obtained through holes in the sea-ice maintained under the floor of small field laboratories (62*B* and 62*E*), similar to but less elaborate than that described by Tressler[2]. The depth of water at the former station was 300 m, and at the latter 25 m. Both stations were off Cape Armitage on Ross Island. During the study the ice was 4–5 m thick, with an additional snow cover of variable thickness. The results in this article refer almost exclusively to 62*E*.

The appearance of blocks of sea-ice, upturned in the passage of ships, gives a most misleading picture of the habitat occupied by the ice flora. *In situ* inspection of the undisturbed ice shows that it consists of two distinct zones, a relatively thick upper layer which is dense and hard, and a lower layer, 0·5–1 m thick, which is easily disrupted and washed away when the integrity of the ice is disturbed. It is this layer which supports algal growth. Unlike the main mass of sea-ice, it consists of a relatively loosely aggregated matrix of large, plate-like ice crystals and a considerable proportion of unfrozen water. Although

the boundary between the matrix of crystals and the main water column is sharply defined and generally plane, there are many local irregularities. The lower surface is not smooth, but composed of more or less uniformly projecting ice crystals.

In such a system it is difficult to determine the precise disposition of the algal community. However, present field and laboratory observations indicate that many species in the flora grow attached to the surfaces of individual ice crystals, while others derive physical support from sessile forms or exist in more or less free suspension in the interstices within the ice matrix. Certainly, it is clear that the organisms are distributed throughout the loosely aggregated layer and are even present in large numbers in the lower few centimetres of the upper dense layer of sea-ice. In fact, it is this thin stratum that can be seen when sea-ice is broken and turned over by ships.

With the nature of the habitat unknown until the first dive was undertaken, and in the face of various practical difficulties, it was not feasible to design appropriate sampling equipment for use in the present study. Instead, it was decided to collect samples by the simple expedient of dislodging, manually, large quantities of ice crystals from beneath the sea-ice in the vicinity of the lower rim of the hole, recovering this material after allowing it to float to the surface. In spite of the obvious deficiencies of this procedure, it is felt that the results obtained at this early stage are of sufficient interest to justify their presentation.

Samples of ice crystals from Station 62*E* were collected at intervals during December 12, 1962–January 4, 1963. As a standard practice, samples were placed in clean plastic buckets and held at −1·5° C. Under these conditions, the ice did not alter state, while interstitial water soon gravitated to the bottom of the bucket. Samples of ice and seepage water were retained separately for the determination of chlorinity and *p*H.

The results of these determinations, together with parallel values for the water immediately below the sea-ice, are shown in Table 1. It has been assumed, for the present, that the water drained from collected ice crystals is representative of the interstitial water of the ice layer *in situ*. Whether or not this is entirely true remains to be verified. However, it may be seen that both the *p*H and chlorinity of this water is lower than that of the water immediately below the sea-ice and that the ice crystals, when melted, give *p*H and chlorinity values considerably lower than both the seepage water and the water below the ice. Thus, it seems likely that, between the surfaces of individual ice crystals and the interstitial water, there may exist appreciable gradients in physico-chemical conditions and in concentrations of various nutrient substances which, in turn, could exert a marked influence on the activities and distribution of the various components of the algal population. Further, the maintenance of equilibrium in the environment must lie in delicate balance, easily upset by quite small variations in temperature.

Table 1. *p*H AND CHLORINITY DATA FOR THE LOWER LAYER OF SEA-ICE AND THE WATER IMMEDIATELY BELOW THE ICE

	14.12.62		24.12.62		30.12.62		4.1.63	
	*p*H	Chlor.	*p*H	Chlor.	*p*H	Chlor.	*p*H	Chlor.
Melted ice	—	6·50	7·20	7·65	7·30	3·30	7·15	3·27
Seepage water	7·60	18·00	7·60	18·00	7·60	18·03	7·70	17·88
5 m water	7·65	19·33	7·70	19·34	7·80	19·32	7·85	19·29

Where sea-ice is re-formed annually, the ice habitat and its flora are, of course, ephemeral. In McMurdo Sound its disruption occurs some weeks in advance of the main break-out, and appears to be associated with sudden, active water movements and slight increases in temperature from around −1·7° C to approximately −1·4° C in the upper strata of the water column. In 1963, this process began about January 7 and was complete by January 13, when all traces of the laver of loose ice crystals and of the epontic community had been swept away.

Although it would have been desirable to collect data earlier in the season, the observations made during December 1962 and early in January 1963 provide a reasonably clear picture of the development of the ice flora, especially towards its peak phase. However, it should be emphasized that the concentrations of algal material reported here do not represent fully the magnitude of the flora. To obtain estimates of productivity, it was considered important to avoid the dilution of water samples with liquid derived from ice crystals. Consequently, cells firmly attached to ice surfaces were excluded from the aliquots of seepage water which were used in productivity studies as well as for pigment determinations and cell counts.

Rates of photosynthetic carbon fixation were measured with a carbon-14 technique described by Jitts[3], the incubation temperature in these studies being −1·5° C. The method of pigment analyses has been given by Humphrey[4]. Cell numbers were obtained by first passing water samples through a high-speed continuous centrifuge and counting aliquots in a Petroff–Hauser counter by fluorescence microscopy.

From Fig. 1, it may be seen that the numbers of chlorophyll-bearing cells, all diatoms, recovered in seepage water increased sharply from 4·5 × 10^6/l. on December 15, 1962, to 16·8 × 10^6/l. when the last samples were gathered on January 4, 1963. The numbers of diatoms in water samples recovered from immediately beneath the sea-ice during the same period were, by comparison, negligible. The remarkable amounts of chlorophyll *a* and *c* from the diatoms in the seepage water showed a pattern of increase very similar to the increase in cell numbers. The final concentration of chlorophyll *a* recorded was 156 mg/m³, and of chlorophyll *c*, 183 milli-specific-plankton units (MSPU)/m³. Although small values were obtained for chlorophyll *b* in several samples, these results should be treated with caution in view of the fact that this pigment is not known to occur in diatoms. Similarly, because of the doubtful reliability of the values for non-astacin

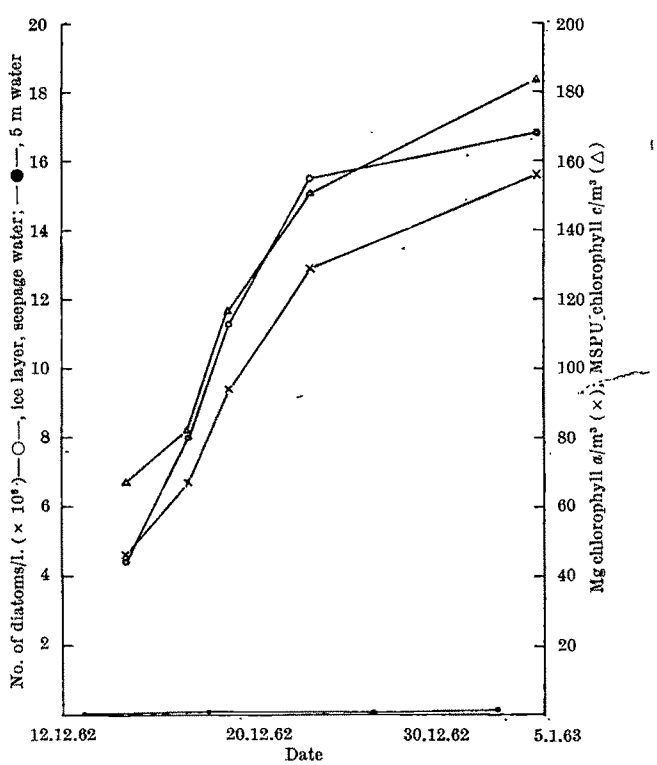


Fig. 1. Numbers of diatoms and concentrations of chlorophylls *a* and *c* in seepage water from the ice habitat at Station 62*E*. Numbers of diatoms in water below the sea-ice for comparison

[redacted]ids, the figures for this group of pigments have been presented.

At the time the peak pigment values were recorded in the seepage water, 617 mg chlorophyll a/m^3 and 543 MSPU chlorophyll c/m^3 were recovered from samples of melted ice crystals. Unfortunately, the ratio of ice crystals to interstitial water in the ice habitat was not then known. Several days later, however, some success was experienced with a hand-held van Dorn sampler during a dive at the Station 62*B*, when it was found that some 20 per cent by volume of the lower ice layer consisted of ice crystals. If this figure be taken as representative, then it may be calculated that, on January 4, 1963, there would have been approximately 248 mg chlorophyll *a* and 254 MSPU chlorophyll c/m^3 of ice layer. Although the photosynthetic activity of the organisms in the melted ice crystals was measured, the results have little meaning because the characteristics of the suspending fluid were so dissimilar to those of the interstitial water to which the cells normally would be exposed. Consequently, it is not at present known whether this component of the flora possesses an activity comparable with that displayed by the organisms in the seepage.

Since this flora was completely dispersed with the loss of the lower layers of the sea-ice soon after these observations were made, it may be assumed that these figures reflect, reasonably well, the total net production within this habitat for the 1962–63 summer season.

In the absence of data indicating the ratio between chlorophyll *a* and organic carbon in these populations, it would be unwise to apply an arbitrary factor for the expression of levels of plant growth in terms of carbon. None the less, it is clear that the production achieved under these extreme conditions is remarkably high and may well lie not far short of the maximum possible value suggested by Steemann Nielsen[5] for planktonic algae.

While the possible existence of heterotrophy cannot be excluded, there seems little doubt that the growth of the diatoms in the ice habitat results almost if not entirely from photosynthesis. In support of this contention is the observation that, at station 62*B*, where the light intensity at the depth of the lower ice layer was too low to be measured with a photocell capable of response at about 0·2 ft.-candles, very few diatoms were found. At Station 62*E*, however, present measurements indicate that the mean light intensity in the sampling zone was around 8 ft.-candles. Experiments to be described elsewhere have shown that, at this level of illumination, the diatoms of the ice flora are capable of roughly 20 per cent of the net photosynthetic activity realized at full light saturation and, therefore, possess extreme 'shade' characteristics. Further, observed rates of photosynthetic carbon fixation under conditions of weak illumination would give a pattern of increase in plant carbon very similar to that recorded for the chlorophyll pigments.

The fact that various species of diatoms are capable of reaching dense concentrations under such marked environmental limitations is not only of intrinsic interest but should also have particular relevance in the field of antarctic ecology. In this connexion, since McMurdo Sound lies so far to the south, it seems probable that the conditions there are rather more severe than those obtaining in similar situations farther to the north. Other things being equal, there seems no reason, therefore, why production, at least equivalent to that in McMurdo Sound, should not be found to occur wherever sea-ice persists into the summer season. With the information available, it is difficult to estimate the extent of sea-ice in antarctic waters at any time, although the data collated by Mackintosh and Herdman[6] suggest that perhaps 1–2 million square miles persist into mid-summer. On this basis, and assuming an average thickness for the ice habitat of 0·5 m, the production of the ice flora, in terms of chlorophyll *a*, could be of the order of $0{\cdot}3–0{\cdot}6 \times 10^6$ tons/year.

It is hoped that the findings of this investigation will lead to more extensive surveys using specially developed techniques so that the importance of primary production in the sea-ice habitat may be investigated with the thoroughness it would appear to deserve.

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BEHAVIOUR OF ACTIVE BACTERIAL ANTIGENS DURING THE INDUCTION OF THE IMMUNE RESPONSE

(I) Properties of Flagellar Antigens from *Salmonella*

THOUGH much is known about the cellular basis of antibody formation[1], the detailed behaviour and role of antigen during the induction of an immune response are still little understood. Sabin[2] first showed that dye-protein antigens were taken up by phagocytic mononuclear cells, and investigation of the function of such cells, now usually termed macrophages, in immunity has ensued[3–10]. Both particulate and soluble antigens enter these cells[2–5], and may persist in them for many months[6]. It has been claimed that antigen in macrophages causes prolonged antibody formation and immunological memory[7,8]; alternatively, it may cause the formation of a ribonuclease-sensitive factor capable of inducing antibody formation in normal lymphoid cells[9,10]. Actually we do not know whether antigen leaves the macrophages and enters lymphoid cells capable of becoming antibody-formers. Two autoradiographic investigations[11,12] have demonstrated antigen in or on antibody-forming plasma cells, but the data do not show whether the antigen entered these cells and induced antibody formation within them or whether it merely became associated with their surface after antibody formation had already begun.

We have been struck by the fact that, in most of the foregoing experiments, milligram quantities of antigens have been used. As previous experiments from our laboratory[13] have shown that 0·01 μg of *Salmonella* antigens could cause antibody formation in rats within 3 days, we wondered how much of the antigen detected by previous workers was directly relevant to antibody production and how much to the disposal of excess antigen. To evaluate critically the role of antigen during the inductive phase we felt that: (1) the antigen to be used should be as pure and as well characterized as possible; (2) the antigen should be a 'powerful' one, so that very small quantities would induce a specific immune response; (3) the organ to be investigated should be a major site of antibody production; (4) the methods used for the detection of

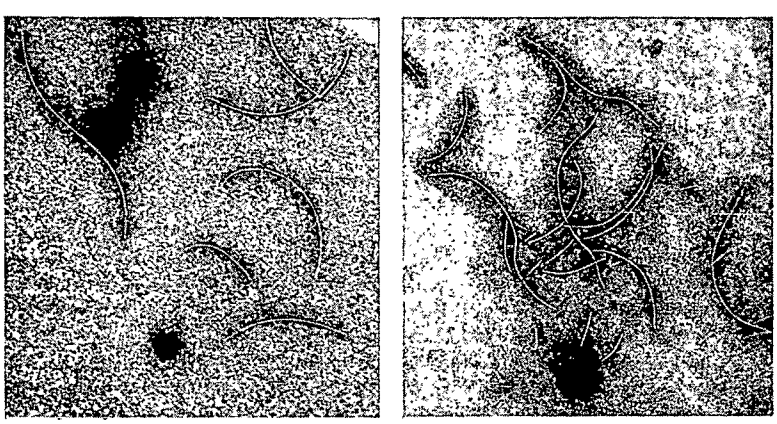
antigen should be sufficiently sensitive to allow demonstration of but a few molecules or particles of antigen.

In this communication we wish to describe investigations of antigen distribution in a system meeting most of these criteria. The antigens used were various flagellar preparations from *Salmonella adelaide* bacteria, namely the intact, isolated flagella; the low molecular weight protein, flagellin, prepared by acid treatment of the flagella[14]; and a polymer prepared from flagellin by treatment with concentrated ammonium sulphate solutions. Despite the fact that iodine-131 does not give particularly good localization in autoradiographs, we chose this isotope to label our antigens. Iodine-131 has a short half-life (8·03 days), so that small quantities can be detected by autoradiography without inordinately long exposure times. Carrier-free material is available also, and trace-labelling methods have recently been devised[15,16] which allow efficient substitution without significant denaturation of proteins. The organ chiefly investigated has been the rat popliteal lymph node, where antibody-forming cells can readily be detected during a primary response to these antigens[13].

Preparation and characterization of antigens. Flagella, the first antigen, were prepared in relatively pure form by slight modifications of Koffler's[14] techniques. The method involves shearing the flagella off the bacterial bodies, and then purifying them by differential centrifugation and extensive washings. The final preparation contained no bacterial bodies when examined in the electron microscope and only trace amounts of soluble protein. Acid treatment of flagella yields a low molecular weight protein, flagellin. Flagella were brought to *p*H 1·5 with hydrochloric acid, held at room temperature for 30 min, and centrifuged at 93,000*g* for 60 min to remove a small amount of acid-insoluble material. The supernatant was neutralized and filtered through a sterilizing pad. This preparation, when chromatographed on hydroxyl apatite, shows a single protein peak. Flagellin may be polymerized by increasing the salt concentration. Flagellin solutions are brought to 60 per cent saturation with ammonium sulphate[14], held at 0° C for 16 h and centrifuged at 15,000*g* for 15 min to deposit a substantial precipitate. This pellet was dissolved in, and dialysed against, distilled water, the final solution representing polymer which we used as the second antigen. The third antigen was monomer (flagellin), which was prepared by acid treatment (as already described) of polymer. Such preparations of monomer could be repolymerized by ammonium sulphate precipitation. When used as an antigen, monomer was injected as soon as possible after preparation.

Flagella, polymer and monomer were examined for their physical and serological properties. The first feature of note is that when monomer is polymerized by ammonium sulphate fractionation, rod-shaped structures similar to the original flagella result. Figs. 1*a* and *b* are electron micrographs of flagella and polymer, stained by phosphotungstate. Both flagella and polymer preparations consist of straight or curved rods of varying lengths; more concentrated preparations of monomer examined in this way contained no such elements. Gradient density ultracentrifugation has shown that flagella and polymer are polydisperse to about the same extent. In the analytical ultracentrifuge, monomer at *p*H 1·5 (0·05 hydrochloric acid) and at *p*H 7·3 (0·1 M sodium chloride, 0·01 M phosphate buffer) showed a single peak with sedimentation constants of 1·91 and 2·25 respe[illegible]. If a molecular weight of about 30,000 for mono[illegible] and a structure for flagella and polymer similar to tha[illegible] described by Kerridge *et al.*[17] are assumed, then strands of flagella or polymer in which the ratio of length to thickness is 25 : 1, the average length found in some of our preparations, would contain about 300 sub-units. One μg of flagella or polymer would thus consist of about 1×10^{11} particles, and 1 μg of monomer, of about 3×10^{13} molecules.

The serological properties of our three antigens were examined in several ways. Rabbits were hyperimmunized with each antigen, and the resulting sera all had high and roughly equal titres of anti-flagellar antibody as measured by a serial two-fold dilution method involving bacterial immobilization[18]. Moreover, each antigen was effective in neutralizing antiflagellar antibody prepared against any of the three antigens. On a weight basis, flagella and polymer were better inhibitors than monomer, but the differences were slight. Iodination at the levels used in these experiments had no significant effect on the inhibiting capacity of the three antigens. Precipitin tests confirmed this basic similarity between the different antigens. Gel-diffusion showed one main common antigen and a second antigen present in small amounts. Immunoelectrophoresis showed one main antigen with sometimes evidence of a minor antigen. Thus the three antigens, and their iodinated counterparts, appear to consist mainly of a single antigen, present as a low molecular weight protein in monomer; as an organized structure held together by non-covalent forces in the case of polymer;



a *b*

Fig. 1. Electron micrographs of flagella and polymerized flagellin (polymer) from *Salmonella adelaide*. Samples were mixed with phosphotungstate and latex spheres (diameter 88 mμ). Concentrated preparations of monomer showed no recognizable structures. *a*, Flagella, *b*, Polymer (both *c.* × 9,200)

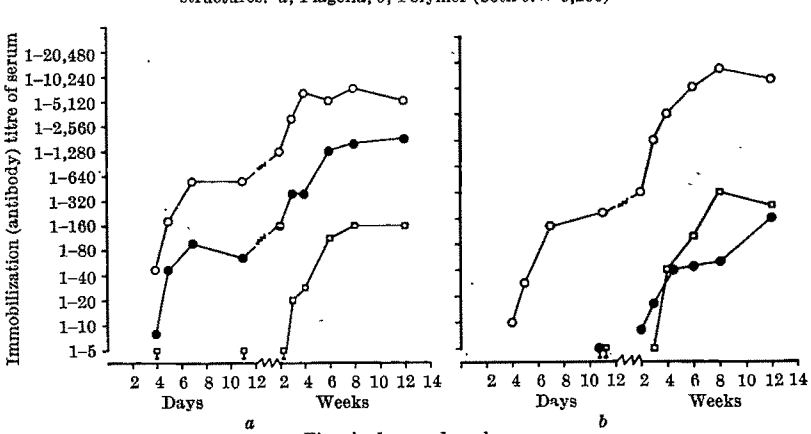


Fig. 2. The immune response of rats following injection of flagella, polymer or monomer. (*a*) 10 μg injected; (*b*) 100 ng (0·1 μg) injected. ○, Flagella; ●, polymer; □, monomer

and as an intact rod-shaped organ which also contains some acid-insoluble material in the case of flagella.

Iodination of antigens. Antigens were iodinated with carrier-free iodine-131 by slight modifications of the direct oxidation technique[16]. Monomer was more readily iodinated than were flagella. The highest specific activity achieved without alteration of antigenic specificity was 125 μc./μg of antigen, or about one atom of iodine-131 to every two molecules of monomer. This specific activity is considerably higher than that used by previous workers in this field, so that the fate of as little as 0·01 μg injected into the rat could be investigated.

Immunogenicity of the antigens. The three preparations, although consisting basically of the one antigen, showed marked differences in their capacity to induce the formation of antiflagellar antibody in adult rats. Amounts of antigens injected will be described in terms of milligrams, (mg, 10^{-3} g), micrograms (μg, 10^{-6} g), nanograms (ng, 10^{-9} g) and picograms (pg, 10^{-12} g). Rats, in groups of three, were given a primary antigenic stimulus into the hind foot-pads, the dose varying from 100 pg to 10 mg, a 10^{8}-fold range. Blood samples were taken at frequent intervals from 3 to 84 days after immunization. Serum samples were titrated for antiflagellar antibody using the immobilization method[18], both before and after treatment with 0·1 M mercaptoethanol (37° C, 1 h). This allowed us to determine the titres, respectively, of total antibody and mercaptoethanol resistant antibody. We presume, but have not proven, that the mercaptoethanol-sensitive antibody represents predominantly 19*S* macroglobulin, and the mercaptoethanol resistant antibody predominantly 7*S* γ-globulin. Figs. 2*a* and *b* illustrate the response of rats to, respectively, 10 μg and 100 ng of each antigen. The chief features of interest derived from these curves, and from examination of the data from the other six dosage-levels which will be reported in full elsewhere, are as follows:

(1) Flagella are more immunogenic than polymer, and polymer more immunogenic than monomer.

(2) These differences are much more striking during the first week after immunization than afterwards. Nearly all the antibody formed by our rats in the first week was mercaptoethanol sensitive ('19*S*'), and most of the antibody formed after 11 days, mercaptoethanol resistant ('7*S*'). Flagella were 100,000 times as powerful as monomer, and 100 times as powerful as polymer, in causing a '19*S*' primary response. By contrast, flagella were 100–1,000-fold as effective as polymer in causing a '7*S*' primary response, monomer and polymer showing little difference from each other.

(3) Remarkably low doses of antigen led to high 7*S* antibody titres. With flagella, 1 ng consistently and 100 pg occasionally caused extensive antibody formation, though peak titres were achieved only after some weeks. This finding confirmed the earlier work of Fitch and Winebright[19]. Increasing the dose above 1 μg had little effect on the immune response.

(4) With all 3 antigens, peak titres were maintained for many weeks.

One possible reason for the greater immunogenicity of flagella is that these preparations are more heavily contaminated with traces of somatic *O* antigen, which has known adjuvant properties. Surprisingly, however, we could demonstrate no significant difference in *O*-immunogenicity between the three preparations. The persistence of traces of *O* antigen in extensively purified monomer remains a puzzling feature.

The distribution of these antigens in the host animal will be discussed in Part II of this communication. The chief conclusions arising from both Parts I and II will be summarized at the end of Part II.

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(II) Cellular Distribution of Flagellar Antigens labelled with Iodine-131

In Part I of this article, the preparation and properties of antigens from *Salmonella* flagella have been described. These were labelled with carrier-free iodine-131; this section describes their cellular distribution in the rat following injection via the hind foot-pads.

Distribution and persistence of antigens in various organs. Labelled antigens were injected into the hind foot-pads of rats in doses of 70 ng–10 μg. Rats were killed at intervals of 5 min–72 days after injection. Lymph nodes (popliteal, aortic, inguinal and mesenteric), thymus, liver, spleen and thyroid were removed and weighed, and serum samples were prepared. Radioactivity was determined by counting γ-rays with a sodium iodide crystal. Fig. 3 shows the specific activities of the popliteal and aortic lymph nodes, liver, and spleen in a representative experiment where 10 μg of labelled flagella (170 μc., iodine-131) was injected. Other organs, with the exception of thyroid, showed minimal labelling. It can be seen that the radioactivity, per wet weight of tissue, is far higher in the popliteal node than elsewhere. The next lymph node draining the injection site, namely the aortic node, showed significant, though considerably less, labelling. Specific activities in liver and spleen were less than 1 per cent of those achieved in popliteal nodes. In all these organs, there was a marked tendency of radioactivity to persist for long periods. Fig. 4 is a composite representation of several experiments to illustrate the differing degrees of persistence of different labelled proteins in popliteal nodes. The proteins used were: 2 different doses (10 μg and 70 ng) of flagella; 2 doses (10 μg and 100 ng) of monomer; and 1 dose (10 μg) of the virtually non-antigenic protein, homologous rat serum albumin. Results are expressed as the percentage of the injected radioactivity found in the popliteal nodes at various times after injection. The most antigenic material, flagella, was trapped to the greatest extent in the popliteal nodes. It also persisted most markedly, the biological half-life in the nodes being 3 weeks. Monomer, a less-efficient antigen, was trapped and retained less efficiently, the half-life in the nodes being 1 week. Rat serum albumin was poorly trapped by the nodes, and also had a short half-life. Despite a 150-fold variation in total dosage, the percentage of the injected antigen retained in the nodes is remarkably constant from animal to animal and from experiment to experiment.

Cellular localization of labelled antigens in popliteal lymph nodes. Autoradiographs of sections, and occasionally cell smears, from the various organs were prepared

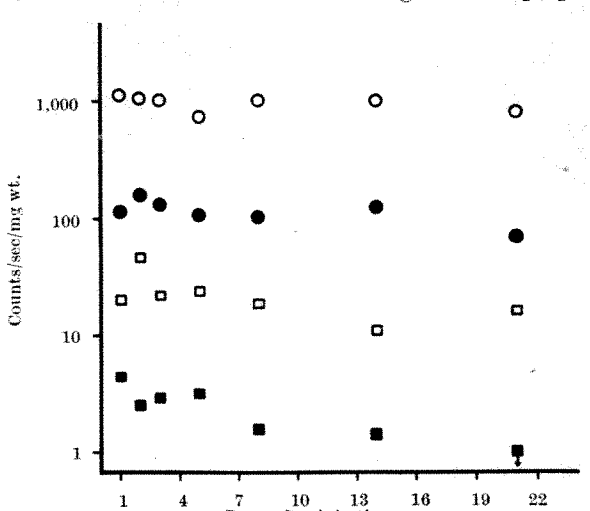


Fig. 3. Specific activity (counts/sec/mg wet weight of tissue) of various organs of the rat after injection via the hind foot-pad of labelled flagella (10 μg protein, 170 μc. iodine-131). ○, Popliteal lymph nodes; ●, aortic lymph nodes; □, spleen; ■, liver

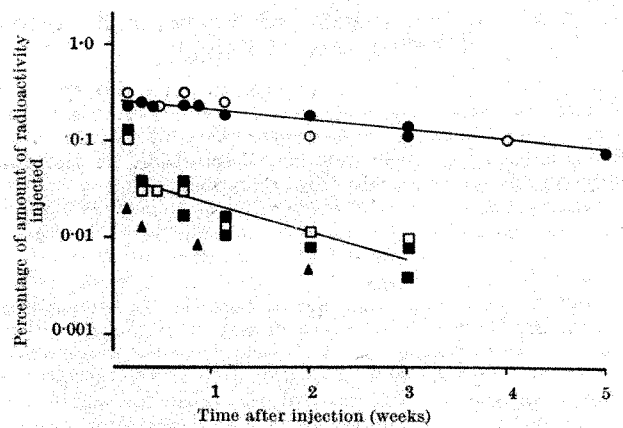


Fig. 4. Persistence of different labelled proteins in the popliteal node. ●, Flagella, 10 μg protein, 170 μc. iodine-131; ○, flagella, 70 ng protein, 8·3 μc. iodine-131; □, monomer, 10 μg protein, 112 μc. iodine-131; ■, monomer, 100 ng protein, 15 μc. iodine-131; ▲, rat serum albumin, 10 μg protein, 120 μc. iodine-131. Regression lines fitted according to the method of least squares

using Pelc's[20] technique and Kodak '*AR* 10' stripping film. Chief attention was focused on the popliteal nodes. The key findings of this part of the investigation were as follows:

(1) Macrophages took up and retained large quantities of antigen.

(2) Lymphoid follicles became heavily labelled with antigenic proteins but not with rat serum albumin. Many of these follicles retained label avidly, and developed into typical germinal centres.

(3) Plasma cells formed as a result of the injection of the labelled antigens showed a remarkable paucity of labelling.

Fig. 5 is a tracing of a section through a normal unstimulated rat popliteal lymph node, somewhat schematized. Lymph enters via the peripheral circular lymph sinus, 'percolates' through the cortex, enters the medullary areas and leaves via the efferent vessels. The cortex contains much diffuse lymphoid tissue, but also discernible rounded collections of small lymphocytes, without detectable germinal centres, the lymphoid follicles. The cortex contains only occasional scattered macrophages, but the layer of reticular cells forming the inner wall of the circular sinus appears to have phagocytic properties (*vide infra*). The medulla of normal nodes can be arbitrarily divided into 'dense' and 'loose' areas. The dense areas contain large numbers of macrophages and mast cells. The looser areas contain the lymph sinuses, scattered macrophages and mast cells and a very small number of plasma cells. In this area, particularly adjacent to the diffuse cortical tissue, plasma cells develop during the immune response.

Five min after injection of iodinated flagella, heavily labelled macrophages can be seen in the medulla. At 30 min, this labelling is more intense and diffuse, and specific labelling can also be seen in the littoral phagocytes of the circular sinus. By 2 h the first traces of label have

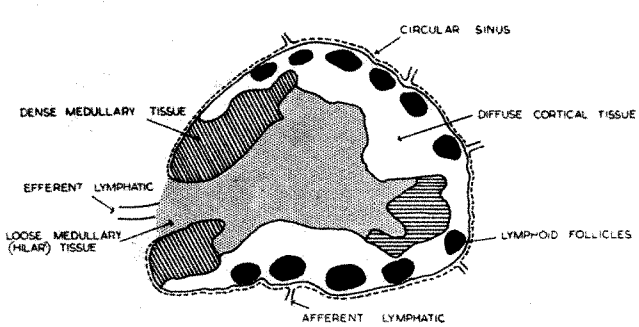


Fig. 5. Schematic diagram of a section through the rat popliteal node

entered the lymphoid follicles, though this localization is much more striking after 24 h when all the lymphoid follicles of the node are heavily and specifically labelled. Fig. 6 is a low-power view of a popliteal node section 24 h after the injection of 10 μg (170 μc. iodine-131) of labelled flagella. The labelling pattern over the follicles is not uniform, but frequently reticulated or variegated, suggesting that the label may be between rather than in the lymphocytes. Smears in which the cells are separated from each other show that most of the label is not in or on the lymphoid cells. We postulate, though without firm evidence, that this label is in cytoplasmic extensions (dendritic processes) of specialized macrophages pre-existent in the lymphoid follicle. At this, as at all subsequent times, the diffuse cortical lymphoid tissue is virtually unlabelled.

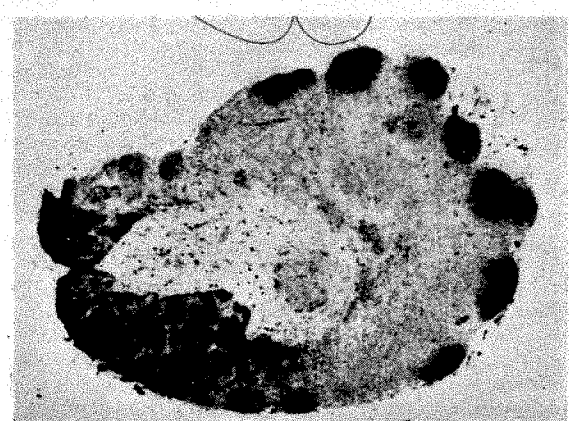


Fig. 6. Autoradiograph of popliteal lymph node section 24 h after injection of 10 μg of labelled flagella (170 μc. iodine-131). Note heavily labelled lymphoid follicles and medulla (×*c.* 51)

By 2 days, the diffuse cortical tissue showed increased cellularity, with an increase in the number of scattered but unlabelled blast cells. By 3 days, accumulations of primitive cells could be seen lying immediately deep to the labelled follicles, and frequently appeared to compress the originally rounded collection of grains into a crescentic shape. By 5 days these primitive cells had formed a typical young germinal centre (Fig. 7). The appearances suggested that germinal centres arose by invasion of pre-existent labelled follicles by blast cells arising in the diffuse cortical tissue, and not, as had generally been believed, by division of primitive cells in the centre of the pre-existent follicle. Possibly many of these blasts migrate to the medulla. At 5 days, extensive sheets of plasma cells, many of them still immature, could be seen in the medulla of the nodes and most of these had become typical mature plasma cells by 8 days. Figs. 8*a* and *b* show two areas in the medulla of a single lymph node taken 8 days after the injection of 70 ng (8·3 μc. iodine-131) of labelled flagella. The areas were about 1 mm apart in the section. The extremely heavy labelling of the macrophages (Fig. 8*a*) contrasts strongly with the virtual absence of label over the antibody-forming plasma cells (Fig. 8*b*). Cell smears confirmed that most plasma cells showed only background labelling.

Over the next few weeks, the general cellularity of the nodes decreased somewhat, but labelled lymphoid follicles, many of them now showing prominent germinal centres, remained an outstanding feature. Medullary cords with only slightly diminished numbers of mainly unlabelled plasma cells could be seen throughout. Macrophages remained heavily labelled, and 4 weeks after antigenic stimulation, many mast cells also showed specific, heavy labelling.

The sequence of events following the injection of labelled monomer differed in a number of important respects. Though extensive phagocytosis by macrophages also

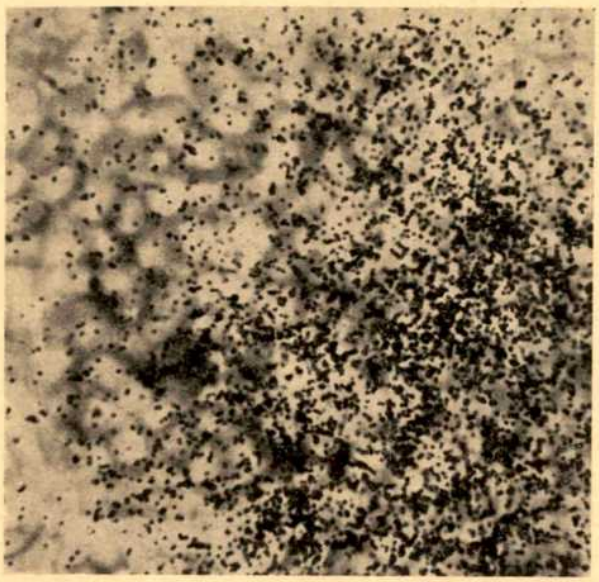


Fig. 7. Autoradiograph of popliteal lymph node section 5 days after injection of 70 ng of labelled flagella (8·3 μc. iodine-131). Note heavily labelled lymphoid follicle (right) and lightly labelled germinal centre (left). (×1,000)

occurred rapidly the low-molecular weight antigen diffused more extensively throughout the cortical tissue. Specific labelling of lymphoid follicles occurred later, was less marked, and more variable. Plasma cells appeared much later (10–14 days), and macrophage labelling decreased more rapidly. Germinal centres were prominent at 14 days and remained so for some weeks.

Labelled homologous rat serum albumin (9 μg protein, 120 μc. iodine-131) was detected in large amounts in the node 1 h after injection; the label was scattered diffusely throughout the node but tended to be heavier in medullary areas. Within 24 h the amount of label decreased rapidly, only small quantities being retained by macrophages. At no stage did selective localization occur in the lymphoid follicles.

To examine quantitatively the presence of labelled antigen in the lymphoid follicles and medullary macrophages, visual grain counting was performed. As labelling of lymphoid follicles was somewhat variable, especially with small doses of antigen, it was decided to count a random area of each follicle seen in a section, using an eye-piece graticule. By contrast, as the medullary labelling was usually very uneven, it was felt that a subjective element in selecting medullary areas for counting could not be avoided. Thus 2 medullary areas, from each section, each considered to have a median degree of labelling, were selected for grain counting. The results in the case of an experiment using 70 ng of labelled flagella are shown in Table 1. Counts are expressed per 1,000 μ^2, an area usually comprising about 40 small lymphocytes or 15 medullary macrophages. Within 2 h after antigen injection, medullary labelling appears to reach a peak while localization in lymphoid follicles is barely detectable. After this period, the amount of label in the medulla appears to stay relatively constant whereas label in the follicles continues to rise for a day or more. Between 8 and 28 days, labelling in both areas fell slowly.

The autoradiographic results are consistent with the following picture. Two sorts of macrophages exist in the lymph node. Medullary macrophages take up all proteins

Table 1

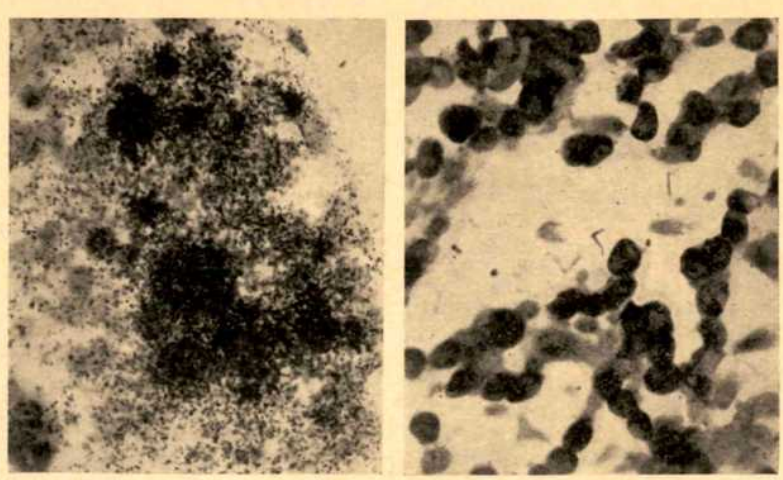
Time killed after injection of antigen	Mean grain count of follicles	Mean grain count of medulla
5 min	2	38
30 min	2	182
2 h	24	168
4 h	38	149
24 h	42	166
3 day	73	186
5 day	63	230
8 day	62	180
14 day	32	124
28 day	46	185

Grain counts from autoradiographs of sections of popliteal nodes from rats at various times after injection of 70 ng of labelled flagella (8·3 μc. iodine-131). All counts refer to areas of 1,000 μ^2. The follicle counts represent the mean values of all follicles in one randomly chosen section from each node. The medulla counts represent the means of two areas with apparent average numbers of grains. Each count was adjusted to allow for decay of isotope. The adjusted counts over diffuse cortex were background level (about 5 grains).

used but retain avidly only the bacterial antigens. More specialized, dendritic macrophages in the lymphoid follicles take up only the bacterial antigens and thus appear to exhibit some capacity to recognize 'foreignness'. The presence of antigen in the node somehow causes the appearance of blast cells in the diffuse cortical tissue. Some of these collect immediately deep to the antigenic depot in the lymphoid follicle and form a germinal centre. Meanwhile, primitive plasmablasts appear in the medullary cords and in time develop into antibody-forming cells. It is tempting to think that germinal centre formation in the cortex and plasma cell formation in the medulla are related in some way, particularly during the period of '7*S*' antibody formation, but evidence for this is lacking. The plasma cells formed as a result of antigenic stimulation are largely unlabelled. From the dose of antigen given and the distribution of label in the node, it can be calculated that antibody-forming cells contain a smaller (and possibly much smaller) number of antigen-derived amino-acid residues than there are ribosomes in the cell. This suggests that antibody formation is not due to a direct template mechanism. Immune induction may involve a transfer of some material from macrophages to primitive lymphoid cells, which have perhaps been rendered more reactive in some way. This material might be minimal quantities of antigen acting as an inducer or a nucleic acid fraction.

Our findings from Parts I and II may be summarized as follows:

(1) We have described an antigen which exists in three different physical states: as a monomer, as an organized polymer, and as the native, bacterial flagellum. Though the three preparations show little difference in their *in vitro* immunoligical behaviour, they differ markedly in



a *b*

Fig. 8. Autoradiographs of two adjacent areas in the medulla of the same popliteal lymph node section, 8 days after the injection of 70 ng of labelled flagella (8·3 μc. iodine-131). *a*, Shows heavily labelled macrophages; *b*, shows absence of label in plasma cells

their capacity to cause antibody formation, particularly '19*S*' antibody formation, *in vivo*.

(2) As 100 ng of flagella is virtually a maximal primary antigenic stimulus, the use of larger amounts introduces the danger of obscuring the basic problems of immune induction.

(3) This consideration makes it essential to use, as a label, a carrier-free isotope of short half-life.

(4) Although the popliteal node traps less than 1 per cent of antigen injected into the foot-pad, the specific activity of this organ is 100 times higher than that of organs such as liver and spleen, which were commonly used in this type of investigation by previous workers. With critically small doses of antigen, the cellular distribution of labelled material is thus best investigated in the popliteal node.

(5) Flagella are trapped and retained by the popliteal nodes to a greater extent than is monomer.

(6) Antigens (flagella and monomer) are primarily localized in macrophages and lymphoid follicles. A minimally antigenic protein, such as homologous serum albumin, shows slight localization in macrophages but none in lymphoid follicles.

(7) Plasma cells resulting from antigenic stimulation show little or no labelling.

We feel that the system here described will prove to be valuable in quantitative investigations on the role of antigen in various immunological events. The work reported will be published in detail elsewhere. Present investigations are also directed towards the role of the antigen in immunological memory, in immunological tolerance and the general problem of neonatal incompetence and in immunological paralysis.

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A NEW REPEAT STRUCTURAL ELEMENT OF MITOCHONDRIAL AND CERTAIN CYTOPLASMIC MEMBRANES

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THE mitochondrial outer surface membrane as well as inner membranes are paired structures with two membrane elements forming the outer membrane as well as each inner membrane. Each constituent membrane element appears triple-layered after potassium permanganate fixation, as shown by Robertson[1], who has included the mitochondrial membrane elements as part of his unit membrane system.

The same triple-layered unit membrane structural pattern has been observed in the α-cytomembranes ("rough-surfaced membranes") and the Golgi membranes ("smooth-surfaced membranes").

The triple-layered structure of the unit membranes has been assumed to reflect their basic structural feature, a bimolecular leaflet of lipids sandwiched between two thin layers of protein (or of non-lipid material), according to the membrane model by Danielli and Davson[2], Robertson[3], and myself[4,5].

At the Gordon Research Conference on "Structure and Function of Multilayer Systems in Cells", June 1962, as well as at the International Electron Microscopy Congress in Philadelphia, August 1962, I presented material indicating a more complex membrane structure in mitochondria and in certain cytoplasmic membranes. After osmium fixation and section staining, each membrane element appears triple-layered, but a considerable amount of the material in the middle light layer is stained. The total thickness of the triple-layered membrane element is about 50 Å, corresponding to the thickness of the single osmiophilic layer observed in the classical osmium-fixed material that had not been section-stained.

After potassium permanganate fixation, the mitochondrial membrane elements show a more complex structural pattern with opaque layers at the two surfaces of each membrane element and opaque cross-bridges connecting these surface layers (Fig. 1). The membrane elements appear to consist of minute globular components with a diameter of about 50 Å. The patterns observed both in cross-sections and tangential sections through the membrane elements could be interpreted on the same basic assumption that the membrane represented a two-dimensional aggregate of globular particles.

Smooth-surfaced cytoplasmic membranes in the tubular cells of the kidney show the same structural pattern as the mitochondrial membrane elements (Fig. 1). However, the total thickness of these membranes is distinctly greater (60–70 Å) than that of the mitochondrial membrane elements as is obvious from the direct comparison of the dimensions which can be made on pictures where they are located side by side.

The plasma membrane appears geometrically asymmetric, triple-layered[6–8] after section staining of osmium-fixed and mostly also of potassium permanganate-fixed tissue. The stained cytoplasmic layer is thicker and frequently also more intensely stained than the peripheral opaque layer.

In the material discussed here, the plasma membrane in the potassium permanganate-fixed material appears triple-layered without any such sub-structure observed in the mitochondrial and cytoplasmic membranes. The total thickness of the plasma membrane (90 Å) furthermore exceeds that of both the mitochondrial membrane element and the cytoplasmic membranes (Fig. 2).

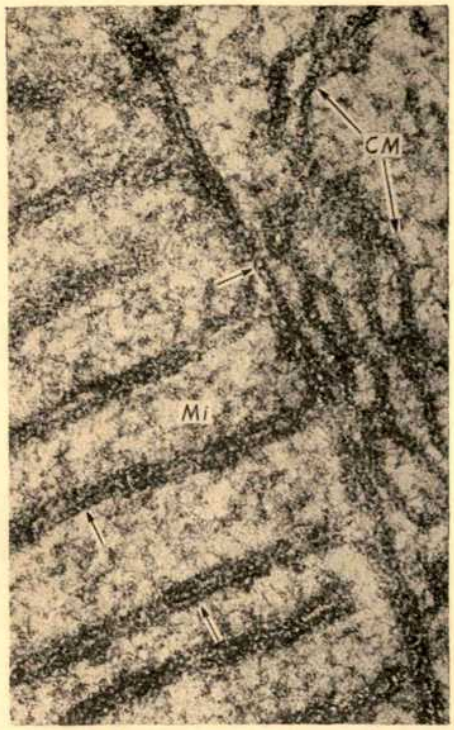


Fig. 1. Part of mitochondrion (*Mi*) and some cytoplasmic membranes (*CM*) in potassium permanganate-fixed mouse kidney. Arrows point to regions where membrane elements are favourably oriented to reveal the globular substructure. (×200,000)

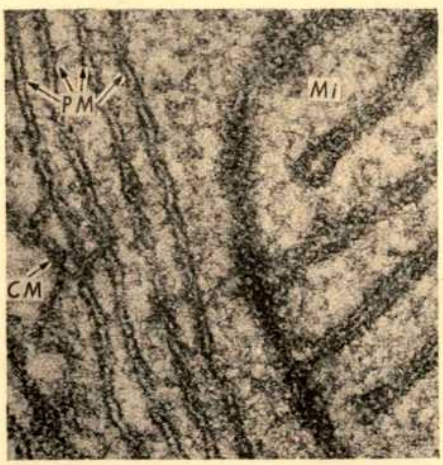


Fig. 2. Part of mitochondrion (*Mi*), some cross-sectioned plasma membrane (*PM*), and some cytoplasmic membranes (*CM*). Notice the difference in dimensions between mitochondrial membrane elements and the plasma membrane. The plasma membrane appears symmetrical owing to incomplete staining. In spite of this the difference in dimensions is clearly shown. (×200,000)

Recent observations made on frozen-dried pancreas tissue[9] support the conclusion that certain types of membranes are characterized by a globular substructure.

The cytoplasm of the exocrine pancreas cells is filled with α-cytomembranes between mitochondria, Golgi regions, and in the apical cell region, zymogen granules. Any section through an exocrine pancreas cell, therefore, will cut through a large number of cytoplasmic membranes at different orientations—transversally, obliquely or tangentially. This simple cytology of the exocrine cells is very helpful when trying to identify structural components in frozen-dried material with its low contrast and with contrast patterns which are different from the conventional ones observed after chemical fixation.

In cross-sections at low magnification the various membranes appear as a light line according to the description by Sjöstrand and Baker[10]. In the mitochondria each membrane element is represented by one such light line with a thickness of 40–50 Å. In areas in the cytoplasm where such light lines are absent, the membranes must be tilted in relation to the plane of the section since no parts of the cytoplasm of these cells are lacking membranes. In such areas where the membranes must be oriented more or less parallel to the plane of the section a very regular crystalline-like pattern can be observed (Figs. 3 and 4). This pattern seems to consist of regularly arranged rows of lightly stained or unstained particles, the individual particles being separated by more intensely stained material. These rows alternate with rows of intensely stained spots which range in diameter between 10 and 20 Å. The separation between the centres of the parallel rows of light particles is 45 Å.

Since the dimensions of the globular substructure are similar in both potassium permanganate-fixed and in the

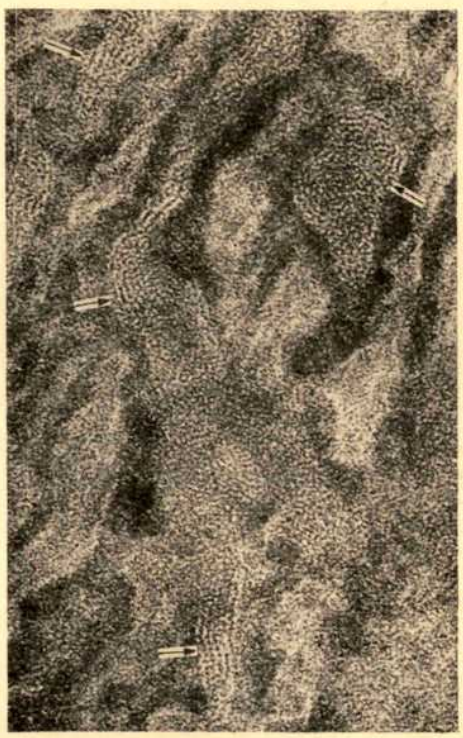


Fig. 3. α-cytomembranes in exocrine cell of the mouse pancreas fixed by means of freeze drying. The orientation of the membranes in relation to the plane of the section varies considerably. When the membranes have been sectioned obliquely a regular pattern of globular sub-units arranged in rows can be seen (arrows). (×200,000)

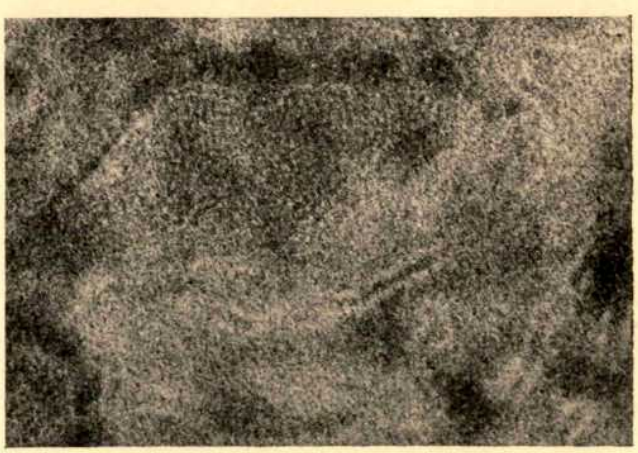


Fig. 4. Close-to-focus picture of mitochondrion in frozen dried exocrine pancreas cell. Some mitochondrial membranes are oriented perpendicular to the plane of the sections. In the upper part of the picture mitochondrial membrane oriented parallel to the plane of the section showing a granular pattern. (×300,000)

frozen-dried material, and since the dimensions correspond to the total thickness of these membranes, it is assumed that both methods of preparation reveal the same structural components. In frozen-dried material the arrangement of globular sub-units is frequently strikingly regular. This regularity has not been observed in potassium permanganate-fixed material. We would, however, expect a higher probability for distortion of such a pattern in connexion with chemical fixation than during freeze-drying.

The fact that a similar sub-structure can be observed in both permanganate-fixed and frozen-dried material makes it justifiable to discuss this structural pattern as possibly a real pattern reflecting the structure of these membranes in the living cell.

Recently (at the Biophysical Society meeting in San Francisco), Robertson reported having observed a sub-structure showing a geometric regularity in the plasma membrane confined to certain special localized contact regions (presumably synaptic) in the central nervous system of the goldfish. The two plasma membranes in contact in those regions are closely packed. This pattern was not observed in other parts of the plasma membrane. He also observed a similar pattern in the isolated disk membranes from the outer segments of retinal rods. The pattern observed in the investigation reported here has not so far been observed in the plasma membrane.

This structural pattern is not related to the globular structure observed by Fernández-Morán[11], Parsons[12], and Stoeckenius[13] in fragmented negatively stained mitochondria. The globular components observed by that technique are of a different size (about 80 Å in diameter) and represent globular components associated with the surface of transformed and fragmented mitochondrial membrane material.

The interpretation of this structural pattern in terms of molecular architecture is difficult considering the uncertainties with respect to what appears stained in the patterns. Tentatively, it is proposed that the observed patterns reflect a regular arrangement of globular units or micelles consisting of lipids. The membrane would then contain as a basic structural component lipids in a globular form like a two-dimensional flocculated emulsion. Between the lipid globules protein molecules are arranged in rows and also extend between the rows, thereby delimiting the territories of the individual lipid globules. This protein structure would represent the stabilizing factor which would prevent the lipid globules from fusing to form either a bimolecular leaflet or large lipid droplets.

With respect to the mitochondrial membrane elements, it is tempting to propose that the components of the electron transport chain constitute at least part of the proteins of the membrane element, that they are kept in orderly array between the lipid droplets and, with respect to movement, are more or less restricted to rotational movement by this association.

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SOLUTE AND WATER ABSORPTION BY THE HUMAN SMALL INTESTINE

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INTESTINAL absorption was studied in normal subjects and patients with non-tropical sprue by transintestinal intubation[1]. Solutions pumped into the nasal end of the tube at 15 ml./min entered the gut through a proximal hole in the tube (Fig. 1). After perfusing the intestine the intraluminal contents are sampled through an opening 15 cm distal to the entry site by siphonage from the anal end of the tube. As the tube progresses distally, the entire small intestine is studied as segments of gut included between solution entry and sample exit holes 15 cm apart[2]. A steady-state is reached after 30 min of perfusion. Net transfer is measured by analysis of intraluminal contents. Transfer of a component is calculated from the difference between initial concentration in the solution perfused and final concentration in the sample of perfusate. Net fluid transferred is corrected for by the non-absorbed indicators polyethylene glycol (PEG) and phenol red. The relation of initial to final non-absorbed indicator concentration, $C_{initial}/C_{final}$, is the volume in the bowel lumen relative to the volume infused at the time the sample is obtained. During each investigation at least three successive 5–15 min samples are collected. Transfer rates during each of these periods are similar, in agreement with steady-state kinetics, and the data are means of rates of the periods. The bowel lumen is the point of reference. Addition to the luminal contents (secretion) is designated by numbers greater than 0, and subtraction from the bowel contents (absorption) by numbers less than 0.

* Holder of the Lederle Medical Faculty Award.

Sodium and potassium are analysed by internal standard flame photometry, chloride by amperometric titration, osmolality by freezing-point depression, glucose by glucose oxidase and PEG turbidimetrically[3]. Phenol red analysis and the preparation of Ringer's and glucose-Ringer's solutions have been described[2,4].

Transfer data are calculated as follows:

Net transfer, m.mole/l. =
(Initial conc., m.mole/l.) −
(final conc., m.mole/l.) $\left(\frac{C_{initial}}{C_{final}} \text{ of indicator}\right)$

Net transfer, m.mole/h. =
net transfer, m.mole/l. (vol. perfused, l./h)

Transfer data obtained during distilled water perfusion are given in Table 1. Secretion of sodium and chloride is observed in both normal subjects and the patient with non-tropical sprue. A consistent proximal to distal gradient is not observed in the normals. Secretion of sodium and chloride in a normal is lower in the colon than in the small intestine. Potassium secretion in both small intestine and colon is low, although colonic rates are slightly higher. In normal subjects, absorption of water is greater in the proximal small gut. In the patient with sprue, absorption of water is the same throughout the small gut, but secretion of sodium, chloride, potassium and total solute shows a slight distal increase. The sum of net transfers of sodium, chloride, and potassium

(Σ column) approximates net transfer of total solutes (mOSM column).

Table 1. DISTILLED WATER

Distance (cm. from nares)		Net transfer* m.mole/h Na⁺	Cl⁻	K⁺	Σ‡	mOSM/h	Volume change (%)† PEG	Phenol red
S.S., Normal control,								
140	male	28	—	1·4	57·4§	55	−38	—
230	,,	29	—	1·6	59·6§	61	−5	—
350	,,	17	—	1·0	35·0§	32	−9	—
E.M., Normal control,								
111	male	32	30	1·4	63·4	69	−13	−12
188	,,	45	41	1·8	87·8	94	1	5
200	,,	48	45	2·0	95·0	103	−1	3
320‖	,,	11	9	3·9	23·9	27	−1	−3
G.H., Non-tropical sprue								
59	male	15	—	0·7	30·7§	31	−5	—
109	,,	21	20	0·9	41·9	43	−5	—
344	,,	27	23	1·9	51·9	68	−5	—

* Net transfer is the resultant of secretion (entry into the gut) and absorption (exit from the gut) during steady-state perfusion of the indicated solutions. Secretion increases gut contents and is indicated by numbers greater than zero. Absorption decreases gut contents and is indicated by numbers less than zero.

† Calculated as 100 (indicator ratio 1:1), where PEG and phenol red are the indicators, and the ratio is that of initial to final indicator concentration.

‡ Algebraic sum of the transfer of Na^+, Cl^-, and K^+.

§ Sum is calculated assuming that the chloride transfer, which was not determined, is equal to the sodium transfer.

‖ Colon.

Small intestinal transfer data derived from perfusion of Ringer's solution are given in Table 2. Sodium concentration in Ringer's (approximately 145 m.equiv./l.) is essentially the same as in extracellular fluid (ECF). Chloride concentration (approximately 150 m.equiv./l.) provides a 45 mM. gradient for chloride absorption. In normal subjects, water, sodium, chloride and potassium are absorbed proximally and secreted distally. Total transfer rates are much lower than with distilled water (Table 1). In the patient with non-tropical sprue, water, sodium and chloride are secreted proximally and absorbed distally, that is, the net transfer relationship of proximal to distal small intestine is the reverse of normal in the absorption and secretion rates are great. The sum of normal subjects under these con[illegible] the net transfer net transfers of electrol[illegible] of total solutes.

[illegible] RINGER'S SOLUTION¶

[illegible] nares)		Net transfer* m.mole/h Na⁺	Cl⁻	K⁺	Σ‡	mOSM/h	Volume change (%)† PEG	Phenol red
P.O., Normal control,								
90	male	−8·5	−13·9	−0·1	−22·5	—	−5	−3
143	,,	−0·3	−6·5	−0·3	−7·1	−9·0	−3	—
184	,,	1·6	0·8	0·2	2·6	—	2	2
338	,,	1·0	0·4	0·3	1·7	5·6	2	1
387‖	,,	−8·7	—	3·0	−14·4§	−8·3	−2	—
E.M., Normal control,								
111	male	−3·9	−5·4	−0·1	−9·4	−5·7	−1	—
188	,,	0·2	0·7	0	0·9	4·6	2	—
G.H., Non-tropical sprue								
89	male	42·4	42·8	—	85·2	90	30	25
95	,,	39·0	47·0	—	87·0	92	33	31
249	,,	−9·1	−7·0	—	−16·1	−14·5	−5	−1
253	,,	−23·8	−18·7	—	−42·5	−31·7	−9	−6
385	,,	−11·3	−16·7	—	−28·0	−17·5	−4	−9

¶ See Table 1 for footnotes.

Transfer data from 1 per cent glucose-Ringer's solution in Table 3 show absorption throughout the small gut in normal subjects. (The changes in net potassium transfer are small, and there is a small distal secretion.) The gradient is marked: absorption of water, sodium, chloride, glucose, and total solute is very high proximally and progressively decreases distally. The concentrations of sodium and chloride are 27 mM lower in 1 per cent glucose-Ringer's than in Ringer's. This reduces correspondingly the gradient from bowel lumen to ECF for chloride absorption. Sodium concentrations are essentially equal between ECF and Ringer's (Table 2), but now have a 27 mM gradient for secretion from ECF to glucose-Ringer's in the bowel lumen. Despite reduction in the chloride and reversal of the sodium absorption gradients, absorption is much greater from 1 per cent glucose-Ringer's than from Ringer's, even though both are isotonic.

In contrast with the normal subjects, the patient with non-tropical sprue again shows high absorption distally. Distal absorption in sprue with glucose-Ringer's is as great as proximal in normal subjects. Secretion occurs in the proximal small gut of the patient with sprue even with glucose-Ringer's, although it is lacking throughout in normal subjects, except for a low distal potassium secretion.

Net transfer by the small intestine during perfusion with distilled water and Ringer's solution is the resultant of concentration gradients between bowel lumen contents and ECF. Thus, high rates of sodium and chloride and low rates of potassium secretion during distilled water perfusion parallel concentration gradients (Table 1). With Ringer's solution, normal subjects show little net transfer in agreement with minimal gradients (Table 2). The thermodynamic activity gradients of water and ions between ECF and Ringer's solution is minute by comparison with the ECF-distilled water gradient that promotes secretion of solute and absorption of water. The small gradient for chloride accounts for the slight absorption in the proximal gut. If the proximal small gut were more permeable than the distal, the proximal absorption of sodium and chloride would be explained. A low rate of distal secretion of sodium, chloride and potassium is observed.

Concentration gradients cannot explain absorption from 1 per cent glucose-Ringer's. Proximal sodium and chloride absorption is much greater than predicted from data with Ringer's. Despite the decrease in chloride gradient, chloride absorption increased 3–5-fold. Sodium absorption increased [illegible]-fold despite the change from Ringer's to a gradient for sodium secretion to no gradient glucose-Ringer's. Rates are remarkably high: one-third of the total solute and water are absorbed. The transported solutes comprise a little less than one-third of the total sodium and chloride and more than half the glucose perfused. The high sodium and chloride absorption is related to the presence of glucose, since it does not occur without glucose. Glucose, sodium and chloride are absorbed in equimolar proportions, and appear to be inextricably linked in their transport under the conditions of study.

Bulk solution other than that perfused could enter the perfusion zone either through secretion by the mucosa of this region or by entry from above or below. The final concentration of non-absorbed indicator would be lowered by these entering bowel fluids. Absorption of components of entering fluids, for example, water, sodium and chloride, would be underestimated. Thus, even during high absorption, if a slightly higher rate of secretion of such extraneous fluids were to occur, only a slight secretion would be detected. For this reason the terms 'net transfer', 'absorption', and 'secretion' do not indicate sources of transported materials, but refer merely to the resultant of the total process. Since isotopic data are not presented, fluxes are not relevant to this discussion.

During absorption of sodium, chloride and glucose in concert, bulk entry of solution other than that perfused would distort interrelationships. Such contaminating solutions would contain higher sodium concentrations than 1 per cent glucose-Ringer's and lack glucose. This would oppose the demonstration of simultaneous sodium and glucose transport: dilution of glucose would be detected by change in indicator concentration, glucose transport would be estimated reliably, but net sodium transport would be underestimated. Extraneous sodium could not be differentiated from that infused. Since simultaneous sodium and glucose transport is demonstrated, the role of this artefact must be considered.

The following argument supports the contention that bulk entry of extraneous solution influences data in normal subjects only minimally at the infusion rate of 15 ml./min.

Table 3. 1 Per Cent Glucose–Ringer's Solution¶

Distance (cm from nares)	Net transfer* m.mole/h Na+	Cl-	Glucose	K+	Σ‡	mOSM/h	Volume change (%)† PEG	Phenol red
P.O., Normal control,								
90 male	−33	−36	−37	−0·4	−106·4	—	−32	—
160 ,,	−32	—	−32	−0·5	−96·5§	−92	−29	—
160 ,,	−24	−26	−27	−0·3	−77·3	−76	−22	—
161 ,,	−26	−29	−30	−0·3	−85·3	−75	−24	—
195 ,,	− 6	− 9	−12	0·1	−26·9	—	− 6	—
238 ,,	− 2	− 5	− 9	0·1	−15·9	−13	− 3	—
273 ,,	− 3	—	−10	0·5	−15·5§	−22	− 3	—
338 ,,	− 6	− 9	−11	0·2	−25·8	−25	− 7	—
358 ,,	−14	−21	−14	0	−49·0	−33	−10	—
S.S, Normal control,								
140 male	−40	—	−34	—	−114·0§	−105	−35	—
230 ,,	− 3	—	− 6	0·2	−11·8§	−15	− 3	—
B.J., Normal control,								
86 female	−18	—	−29	−0·2	−65·2§	−64	−20	−18
135 ,,	− 7	—	−14	0·3	−28·7§	−27	− 6	− 8
234 ,,	− 1	—	−13	—	−11·0§	−14	− 2	—
238 ,,	−11	—	−12	—	−33·0§	−25	− 6**	− 5**
G.H., Non-tropical sprue								
103 male	25	24	− 7	—	42·0	42	14	—
105 ,,	19	18	− 5	2·5	34·5	41	13	8
113 ,,	9	8	−15	—	2·0	6	3	—
219 ,,	−25	−25	−35	−0·1	−84·9	−81	−27	−26
255 ,,	−21	−24	−36	—	−81·0	−83	−29	—
258 ,,	−23	−24	−31	—	−78·0	−75	−26	—
389 ,,	−30	−34	−33	—	−97·0	−81	−24	—

¶ See Table 1 for footnotes.
** Water injected to unplug tube and obtain samples.

During fasting, 7–8 l. of fluid are secreted into the small intestine each day. Assuming even distribution over the 24 h, this is an entry-rate of 4·9–5·5 ml./min. If it is further assumed that this secretion rate is evenly distributed over the 3 m of small gut, and the perfusion area is but one-tenth to one-twentieth of total small bowel, the contribution of non-perfused bulk solution would approximate at most 0·5 ml./min. The method is of the order of magnitude of errors inherent in the method and is less than cumulative results from several [illegible]. Actually, on the average the volume of fluid present intraluminally at any one time must be greater in the proximal than the distal small gut, since most of the secretion originates proximally. Continuous absorption by the gut, and the predominance of high absorption rates in the proximal gut would further contribute to an average proximal intraluminal volume greater than the dis Thus there should be a greater interference by extrane solutions in the proximal small gut. Nevertheless, c current transport of sodium, chloride and glucose equimolar proportions is readily demonstrated. Th there is at least enough sodium absorbed to give equimolar relation between sodium and glucose transp It cannot be excluded that, as a result of errors of unc estimating sodium absorption, absorption of sodium excess of glucose might not occur.

con[illegible]n though this source of error is present in relat transpo[illegible]ransport, conditions of greater and lesser by this techn[illegible]ous substances are readily defi of at least three [illegible]e each rate is the mean of ra inherent error of underest[illegible]ch agreed quite well, t present in the bowel causes study. Artefact from this source [illegible]ption of substan in normal subjects. [illegible]ation during

Certain data on a patient with sprue are n[illegible] minir with findings in normals. Thus, with Ringer's, h proximal secretion occurs in the presence of a small c centration gradient for absorption. The distal absorp rate is so much greater than normal that increased meability, prolonged gut contact time, or an electro conservation mechanism are suggested. Furtherm the patient with sprue shows dissociation of sodium chloride from glucose transport. Thus, with gluc Ringer's, high proximal rates of sodium and chlo secretion occur during low rates of glucose absorpt Increased entry of extraneous solution into the perfu area shown by the increase in net intraluminal vol explains this. Increased permeability or impairmen sodium conservation in the proximal gut in sprue allo leak of sodium down its concentration gradient ma present. In the distal small gut, sodium, chloride glucose absorptions are equimolar and are as high as proximal small gut in normals.

During perfusion of the colon with distilled w sodium and chloride, secretions are lower but potass secretion is higher than in the small intestine. Ringer's, sodium is absorbed, potassium secreted. accords with the finding of sodium retention and potass secretion by the colon[5] and suggests a colonic permeab lower than that of the small intestine to the ionic gradi produced by distilled water, despite a longer contact under conditions we have used.

Net transfer in the small intestine in normal sub and patients with non-tropical sprue has been studie

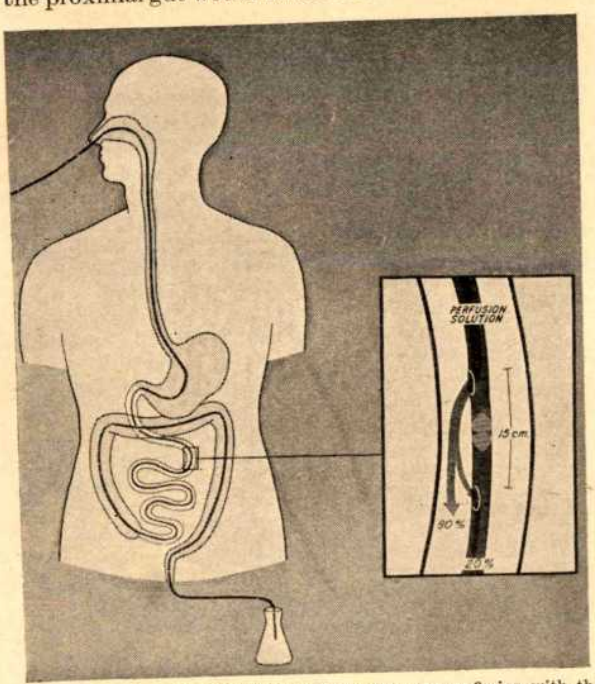


Fig. 1. Technique for investigating absorption by perfusion with the transintestinal tube. Steady-state perfusion of the small intestine is induced by pumping solution into the bowel through the proximal hole in the transintestinal tube at 15 ml./min. The solution enters at a known distance from the nares, and the perfusate is sampled 15 cm distal to the entry site by siphonage from the anal end of the tube. A solid metal connector separates entry from exit holes

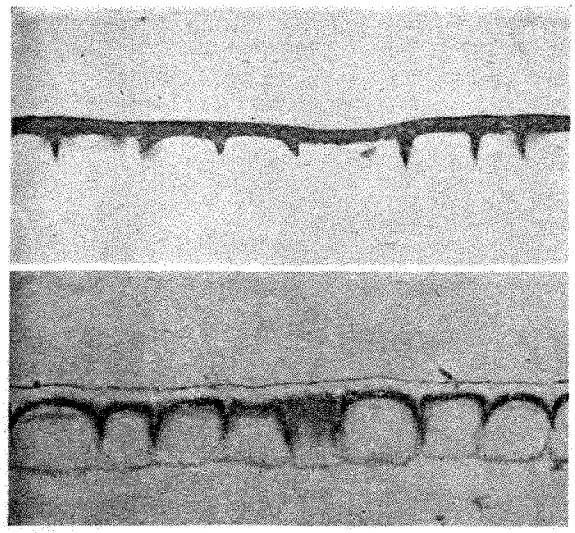


Fig. 1. Cuticular membranes of leaf of *Euonymus japonicus* L. *A*, cutin, stained with sudan IV; *B*, pectin near epidermal cells, stained with ruthenium red

obtained from minced leaves but the method of separation suggests that they were derived from the leaf cutin. Our limited information on the nature of cutin raised the question of the extent to which the cutin of plants is constant in composition. An examination was therefore made of the cutin of leaves and fruits of different species.

Isolation of cuticular membranes and constituent acids. Cutin was obtained, in large-scale preparations, from fruits of apple (Dabinett cider variety) and gooseberry (var. Careless) and leaves of *Euonymus japonicus* L. (an evergreen shrub), selected because of their well-developed cuticles. The apple fruits were boiled in water to loosen the skins, which were then boiled in successive portions of 1 per cent sulphuric acid to free them from flesh. The cuticular membranes were extracted with methanol to remove waxes, and dried. The gooseberry fruits were shaken with heptane–methanol to remove surface waxes, and the skins were separated from the flesh by hot water and acid treatment. The membranes obtained were extracted with methanol, treated with zinc chloride–hydrochloric acid solution to remove adhering remnants of epidermal cells, washed with dilute sodium carbonate solution, water, alcohol and dried. The *Euonymus* leaves were extracted with ether to remove waxes, and laminæ cut from the leaves were treated with ammonium oxalate–oxalic acid solution[5,19] to liberate the cuticular membranes. These were treated with zinc chloride–hydrochloric acid solution, washed and dried.

Cutin in the membranes from apple fruits was saponified, at room temperature, with 3 per cent ethanolic potassium hydroxide. The alkaline extract was almost neutralized, precipitated salt was removed, and the alcohol was taken off under reduced pressure. Acids liberated on acidification were extracted with ether, washed out with potassium bicarbonate solution, and again taken up in ether. The pale yellow solid (19 g) obtained on removal of the solvent was dissolved in a minimum amount of ethyl acetate. On keeping the solution in the refrigerator, two crops of crystals were obtained and recrystallized to constant melting points from ethanol–petroleum ether (compounds I and II). Infra-red analysis showed them to be hydroxylated acids; equivalent weights were determined by titration of benzene–methanol solutions, under nitrogen, with potassium methoxide[20]. Compound I was shown to be a saturated acid, m.p. 99°–100° (found: C, 64·9; H, 11·1 per cent; equiv. 324: calc. for $C_{18}H_{36}O_5$: C, 65·0; H, 10·9 per cent; equiv. 332). The m.p. was undepressed by admixture with 9,10,18-trihydroxyoctadecanoic acid, m.p. 102°–103°, kindly supplied by Dr. M. Matic. Compound II also was a saturated acid, m.p. 75°–76° (found: C, 66·1; H, 11·3 per cent; equiv. 292: calc. for $C_{16}H_{32}O_4$: C, 66·6, H, 11·2 per cent; equiv. 288). The m.p. was undepressed by admixture with 10,16-dihydroxyhexadecanoic acid, m.p. 76°, supplied by Dr. Matic. Compound I amounted to 25 per cent and compound II to 15 per cent of the ether–soluble acids obtained from the apple fruit cutin; in comparison, Matic[17] found 43 per cent of 9,10,18-trihydroxyoctadecanoic acid (compound I) in the *Agave* acids. The residual fraction of the apple ether-soluble cutin acids was an oil which showed additional hydroxy-fatty acid components and some unsaturation.

10,16-Dihydroxyhexadecanoic acid crystallized readily from the ethyl acetate solution of the ether-soluble acids (4·9 g) isolated from the gooseberry fruit cutin, and comprised more than 70 per cent of the acids obtained. The *Euonymus* leaf ether-soluble cutin acids (27 g) yielded both 10,16-dihydroxyhexadecanoic and 9,10,18-trihydroxyoctadecanoic acids, but in differing proportions from those obtained from apple. The 10,16-dihydroxyhexadecanoic acid predominated, to the extent of 45 per cent of the cutin acids.

Examination of the cutin of different plants. The isolated 9,10,18-trihydroxyoctadecanoic and 10,16-dihydroxyhexadecanoic acids and samples of 10,18-dihydroxy- and 18-hydroxyoctadecanoic acids obtained elsewhere were used as reference compounds in the identification and assessment of the ether-soluble acids in the cutin of different plants. Reversed phase chromatography using castor oil-impregnated paper and 60 per cent acetic acid as the mobile phase[21] was used, and the acids were detected with lead acetate and sodium rhodizonate[22]. R_F values at 25° C were as follows: 9,10,18-trihydroxyoctadecanoic acid 0·64, 10,16-dihydroxyhexadecanoic acid 0·52, 10,18-dihydroxyoctadecanoic acid 0·33, and 18-hydroxyoctadecanoic acid 0·02.

Waxes were removed from disks of leaves (about 1,000 cm² surface area) or of fruits (about 100 cm²) by extraction with chloroform, and the cuticular membranes were isolated by ammonium oxalate–oxalic acid treatment. Attached cellulosic remnants were removed with zinc chloride–hydrochloric acid, and the membranes were refluxed with successive portions of 1 per cent potassium carbonate solution until the extract was no longer coloured. The membranes were washed with water and alcohol and dried, treated with 3 per cent ethanolic potassium hydroxide at room temperature, and the liberated ether-soluble acids isolated. The acids were applied to the paper in solution in methanol (up to 50 μg/5 μl.). The occurrence and relative proportions of the cutin acids assessed from comparisons with standards are shown in Table 1.

Table 1. Ether-soluble Cutin Acids in the Cutin of Plants

	9,10,18-trihydroxy-octadecanoic acid	10,16-dihydroxy-hexadecanoic acid	10,18-dihydroxy-octadecanoic acid	18-hydroxy-octadecanoic acid
Leaves				
Potato		+ + +		+
Lime (citrus)	+ + +			+
Euonymus	+ +	+ + +	+	+
Fruits				
Gooseberry		+ + +		+
Blackcurrant		+ + +		+
Pear (Conference)	+ + +	+ + +		+ +
Pear (Oldfield)	+ + +	+ + +	+	+ +
Apple (Bramley)	+ + +	+ + +		+ +
Apple (Cox)	+ + +	+ + +	+	+ +
Apple (Worcester)	+ + +	+ + +	+	+ +
Apple (Dove)	+ + +	+ + +		+
Apple (Dabinett)	+ + +	+ + +	+	+

Earlier work[4,16] has shown that purified cutin yields about 80 per cent of ether-soluble acids. Indications were obtained of the presence, among the ether-soluble acids from *Euonymus*, potato, apple and pear cutins of small amounts of an unsaturated acid of R_F 0·10. The 10,16-dihydroxyhexadecanoic and 9,10,18-trihydroxyoctadecanoic acids clearly were major components of the cutin of the plants examined.

The results shown in Table 1 provide interesting comparisons. The cutin of the apple and pear, of the botanical sub-family Pomoideae, yielded approximately the same proportions of the four acids, with 9,10,18-trihydroxyoctadecanoic and 10,16-dihydroxyhexadecanoic predominating, while that of the gooseberry and blackcurrant, of the Ribesioideae, gave largely 10,16-dihydroxyhexadecanoic acid. Eglinton *et al.*[23] have shown that the composition of the paraffin fraction of the cuticular wax is characteristic of a species and may be useful for taxonomic purposes. It may be that cutin composition also follows botanical classification.

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EFFECT OF GIBBERELLIN ON VARIETAL IDENTITY IN RED CLOVER (*TRIFOLIUM PRATENSE* L.)

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In earlier investigations of the effect of gibberellin A_3 applied to late-flowering genotypes of red clover, pronounced changes in rate of tillering, stem extension and leaf shape were noted[1]. It became evident that gibberellin could modify many of the morphological characters which are commonly used to distinguish between early- and late-flowering varieties of red clover so that the overall result of gibberellin treatment of a late-flowering genotype was the production of a plant which in most respects phenotypically resembled an early-flowering variety. Interest was centred, therefore, on the effect of similar treatment on early-flowering genotypes.

Clonal propagants of Dorset Marl (an early-flowering variety) and Aberystwyth *S*.123 (late-flowering) were established in vermiculite, under a 10-h day-length, at 15·5° C for one month in order to produce vigorous vegetative growth. After this pre-treatment the plants were placed in a growth chamber which provided an inductive day-length of 16 h at 18° C. A completely randomized layout was adopted. Half the propagants of each variety received gibberellin A_3 (500 μg/plant/application) at the first and sixth internode stages and the remaining plants were sprayed with solvent only (0·1 per cent ethanol).

All plants showed significant responses to gibberellin in terms of stem elongation, tillering, leaf shape and general growth habit, but the response was considerably greater in the late-flowering variety. Comparison of leaves produced at a given stem internode in the two varieties showed that the characteristic difference in length/breadth could not be detected in the presence of gibberellin. Similarly, the profuse basal vegetative tillering characteristic of late-flowering varieties was completely inhibited by the first gibberellin treatment, giving rise to an open crown habit typical of early-flowering types. The close similarity in appearance between untreated Dorset Marl and treated *S*.123 is clearly shown in Fig. 1, and at this growth stage using normal diagnostic methods it was not possible to distinguish between the varieties.

At flowering certain varietal characteristics persist, notably the greater number of extended stem internodes produced before terminal inflorescence emergence in *S*.123 and the earlier date of flowering in Dorset Marl, but the time difference is considerably reduced by gibberellin treatment.



Fig. 1. Response of early and late-flowering red clovers to gibberellin A_3. Top, *S*.123 and Dorset Marl (control);—bottom left, Dorset Marl (control); bottom right, *S*.123 treated with gibberellin

perfusion of distilled water, Ringer's solution, and 1 per cent glucose-Ringer's solution. In normal subjects absorption of sodium, chloride and potassium occurred proximally and secretion distally when Ringer's solution was perfused. This proximal-distal relationship was reversed in a patient with non-tropical sprue, in accord with greater intensity of damage to the mucosa in the proximal small gut.

Substitution of 1 per cent glucose in equimolar proportions for sodium and chloride greatly augmented absorption rates of normal subjects, especially in the proximal small gut, where glucose absorption is greatest. The 4–100-fold increase in sodium absorption occurred despite a gradient for sodium secretion. Glucose also caused sodium absorption to occur in the distal small gut, where secretion occurred with Ringer's.

In a patient with non-tropical sprue, the proximal-distal net transfer relationships of the small intestine for 1 per cent glucose-Ringer's were the reverse of normal. Absorption of glucose and electrolytes in the distal small gut in sprue was as high as in the proximal small gut of normals. Thus, in sprue the distal small gut has adapted to the damage and loss of absorbing capacity in the proximal small gut. The distal has in part taken over the proximal small gut functions.

Glucose, sodium and chloride tend to be transported in equimolar proportions across all regions of the small intestine in normal subjects, and the distal in sprue. This is the first demonstration of such a linkage in transport under physiological conditions in man.

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X-RAY INHIBITION OF URETHAN-STIMULATED PROLIFERATION OF LUNG CELLS OF THE MOUSE AS ESTIMATED BY INCORPORATION OF TRITIATED THYMIDINE

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IN an effort to extend our recent observation that 880 rads of whole-body X-irradiation inhibits the well-known induction of pulmonary tumours by urethan in mice[1], we have investigated the uptake of tritiated thymidine by cells of the mouse lung during the first 10 days following a single injection of urethan and/or total body X-irradiation. The purpose was to determine whether increased cell proliferation induced by urethan[2,3] was altered by tumour-inhibiting doses of X-rays. Previous reports[1,4,5] have emphasized the paucity of discrete pulmonary histopathological changes following urethan injection during the first several weeks, while Shimkin and Polissar[2] found a progressive increase in cellularity during this early period.

Twenty 5-week-old $(C57L \times A)F_1$ male mice were given 0·2 ml. of a 10 per cent solution of urethan intraperitoneally. This dose alone is sufficient to cause multiple pulmonary tumours in every animal[1]. Twenty-four hours later, 10 of the urethan-treated mice plus an additional group of 10 normal mice were exposed to 880 rads of whole body X-irradiation (250 kVp.; 15 m.amp; filter 0·5 mm copper plus 1·0 mm aluminium; half-value layer 1·28 mm copper; 100 cm target to skin distance; dose rate, 30 rads/min). This radiation dose is approximately 100 rads higher than the LD_{99} and is the same dose we used previously[1]; bone marrow cells to afford protection against radiation death were not injected in this work. Non-irradiated, untreated mice served as controls.

Tritiated thymidine (specific activity 1·9 c/m.mole) was diluted with physiological saline to give a concentration of 100 μc./ml. and 0·2 ml. of this solution was given intraperitoneally to each animal at 6 h and at 3 h before killing. Mice were killed at the same time each day by cervical dislocation, the lungs were fixed whole, cut transversely into multiple blocks for routine dehydration and paraffin embedding, and sectioned at 5μ. After Feulgen hydrolysis and staining, sections were covered with Kodak '*AR*-10' stripping film and exposed for 7 weeks. Unstained sections gave the same proportion of labelled cells as sections subjected to the hydrolysis of the Feulgen method.

Labelled cells were counted in sub-pleural and deeper fields over alveolar zones only, using an ocular grid. Sections of lung from control and experimental animals were alike in cellularity in these experiments and the ocular grid enclosed an average of 68 nuclei. The number of labelled cells per 100 grid areas was recorded (Table 1) after at least 150 grids had been counted for each animal. Labelled cells were not localized in sub-pleural or deep alveolar zones, and no discrete histological differences were noted between treated and control mice.

Table 1. EFFECT OF URETHAN AND X-IRRADIATION ON PROLIFERATION OF LUNG CELLS OF THE MOUSE AS ESTIMATED BY UPTAKE OF TRITIATED THYMIDINE

Time after treatment (days)	No. of labelled cells per 100 grid fields: Urethan only	Urethan 24 h before X-rays	X-rays only	No treatment (normal controls)
1	8·9	1·2	1·0	26·4
2	27·0	1·3	0·6	—
4	86·0	3·3	22·0	—
7	94·0	12·0	34·6	—
10	259·0*	—	—	33·0*

Five week-old male LAF_1 mice (14–17 g). Figures represent averages of two animals, except where indicated, in which case only one animal was counted.
Urethan—0·2 ml. of 10 per cent solution intraperitoneally.
X-rays 880 rads whole-body exposure; 250 kVp., 15 m.amp.

In 3 control animals (Table 1, last column) there was an average of about 30 labelled cells per 100 grid areas. At 68 cells per grid area, this represents a labelling rate of 0·44 per cent, which is lower than the rate obtained by Schultze and Oehlert[6] although it is in the range of values for growing cell populations reported by Leblond *et al.*[7].

Urethan decreased the proportion of nuclei labelled on the first day after injection, but the proportion of labelled nuclei rose in one week to three times the control value (Table 1, column 2). The very high proportion of labelled nuclei at 10 days occurred in the only animal available; it may indicate a more extensive effect than suspected at present and would require future evaluation.

The rising proportion of labelled nuclei in the alveolar zone of the lungs of the urethan group agrees with the 3-fold increase in labelling and mitotic rate found 13 days

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after urethan by Crocker and Hill[3] and with the rapid increase in general cellularity of the lung reported by Shimkin and Polissar[2].

The initial decline in proportion of labelled nuclei one day after urethan suggests a brief inhibition of cell multiplication. The mechanism for such an effect is not known, but might be similar to suppression of cell multiplication demonstrated in other investigations[8,9]. Rogers's observations[10] that injections of DNA or of various nucleic acid precursors antagonize pulmonary tumour induction by urethan suggest that urethan interferes with DNA metabolism. The evidence that ultra-violet radiation at a wave-length of maximal DNA absorption will produce pulmonary tumours similar to those obtained with urethan[11], and the fact that urethan is known to elicit chromosome abnormalities in cells of many species[9], suggest that the induction of pulmonary tumours by urethan involves events at chromosomal or sub-chromosomal levels.

X-irradiation alone sharply reduced the proportion of labelled nuclei during days 1 and 2, but the labelling rates had returned to control values by day 7 (Table 1, column 4). The effect of urethan followed in 24 hours by X-irradiation (880 rads) was a marked and persistent reduction in the proportion of cells in DNA synthesis (Table 1, column 3). This effect was greater and lasted longer than the suppression of cell replication produced by X-irradiation alone, suggesting that urethan increased the radiosensitivity of pulmonary cells.

Inhibition of cell multiplication by X-rays is well known, but the mechanism is only partially understood. X-irradiation inhibits thymidine kinase and DNA polymerase activity in rat liver following partial hepatectomy[12], and in the kidney following unilateral nephrectomy[13]. Both these biological systems are in a state of cellular proliferation aimed at replacing a functional mass of tissue. The corresponding finding here is that X-irradiation (880 rads) suppressed urethan-induced cellular proliferation.

In previous longer-term experiments, exposure to 880 rads of whole-body X-irradiation 3 h before giving urethan, or at 3 h or 24 h after giving urethan eliminated the lung tumour-promoting property of urethan while X-irradiation as late as 7 days after giving urethan reduced tumour incidence markedly[1]. The results presented here demonstrate that the same X-irradiation dose which eliminated tumour emergence suppresses the early widespread rapid cell proliferation characteristic of the early response to urethan.

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CUTIN OF PLANT CUTICLES

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THE structure of the cuticle, or surface skin, of the higher plants has been established during the past century by anatomical and chemical work which has recently been reviewed[1,2]. The cuticle assumes the form of a non-cellular membrane covering the cellular tissue. The chief structural component of the membrane is cutin; this occurs throughout the membrane, but becomes diluted with pectin (and cellulose) as the membrane merges into the outer walls of the epidermal cells (Fig. 1). Waxes embedded in the membrane and extruded from its surface give plants their water-repellent properties. The cuticular membrane has been likened to a sponge with capacity to absorb water and swell. In the apple leaf, the cutin is deposited as lamellæ parallel to the surface with pathways through the membrane facilitating the passage of water-soluble substances[3]. Wide variations have been found in the levels of cutin in the cuticles of different plants[4,5] and in those of leaves and fruits of one species; the mature apple fruit, for example, carries ten times more cutin per unit area of surface than the fully formed leaf[6]. The cuticle contributes much to the well-being of the plant; it protects the cellular tissues, absorbs ultra-violet light, conserves water and nutrients, and participates in respiration. Interest in the plant cuticle has been stimulated in recent years by the recognition of its influence on the behaviour and efficiency of crop protection chemicals and of the part it may play in providing a mechanical barrier to the invasion of plants by fungi[1,2,7,8]. Heinen[9] has made the interesting observation that cutin is broken down by 'cutinase' produced by a leaf-rotting fungus.

Chemical nature of cutin. By contrast with the cuticular waxes, the identities of which are now well known, little information is available on the chemistry of cutin. Up to 1885, Fremy[10] obtained two acids, stereocutic and oleocutic, from the cutin of apple, ivy and a species of *Agave*. Lee[11] in 1925 concluded from work on flower petals and rhubarb petioles that cutin was a complex mixture of fatty acids, free and combined with alcohols, that had undergone condensation and oxidation. This supported the view of Lee and Priestley[12] that the cuticle was formed from lipoidal substances, formed or mobilized in the epidermis, that migrated to the surface and oxidized to give a 'varnish-like' covering. Legg and Wheeler[13] saponified the cutin of leaves of *Agave americana* and *A. rigida* and isolated cutic and cutinic acids; cutic acid comprised 65 per cent of the acids obtained. Markley and Sando[14] considered that cutin was an ester-like substance yielding fatty acids on hydrolysis, and Roelofsen and Houwink[15] referred to cutin as "a polymolecular network of several dicarboxylic and hydroxycarboxylic acids connected through ester and ether bridges".

More recently Huelin[16] has suggested that cutin, a polyester of hydroxylated acids and other substances, is probably formed under the control of a specific and localized enzyme. An important step forward was made in 1956 by Matic[17], who isolated 9,10,18-trihydroxyoctadecanoic, 10,18-dihydroxyoctadecanoic, 18-hydroxyoctadecanoic, 18-hydroxyoctadec-*cis*-9-enoic and 10,16-dihydroxyhexadecanoic acids also from the leaf cutin of *A. americana*. The first-named predominated, and the five acids accounted for more than 80 per cent of the ether-soluble acids liberated from the cutin. The 9,10,18-trihydroxyoctadecanoic and 10,16-dihydroxyhexadecanoic acids have also been isolated[18] from olive leaves; they were

Assays of endogenous gibberellin-like activity were performed on extracts from Dorset Marl and *S.*123 made at different growth stages during early stem extension. The extraction was carried out on 500 g fresh weight samples with 1 : 1 acetone/water for 20 h at 0° C after which the extract was reduced to the aqueous fraction by *in vacuo* distillation. The gibberellin-like activity was adsorbed on activated charcoal at *p*H 3 and eluted with acetone, the final eluate being concentrated for chromatography by rotary film evaporation.

Each extract was loaded on to strips of Whatman 3 mm paper and chromatographed in a descending solvent system of 80 per cent isopropanol. Chromatogram sections were eluted and assayed for activity using the d1 dwarf maize assay[2]. It was generally possible to detect two active zones on each chromatogram, one at R_F 0·3–0·4 and the other at R_F 0·6–0·7 which varied according to growth stage (Table 1)

The possible functions of the activity in the two zones have been discussed in an earlier publication[3], and it was concluded that both play a part in stem extension although the R_F 0·6–0·7 activity also appears to have some correlation with floral initiation. In terms of total activity it is evident that at any given stage during early stem extension less gibberellin is present in the late-flowering type, which fits the observation that the rate of stem extension is initially slower in late varieties. The results suggest that the effects of applied gibberellin on a late-flowering variety result from the supplementation of a system in which gibberellin is limiting, whereas the lesser response of early-flowering types could be due to the presence of a near-optimal gibberellin-level at an early growth stage.

Table 1. GIBBERELLIN-LIKE ACTIVITY IN EARLY- AND LATE-FLOWERING RED CLOVER

Variety	No. of internodes	GA_3 μg equiv./kg fresh weight R_F 0·3–0·4	R_F 0·6–0·7	Total
Dorset Marl	1	0·40	0·36	0·76
*S.*123	1	0·12	0·00*	0·12
Dorset Marl	2	0·30	0·41	0·71
*S.*123	2	0·20	0·12	0·32
Dorset Marl	3	0·26	0·56	0·82
*S.*123	3	0·22	0·15	0·37
Dorset Marl	4	1·50	0·78	2·28
*S.*123	4	0·25	0·33	0·58

* No significant response in bioassay.

The participation of gibberellins in the expression of genetic differences has been reported previously in dwarfed genotypes of *Pisum sativum*[4], *Zea mays*[2] and *Lolium perenne*[5]. In each instance it was demonstrated that dwarfism due to single gene differences could be overcome by spraying with gibberellic acid, phenotypically normal plants being produced.

Although the aggregate morphological difference between early and late red clovers clearly has a polygenic basis, it is evident from the foregoing data that the morphogenesis of some of the individual characters can be described in terms of differences in gibberellin content.

A simple scheme to explain the effects of exogenous gibberellin and also to account for the more rapid response to inductive day-length in Dorset Marl would be one which suggested that the rate of inception of gibberellin synthesis in response to long-days was more rapid in the early-flowering type.

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CONVERSION OF DOPAMINE TO 3,4-DIMETHOXYPHENYLACETIC ACID IN SCHIZOPHRENIC PATIENTS

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IN a previous investigation, in this laboratory, a biologically active compound, 3,4-dimethoxyphenylethylamine, was identified in the urine of schizophrenic patients[1,2], and it was suggested that this compound might be formed from 3,4-dihydroxyphenylethylamine (dopamine). The present investigation was initiated in order to determine whether 3,4-*O*-methylation of dopamine occurs.

Five hospitalized patients with a confirmed diagnosis of schizophrenia were selected. 3,4-Dimethoxyphenylethylamine had been identified previously in the urine of each of these patients. 3,4-Dihydroxyphenylethylamine-7^{3}H.HCl (specific activity 24·0 mc./m.mole) was dissolved in 200 c.c. of normal saline and approximately 200 μc. of this compound were administered to each patient by intravenous infusion. Urine was collected for a period of 3 h. The urine from each of 3 patients was analysed separately while the extracts of the urines of 2 other patients were pooled in order to increase the concentration of radioactivity.

The urine was extracted by a method previously described[1,2]. In brief, following the infusion, the urine was extracted with chloroform at *p*H 2. The aqueous phase was adjusted to *p*H 9 and again extracted with chloroform. The material extracted at *p*H 9 was co-chromatographed in butanol : formic acid : water (12 : 1 : 6) with 3,4-dimethoxyphenylethylamine and 4-hydroxy-3-methoxyphenylethylamine. Control strips were cut and dipped in ninhydrin-pyridine reagent in order to locate the reference compounds. The chromatograms were scanned in a Tracerlab 4 π chromatogram scanner, and a peak was found that corresponded to 4-hydroxy-3-methoxyphenylethylamine on each chromatogram. A second peak corresponding to 3,4-dimethoxyphenylethylamine was identified on each chromatogram. However, this peak did not represent sufficient activity for further analysis by isotope dilution methods. The radioactivity corresponding to 3,4-dimethoxyphenylethylamine is at present being analysed by other techniques.

The organic phase from the *p*H 2 extraction contained the acidic and neutral metabolites. This extract was evaporated to a small volume and applied for chromatography. The chromatogram was developed in butanol : acetic acid : water (4 : 1 : 1). The chromatograms were scanned for radioactivity. Control strips were cut from the chromatograms of this fraction and were dipped in aniline-xylose reagent[3]. One broad radioactive area with its peak at R_F 0·81 was found. It corresponded to the areas of both 4-hydroxy-3-methoxyphenylacetic acid and 3,4-dimethoxyphenylacetic acid. The material in this area was eluted by wetting the chromatographic paper with 0·01 N hydrochloric acid and extracting it with chloroform. The chloroform was evaporated and the residue was applied for chromatography. The chromatogram was developed in ethanol : concentrated ammonium hydroxide : water (16 : 1 : 3). In each case a scan of the developed chromatogram revealed a radioactive peak at the R_F of co-chromatographed 4-hydroxy-3-methoxyphenylacetic acid (R_F 0·58). A second peak was found that corresponded to 3,4-dimethoxyphenylacetic acid

(R_F 0·69). The urine obtained from patient No. 5 was first extracted at *p*H 9 and then at *p*H 2 to eliminate neutral metabolites from the *p*H 2 extract. The resulting scan of the chromatogram of the acid extract from this patient also displayed the two peaks already described.

An area, 5 mm wide, was cut across the portion of each chromatogram corresponding to the 3,4-dimethoxyphenylacetic acid reference. This area of the paper was wetted with 0·01 N hydrochloric acid and the radioactive material was eluted with chloroform. The chloroform was evaporated and the material was recrystallized from 0·1 N hydrochloric acid with authentic 3,4-dimethoxyphenylacetic acid as the carrier. In addition, radioactive material corresponding to 4-hydroxy-3-methoxyphenylacetic acid was eluted and was also recrystallized with authentic 3,4-dimethoxyphenylacetic acid. The radioactivity of the samples was assayed by means of a Tracerlab gas-flow proportional counter and a Packard Tri-Carb liquid scintillation counter.

The results of the carrier analyses are summarized in Table 1. It will be noted that the activity corresponding to 3,4-dimethoxyphenylacetic acid maintained constant specific activity. This constitutes presumptive evidence that the radioactive material is 3,4-dimethoxyphenylacetic acid. The 4-hydroxy-3-methoxyphenylacetic acid was not carried through 3 recrystallizations with 3,4-dimethoxyphenylacetic acid as the carrier.

Table 1. Results of Carrier Analyses

Radioactive material corresponding to 3,4-dimethoxyphenylacetic acid recrystallized with 3,4-dimethoxyphenylacetic acid as carrier	Specific activity c.p.m./mg*			
	No. recrystallizations			
	1	2	3	4
Patient No.				
1	23·9	24·4	21·0	
2	17·5	16·3	22·1	
3 and 4 (pooled)	75·0	77·0	82·7	68·0
5	24·4	26·9		
Radioactive material corresponding to 4-hydroxy-3-methoxyphenylacetic acid recrystallized with 3,4-dimethoxyphenylacetic acid as carrier				
Patient No.				
1	28·2	13·8	10·4	
2	11·8	8·4	6·6	

* The standard error of the group mean is based on the use of the 'within group variance'. Using paired replicate samples the standard error of the mean of the specific activities of all the samples is ± 0·52.

To obtain further evidence for the metabolic conversion of dopamine to 3,4-dimethoxyphenylacetic acid a liver biopsy was performed on patient No. 5. The liver obtained (0·1 g wet weight) was homogenized with 10 c.c. of 0·5 N phosphate buffer *p*H 7·9. The whole homogenate was incubated at 37° C for 2 h with 0·1 c.c. 0·5 M magnesium chloride, 0·60 μmoles 3,4-dihydroxyphenylethylamine-7^{3}H.HCl and 0·96 μmoles of *S*-adenosyl-L-methionine methyl-^{14}C. The specific activities of dopamine and *S*-adenosyl-L-methionine were 24·0 mc./m.mole and 26·1 mc./m.mole, respectively. After incubation the sample was deproteinized with 15 c.c. of 5 per cent *m*-phosphoric acid and centrifuged. The supernatant fluid was decanted and adjusted to *p*H 2 with concentrated hydrochloric acid and extracted with chloroform. The chloroform was dried with anhydrous sodium sulphate and was then evaporated to a small volume. 3,4-Dimethoxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid were added to the concentrated extract which was then applied for chromatography. The chromatogram was developed in ethanol: concentrated ammonium hydroxide: water (16 : 1 : 3). A control strip was dipped in aniline-xylose reagent. The chromatogram of the acid metabolites was scanned for radioactivity and, as can be seen in Fig. 1, peaks corresponding to reference 4-hydroxy-3-methoxyphenylacetic acid (peak I) and 3,4-dimethoxyphenylacetic acid (peak II) were observed. The other regions of radioactivity have not been identified but may be methylated derivatives of compounds other than dopamine. The region of the chromatographic strip corresponding to peak I and that corresponding to peak II were eluted separately with ethanol. The radioactivity was assayed in a Tri-Carb liquid scintillation counter.

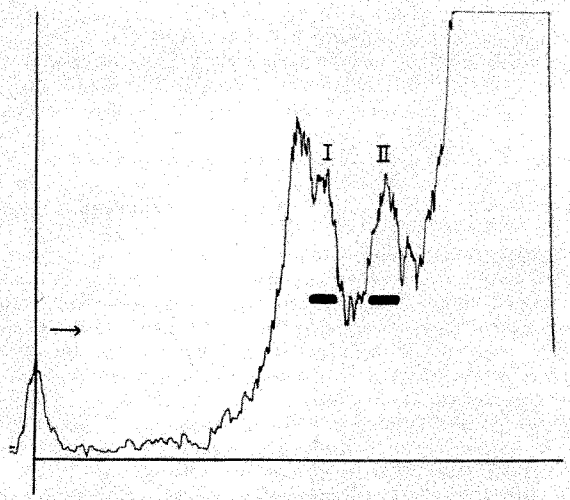


Fig. 1. Scan of a radiochromatogram developed from an extract obtained from the incubation of dopamine-7^{3}H, *S*-adenosyl-L-methionine-methyl-^{14}C and liver from a schizophrenic patient

Each mole of dopamine-7^{3}H will accept one methyl carbon-14 group for each mole of 4-hydroxy-3-methoxyphenylacetic acid formed and 2 methyl carbon-14 groups for each mole of 3,4-dimethoxyphenylacetic acid formed. The theoretical value for the ratio

$$\frac{\text{d.p.m. } ^{14}\text{C} \div \text{specific activity } ^{14}\text{C}}{\text{d.p.m. } ^{3}\text{H} \div \text{specific activity } ^{3}\text{H}}$$

would be 1 for the hydroxymethoxy compound and 2 for the dimethoxy compound. The ratio for the sample eluted from the area of 4-hydroxy-3-methoxyphenylacetic acid (peak I) was found to be: $\frac{1605 \div 26{\cdot}1}{1601 \div 24{\cdot}0} = 0{\cdot}921$.

The ratio for the sample eluted from the area of the 3,4-dimethoxyphenylacetic acid was $\frac{1583 \div 26{\cdot}1}{747{\cdot}7 \div 24{\cdot}0} = 1{\cdot}95$.

These results indicate that 3,4-dimethoxy as well as the usual 4-hydroxy-3-methoxy metabolites of dopamine are synthesized by schizophrenic patients. However, since biological samples from schizophrenic patients, but not from normal controls, were used in this investigation, a relationship between 3,4-dimethoxyphenylacetic acid and schizophrenia has not been demonstrated.

In this work it has been shown that dopamine can be converted enzymatically to a dimethoxy derivative both *in vivo* and in a homogenate made from liver tissue obtained from a schizophrenic patient. It has been shown previously that 3,4-dimethoxyphenylethylamine is readily converted to its corresponding acid derivative by monoamine oxidase[4]. This probably accounts for the large amount of dimethoxy acid excreted as compared with dimethoxy amine. From these results it seems likely that only a small part of the 3,4-dimethoxyphenylethylamine of endogenous origin is excreted unmetabolized, while a large portion of this compound is probably excreted as 3,4-dimethoxyphenylacetic acid.

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Radiation from Jupiter at 178 Mc/s

A NUMBER of observations have been made of the decimeter radio emission from Jupiter at frequencies between 3 cm and 70 cm.

Although there is some evidence that the emission at a given frequency shows some variation with time, the results, when corrected to the same distance for the planet, provide a fairly well defined spectral law (see, for example, the review article by Roberts[1]).

The origin of the emission has been attributed to radiation by relativistic electrons trapped in the Jovian magnetic field in a manner analogous to the terrestrial Van Allen belt[2]. If this interpretation is correct a more accurate determination of the spectral distribution of the non-thermal component, especially at the lower frequencies, is of considerable interest in connexion with the energy spectrum of the trapped particles.

Observations at a frequency of 178 Mc/s have, therefore, been made at the Mullard Radio Astronomy Observatory using the large east–west interferometer[3]. At a frequency as low as this, the expected flux density is small and there is some difficulty in distinguishing between the radiation from Jupiter and the radiation from the numerous radio sources having a comparable flux density. Consequently, it is necessary to develop an observing procedure whereby the records obtained on one day are subtracted from those taken on another day when the position of Jupiter has altered by more than the beam width of the instrument; in this way it becomes possible to detect a source very much weaker than could normally be distinguished if there were no relative motion.

Observations were made both with a fan-beam system and with an interferometer having a separation of 469λ between its elements; a comparison of the two records then enables limits to be set on the angular diameter of the source[4]. The observations were made over the period June 22–July 1, 1963, and the method of subtracting the records was used on both fan beam and interferometric systems to provide a series of ten independent determinations of the apparent flux density of Jupiter on each system.

The mean values derived were:

(*a*) Flux density using fan beam, $3{\cdot}3 \pm 0{\cdot}45 \times 10^{-26}$ W m^{-2}(c/s)$^{-1}$

(*b*) Flux density on interferometer, $3{\cdot}8 \pm 0{\cdot}4 \times 10^{-26}$ W m^{-2}(c/s)$^{-1}$

The day-to-day variations in the measured flux density were close to the values expected from the combined effects of noise and small variations in the phase of the interference pattern caused by ionospheric irregularities; these phase variations lead to a small residual component when subtracting the records from different days.

It can therefore be concluded that over the period of observation the flux density of Jupiter did not vary by more than 20 per cent. Furthermore, since, over this period, the day-to-day variations in electron content of the ionosphere are sufficient to cause variations in the Faraday rotation of more than 45°, the results indicate that at 178 Mc/s any polarized component of the emission is less than 20 per cent.

A comparison of the fan beam and interferometric observations allows an upper limit of 1·5 min arc to be set to the source diameter, on the assumption of a Gaussian strip distribution. With a circular source of this angular diameter the surface brightness would correspond to a brightness temperature $T_s > 1{\cdot}7 \times 10^4$ °K; if the source were the same size as the optical disk $T_s = 8{\cdot}6 \times 10^4$ °K.

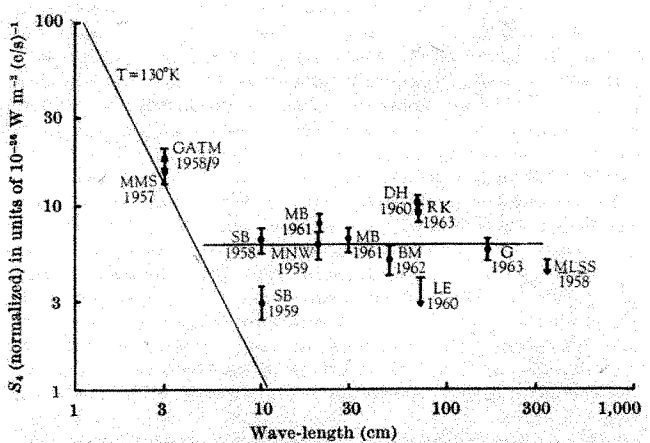


Fig. 1. Flux density of Jupiter corrected to a standard distance of 4·04 A.U. *GATM* 1958/59, see ref. 5; *MMS* 1958, ref. 6; *SB* 1958 and *SB* 1959, ref. 7; *MB* 1961, ref. 8; *MNW* 1959, ref. 9; *BM* 1962, ref. 10; *RK* 1963, ref. 11; *DH* 1960, ref. 12; *LE* 1960, ref. 13; *MLSS* 1958, ref. 14. '*G*1963' refers to the present observations.

The flux densities derived from these and a number of other observations have been corrected to a distance to the planet of 4·04 A.U. and are shown in Fig. 1.

The observed spectrum has been fitted in the figure by the adoption of a thermal contribution corresponding to a temperature of 130° K, as suggested by Roberts[1], and of a non-thermal component which has a spectral index, α, of 0, and for which S equals 6×10^{-26} W m^{-2} (c/s)$^{-1}$.

Although the points show considerable scatter, part of which may be due to temporal variation in the source, the new observations suggest that the spectral index is less than the figure of 0·3 given by Roberts[1].

I would like to thank Dr. P. F. Scott for assistance with the observations.

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GEOPHYSICS

Magnetotelluric and Very Low Frequency Signatures from Small High-Altitude Nuclear Explosions

THIS communication presents a preliminary account of the magnetotelluric and very low frequency signatures associated with the U.S. high-altitude nuclear detonations of October 20 (0830 : 00 G.M.T.), October 26 (0959 : 49 G.M.T.), November 1 (1210 : 06 G.M.T.), and November 4

(0730 : 00 G.M.T.) 1962, observed at our field station near Rochester, New York. Three orthogonal components of dB/dt in the band 0·001 to 3 c/s were sensed with cored loop antennæ of sensitivity 0·07 mV-sec/γ. A pair of electrodes spaced 225 ft. apart sensed the north–south component of the telluric-current field in the same band. A whistler-recording apparatus furnished a broadband very low frequency noise record.

In contrast to the test of July 9, 1962, the four October–November tests displayed generally weak and variable signatures at our station. The magnetotelluric records consisted of the usual continuum background plus a series of short oscillatory transients 0·5–1 sec long. The broadband VLF noise record enabled us to exclude magnetotelluric transients arising from the 'slow tails' of lightning discharge waveforms. A sonagram analysis of this record was scanned for all strong spherics with large amounts of ELF (10–1,000 c/s) energy, and any magnetotelluric transients coincident in time with these spherics were rejected as arising from natural atmospheric effects. The remaining features were assumed to originate with the nuclear detonation, and were classified as either 'prompt' (essentially simultaneous with the instant of detonation) or 'delayed'.

Fig. 1 gives a scheme of the magnetotelluric results from the two low-yield devices detonated on October 20 and November 4. The essential observations are the following: (1) a large prompt magnetotelluric pulse occurred in all channels on October 20; there was no discernible prompt magnetotelluric pulse on November 4; (2) following detonation on October 20 almost two complete cycles of 0·3 c/s signal, with peak-to-peak amplitude of 0·05 γ, appeared in the north–south and east–west magnetic channels; similar frequencies were observed during the first half-minute following the July 9 detonation[1]; (3) delayed pulses appeared at $H+4{\cdot}7$ sec on October 20 and $H+6{\cdot}5$ sec on November 4, in both north–south and vertical magnetic channels; (4) a prompt broadband VLF pulse was observed on October 20.

Fig. 2 shows magnetotelluric results from the sub-megaton-yield tests of October 26 and November 1. The

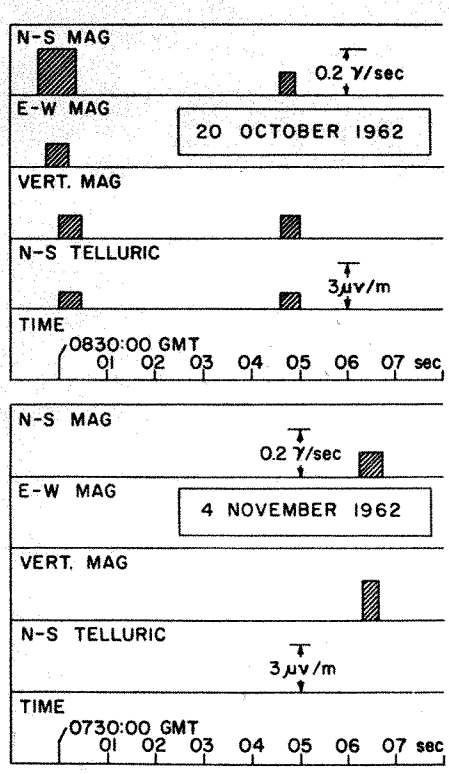


Fig. 1. Magnetotelluric summary diagrams, October 20 and November 4, 1962 tests

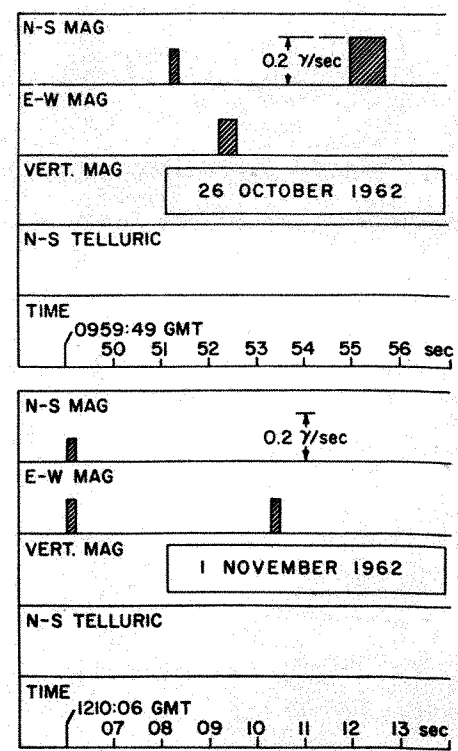


Fig. 2. Magnetotelluric summary diagrams, October 26 and November 1, 1962 tests

data show that: (1) on November 1 a prompt pulse occurred in the north-south and east-west magnetic channels, simultaneous with a prompt broadband VLF pulse, with most of the energy in the latter concentrated above 5 kc/s; (2) no prompt magnetotelluric pulses appeared on October 26; there were, however, two north–south magnetic pulses delayed by 2·5 and 6 sec, respectively; (3) the only delayed pulse on November 1 was a relatively weak east–west magnetic signature at $H+4{\cdot}5$ sec. The large natural background present on November 1 could, however, have obscured other effects.

During the last three tests of the series, we measured phase delays (for a 20-sec period following detonation) on the 18·6-kc/sec signal transmitted from station *NPG* near Seattle, Washington. On November 1, a rapid-onset phase decrease of the neutron-decay type[2,3], with an initial slope of 30°/min, was observed. On November 4 the rapid-onset phase decrease did not exceed 15°/min in the 20-sec observation period. Rapid-onset results on October 26 were inconclusive. However, an interesting coincidence of transient phase excursions was noted; in all three tests observed, the *NPG* signal displayed a rapid, transient phase excursion of 7°–10° peaked at $H+13$, $H+12$, and $H+11$ sec respectively. The probability of chance coincidence is less than 1 in 10. We therefore conclude that the evidence of delayed transient phase excursions is suggestive but scarcely conclusive.

Results of the four tests may be summarized as follows: (1) the prompt magnetotelluric signatures were highly variable; (2) on October 26 a delayed magnetotelluric signature occurred 2·5 sec after detonation; (3) a magnetotelluric signature component delayed by 4–7 sec occurred consistently for all four tests; (4) prompt broadband VLF pulses were observed only on October 20 and November 1; (5) besides the well-known neutron-decay phase delays, there was weak evidence of a transient phase excursion on the VLF signal propagated from station *NPG*, occurring about 12 sec after detonation of the last three tests.

The magnetotelluric and phase-transient signatures show average delays of 5 and 12 sec, respectively. If these delays represent the transit time of fast and slow hydro-

magnetic wave modes, the corresponding mode velocities are 1,700 and 450 km/sec. Both these values are about 40 per cent lower than those reported for the Argus tests of 1958[4,5]. In connexion with the 2·5-sec delay observed for one component of the October 26 signature, we point out that Roquet *et al.*[6] have shown that the main phase of the signature from the July 9 test showed a world-wide delay of about 2 sec following the instant of detonation. Reports from other observers on observations of the later American tests may provide further evidence of this important effect. The paucity of published results on the electro-magnetic effects of the October–November 1962 tests, relative to the July 9 test, is due no doubt to the more spectacular nature of the latter. We suggest, however, that attention devoted to the results of the October–November series may prove equally rewarding.

A fuller account of this work is being prepared for publication elsewhere.

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Detection of Scattering Layers in the Upper Atmosphere (60–140 km) by Optical Radar

This communication reports observations made by us of optical echoes from atmospheric constituents (presumably dust) at heights of 60–140 km. They were detected with an optical radar. The techniques utilized are a development of those already reported by us[1,2].

The optical radar consists of an RCA-designed ruby laser delivering short pulses of approximately 50 nsec, 0·5 joule at $\lambda = 6{,}940$ Å; of a transmitting refracting telescope of 7·5-cm diameter and 201-cm focal length; of a receiving reflecting telescope of 32-cm diameter and 270-cm focal length, and of a photometer utilizing a 20-Å wide interference filter and a cooled EMI 9,558 *A* photomultiplier. The two telescopes are accurately boresighted and rigidly connected on an equatorial mount. All observations reported here were made with the telescopes looking at the zenith. Each time that the laser was fired the return signals picked up by the photomultiplier were displayed on an oscilloscope and photographed. The return signals originating above 30 km were so weak that it was possible to count individual photoelectrons in each 10-km (66-μsec) interval up to 180 km. These experiments were carried out during the months of June and July 1963 at Lexington, Massachusetts. They show the Rayleigh molecular scattering at heights up to 50–60 km. At greater heights—up to 140 km—very weak echoes were detected which we ascribe to dust clouds. These latter echoes appear to come from two main regions: 60–90 km (often about 80 km) and 110–140 km (often about 120 km).

Fig. 1 shows the results of the observations of four consecutive days, July 28–31. This interval covers the period of the δ-Aquarids meteor shower. The sums of the photoelectrons obtained in successive 10-km range intervals are displayed for each night. The large initial counts, resulting from molecular Rayleigh scattering, permit an independent calibration of the apparatus. The noise-level, which is represented by photomultiplier dark current and sky background, is established by taking the average of the returns in the interval 140–180 km. Independent noise measurements taken between laser firings were in substantial agreement with those taken from the extreme ranges. Since the noise has the character of a Poisson process, the standard deviation is taken to be $\sqrt{n}$, where n is the average noise-level count per range-interval; note that the peaks obtained on July 30 and 31 exceed the average noise-level by more than 3 times the standard deviation.

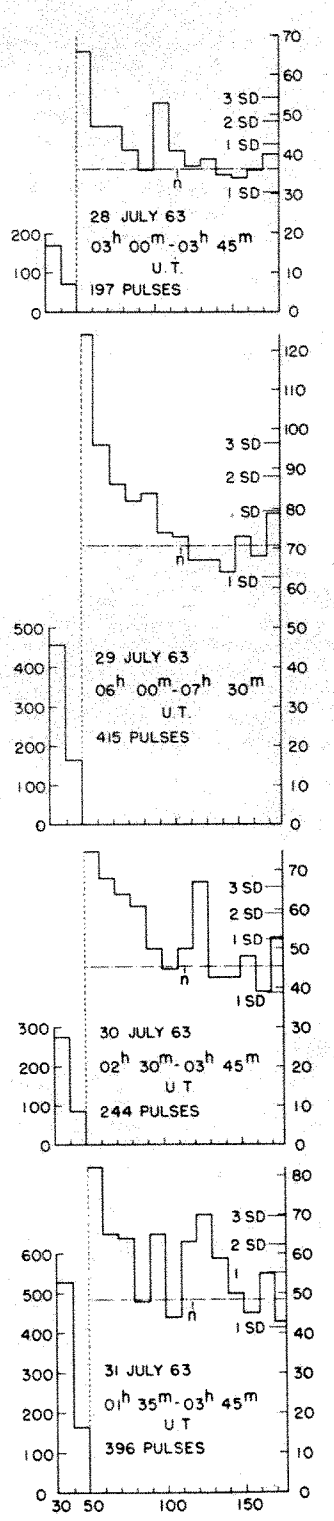


Fig. 1. Accumulated photoelectron counts in 10-km range intervals for July 28–31, 1963; n is the average noise-level

Many similar sets of data have been collected that show similar behaviour. Table 1 was compiled after a pre-

Table 1. Occurrence of Echoes on Nine Nights of Observation

U.T. (1963)	Range (km) 60	70	80	90	100	110	120	130	140
July 17				X		X		X	
July 24		X							
July 25			X	X					
July 26		X							
July 27						X			
July 28									
July 29									
July 30								X	
July 31								X	

liminary analysis of 9 days of observation. The X's show the occurrence of peaks exceeding the average noise-level by a factor of $3\sqrt{n}$. The echoes obtained on July 31 correspond to a backscattering differential cross-section per unit volume (averaged between 120 and 130 km) of $2 \cdot 10^{-13}$ cm^{-1} steradians^{-1}.

In the absence of independent methods of observation we cannot say what causes these echoes. However, one is tempted to compare the lower echoes (~ 80 km) with the observed heights of noctilucent clouds. It has been speculated that more distant echoes (~ 120 km) correspond to the region of meteoric break-up.

The assistance of F. W. Barrows, H. B. Gay, G. A. Garosi, and G. S. Misail in the construction of the apparatus and of H. C. McClees in the conduct of the experiment is gratefully acknowledged. We thank Prof. A. H. Barrett for many helpful discussions. This work was supported in part by the U.S. Army, the Air Force Office of Scientific Research, and the Office of Naval Research; and in part by the National Aeronautics and Space Administration (Grant *NsG*–419).

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Gravity Measurements over the Rhum Tertiary Plutonic Complex

During the past two years, sixty-six new gravity stations were established, using the Imperial College Worden gravity meter, on the islands of Rhum, Eigg, Canna and Sanday in the Inner Hebrides. Local bases on the islands were linked to Pendulum House, Cambridge, through the Mallaig gravity base established by Bullerwell and Phemister (private communication). The results were reduced to the International Gravity Formula, and after correction for terrain effects, the Bouguer anomalies were plotted as a gravity contour map (Fig. 1).

The contours emphasize the intensely local nature of the anomaly, a large positive Bouguer anomaly being centred over the Rhum Tertiary plutonic complex. Over the central ultra-basic mass, the Bouguer anomaly rises to +76·3 milligals, a value which exceeds by 2·9 milligals the largest positive Bouguer anomaly in the British Isles previously recorded by one of us (J. T.) over the Skye Tertiary centre. As all the known Tertiary centres have now been surveyed, it seems unlikely that this value will be exceeded on the remaining unsurveyed land areas within the British Isles. There is, however, always the possibility that similar high positive anomalies may be found over as yet undiscovered Tertiary centres in the sea areas off Northern Ireland and the west coast of Scotland.

Away from the Rhum centre, the Bouguer anomaly decreases rapidly with horizontal gravity gradients exceeding 15 milligals/mile to relatively low values of 5–10 milligals on Canna and Eigg. These values are much lower than the average background anomaly over the

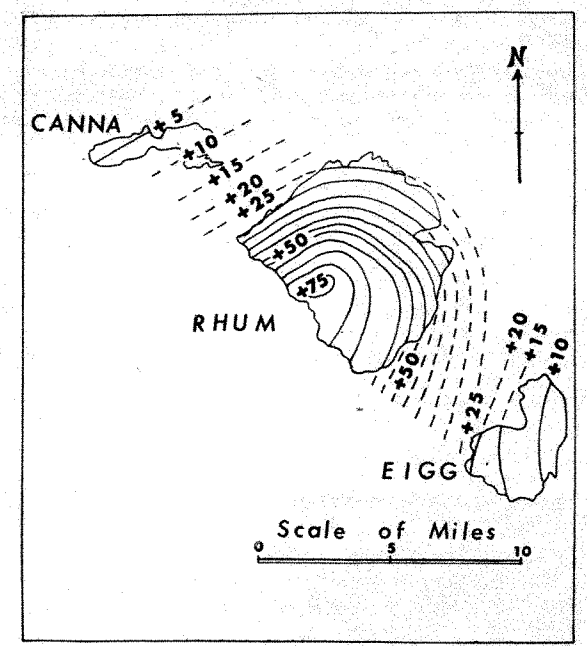


Fig. 1. Bouguer anomaly map of the Islands of Rhum, Eigg, Canna and Sanday in the Inner Hebrides. Contour interval, 5 milligals

Western Isles, and the assumption of a background value of 10 milligals leaves a positive anomaly of 65–70 milligals which requires explanation.

The origin of the anomaly can be interpreted in physical terms by calculating the dimensions and specific gravities of simple models of the gravitating mass and so obtaining a range of theoretical structures capable of producing the observed anomaly. The space form deduced in this way must not only satisfy the numerical characteristics of the anomaly, but must also be consistent with what is definitely known of the geological structure in the area.

As a first step it can be shown, by using the formula for a point mass, that the anomalous mass has a near surface origin, and the most likely cause of the anomaly is a direct density contrast between an extension at depth of the ultra-basic complex seen at the surface and the lighter metasedimentary and granitic layers of the crust. Estimates of the minimum depth to the base can be obtained by assuming: (1) a density contrast; (2) that the anomalous mass is in the form of a vertical cylinder. Calculations were made for vertical cylinders with top surface at datum-level (sea-level) and depth to bottom ranging from 10,000 ft. to 100,000 ft. and for a range of diameters of 5–8 miles. Also, for truncated cones, top surface at datum-level, depth to bottom 44,000 ft., diameter at surface ranging from 5 to 6 miles and a range of inclinations of side.

The results of this analysis were compared with the observed gravity fields, and although further analysis by computer is desirable, it seems probable that the positive gravity anomaly is the result of a dense (and presumably basic or ultra-basic) intra-crustal intrusion having an extension in depth of the order of thickness of the Intermediate layer of seismology (15 km), but having no important horizontal extension beyond the limits indicated by the exposed rocks of the Rhum Tertiary igneous complex.

A better idea of the shape of the mass can be obtained by making certain geological assumptions, namely, that the thickness of Torridonian in the area is 10,000 ft. and that the density of the crust below this depth is 2·75 g/c.c. This analysis indicates that the gravitating mass is best represented by either a vertical cylinder with surface diameter 6 miles, extending downward at least 44,000 ft. (15 km) and having a specific gravity of between 3·05 and 3·10; or, by a truncated cone with a surface diameter

5 miles, extending downward at least 44,000 ft., side inclined to the horizontal at 77°, and having a specific gravity of between 3·05 and 3·10. The surface diameter of the truncated cone model is similar to that of the exposed ring fault, and this model is thought to give a better representation of the probable geological structure.

The lowest permissible specific gravity of 3·05 would indicate that the intrusion is gabbroic; however, by analogy with Skaergaard, it is possible that the body is a heterogeneous complex of a number of rock types, some ultra-basic, some more acid than gabbro.

Gravity surveys by Cook and Murphy[1] and Tuson[2] over other Tertiary centres have yielded similar results, the observed maximum Bouguer anomalies being as follows:

	Milligals
Carlingford-Mourne	58·8 60·8
Skye	73·4
Ardnamurchan	42·0
Mull	71·7
Arran	40·8

This work was carried out with a grant-in-aid from the Royal Society and we thank the Society and Prof. J. G. Bruckshaw of the Imperial College of Science and Technology for the loan of a Worden gravity meter, and Dr. Bullerwell of the Geological Survey and Museum for permission to use some of his unpublished gravity data.

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GEOLOGY

Isostatic Recovery in Scotland

The article by Dr. D. F. W. Baden-Powell, on "Isostatic Recovery in Scotland"[1], claims that the evidence there presented makes it possible to decide "between the rival theories of W. B. Wright and Depéret". Dr. Baden-Powell appears to be challenging the theory of isostatic elevation of Scotland and the north of England following removal of the burden of ice in those regions during the glacial period; but he also makes reference to "the supposed recent tilting of the southern parts of Britain".

The evidence is given (albeit perhaps slender) that the marine faunas of certain raised beaches in the south of England and on the west coast of Scotland are similar and that they occur at very broadly similar heights. Although the evidence of the similarity of age and of beach-level is not at all conclusive, it should be pointed out that even unchallenged evidence of this kind would scarcely suffice to support the far-reaching conclusion which Dr. Baden-Powell places on it.

Dr. Baden-Powell appears to be considering the isostatic recovery of Scotland as a single process extending over a period of time which embraces that of the Tyrrhenian beaches. I think that the more generally accepted view would be that during each major glacial period the accumulation of ice over the north of Britain caused an isostatic subsidence of the crust and that in the ensuing warm interglacial period, on the melting of the ice sheets, there followed an isostatic uplift. There have hitherto been recognized in Britain three major glacial periods alternating with interglacials which are referred to as Ipswichian, Hoxnian and Cromerian, and not only the evidence to which attention is directed, but also the more decisive pollen analytic evidence, refers the Nar Valley clay and the Clacton channel deposits to the Hoxnian interglacial. More recent work, however, by West and Funnell in East Anglia has given very strong grounds for supposing that at least two interglacial periods of comparable magnitude preceded the Cromer Forest Bed and were separated from that and from one another by glacial episodes.

The pollen analytic evidence permits an effective reconstruction of the climatic sequences during each of these interglacial periods and in addition leaves very little doubt that the so-called 'Post-glacial period' in which we live has all the characteristics itself of an interglacial period. My own reading of W. B. Wright's theory of isostasy would suggest that during each interglacial the glaciated north of Britain was depressed and that in the ensuing interglacial it experienced isostatic recovery. Correspondingly, during each glaciation the withdrawal of water from the oceans of the world caused a depression of ocean-level of the order of a few hundred feet, recovery from which followed when the general melting of the ice sheets followed at the end of the glaciation and in the early part of the ensuing interglacial period.

Apart therefore from any longer term tectonic processes, one might expect that in the middle and later part of each interglacial both isostatic and eustatic recovery would have been largely accomplished and that there would be time for the cutting of beaches during a period of prevailing warmth. This is the present position, and one can suppose that this has been approximately reached in each of the interglacials. It need not therefore surprise us to find that beaches in the north and south of Britain which are referable to the warm stages of the interglacials should occur at approximately similar heights in relation to present sea-level: to find this is not to invalidate the theory of isostatic elevation.

If we desire to examine the process of isostatic elevation, it is extremely advantageous to concentrate on the evidence of events since the last glaciation, that is to say within the present interglacial period. Here we have not only the advantage that the geological features are largely undestroyed by subsequent events, but we are within the period in which the powerful tools developed in the study of Post-glacial Quaternary geology can give us most assistance, particularly pollen analysis and radiocarbon dating. The Sub-department of Quaternary Research in Cambridge has been occupied for some time past, in association with numerous geologists interested in problems of relative movement of land- and sea-level, in securing precise evidence of the progress and extent of such changes. Some attention has been directed to preliminary findings in the Croonian Lecture[2] and others are in course of preparation for publication. It seems incontestable that major eustatic restoration of ocean-level after the last glaciation had been completed by about 3000–4000 B.C. and that the so-called "25 ft raised beach" of Northern Ireland and southern Scotland was being formed in the period broadly between 6000 and 3000 B.C. during which the rates of isostatic recovery and eustatic rise of sea-level were very similar. It is further apparent that whereas in south-west England and central Wales there has been little uplift since that time, in Scotland and Northern Ireland the beach has been elevated to its present height above sea-level.

Since Dr. Baden-Powell brings in as a "nearly related problem" the tilting of the southern parts of Britain, it may be added that since the end of the maximum eustatic rise of ocean-level, pollen analysis, radiocarbon dating and archæology alike indicate that there must have been substantial relative downwards tilt of the Fenlands and the southern North Sea relative to the west of Britain. That such a tilting may also be, in part at least, due to isostasy, is suggested by the fact that in the region of The Netherlands three phases of marine transgression are recorded for the last three interglacials, and all have deposits at overlapping levels. These are represented by the Holstein Sea, the Eem Sea and the Post-glacial inundations recorded in the Dutch coastal regions and finally by the formation of the Zuyder Zee.

This evidence sufficiently indicates, I believe, the way in which the reality and character of the isostatic uplift of

Scotland can be most effectively explored. It is quite true that hitherto our evidence for the stages of land- and sea-level adjustment prior to the formation of the 25-ft. beach remain largely unexplored; but the new dating techniques, applied to situations where the field geology is adequately known, should give us the means of extending this knowledge to the earlier stages. I believe that it is against the background of this kind of information that Dr. Baden-Powell's most valuable analyses of the interglacial littoral faunas should be viewed.

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Prof. Godwin's discussion of my article on the isostatic recovery of Scotland is most welcome, and I am grateful to him for raising several points of interest. But I must start by assuring him that I am not considering this isostatic recovery as "a single process extending over a period of time which embraces that of the Tyrrhenian beaches" as he puts it. On the contrary, I am only discussing crustal movement which has occurred since the last glaciation in Britain, emphasizing the effect this movement would have on the very much earlier Tyrrhenian beaches. (The possibility mentioned by Prof. Godwin of isostatic recovery during interglacial periods raises interesting theoretical questions, but I have never yet seen evidence for such a thing in the field, though of course this does not mean that such events have not taken place.)

As regards Prof. Godwin's reference to the so-called 25-ft. raised beach of Scotland and its relative height farther south, it is well known that the Sub-Department of Quaternary Research in Cambridge has been working hard on this problem for some time; but the height of the 25-ft. beach is not exactly what I was discussing in my article. The point I was trying to make was that W. B. Wright, in his original work reported in the *Geological Magazine* (1911), emphasized the distribution of the 100-ft. beaches in Britain as an important part of his theory about isostasy, believing these beaches to be "grouped round a centre in the Scottish Highlands"; but the work described in my article shows that part of Wright's theory was based on incomplete evidence. For example, according to Wright's map (p. 99), the distribution of both the 'preglacial' and the 'late glacial' 100-ft. beaches is restricted to Scotland, indicating that there is no such thing as a '100-ft. beach' in England. One look at such deposits as the Sussex raised beaches and the Nar Valley clay shows us that we have evidence which was never used by Wright; in this case he was wrong in thinking that a 100-ft. beach ('preglacial') in Scotland descends abruptly to a 10-ft. 'preglacial' beach in southern England.

A minor point made by Prof. Godwin rather mystified me, when he writes that beaches formed during warm interglacial periods could have similar heights in relation to present sea-level, and that this does not invalidate the theory of isostatic elevation. How can this be? Surely uneven uplift of the 25-ft. beach must also tilt the earlier beaches, and the answer must be that so far the measurements of the Tyrrhenian beaches have been too coarse to show the relatively slight movements which have taken place since the 25-ft. beach was formed.

In conclusion, I agree with Prof. Godwin that there has been some differential movement between Scotland and parts of England during the Holocene. I must stress this particularly, as we know that the 25-ft. beach of Scotland is a common feature in the north, whereas this beach is conspicuously absent at that height in southern England.

The single point which I was trying to make in my article was that whatever crustal movement has taken place during the Holocene has been much less than the 100 ft. or so suggested by Wright. It may seem strange to divide a problem like this into parts and try to solve each part in turn, but this seems the best way to set about this complicated subject.

Sir Archibald Geikie once wrote an article with the amusing title: "The Raised Beach of Britain and how Scotland has risen in the World". Perhaps what we are really discussing is whether Scotland has been going up or whether parts of England have been sinking. Presumably W. B. Wright and others have been too ready to assume that both movements have occurred at the same time. As I understand it, Depéret thought that Scotland is at its normal level, with the '25-ft. beach' re-appearing at that height in neighbouring parts of Europe and in the Mediterranean area. In that case a slow abnormal sinking of part of England may be nearer the truth than an extensive rise in the north.

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MINERALOGY

Strength and Structure of Flint

In view of the widespread prehistoric use of flint for making tools and weapons, it is of interest to compare its structure and strength with those of present-day ceramic materials. The physical properties of stone tool materials have been investigated by Mary Ellen Goodman[1], who examined and compared the relative density, toughness, resilience and hardness of limestone and a number of siliceous materials. However, the transverse bending strength, needed for quantitative comparison with ceramic materials, was not determined.

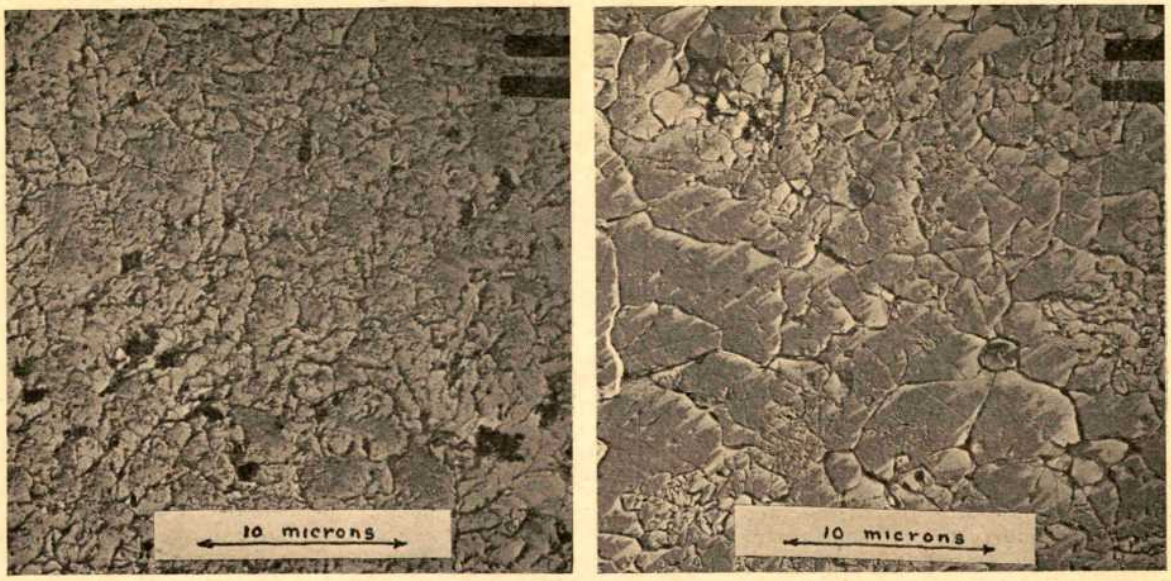
Flint is described as a "tough, very compact, microcrystalline material"[2]. The colour varies from light, almost transparent, to grey or black. Weymouth[3] has shown that flint has a typical density of 2·62 g/c.c., and shows only the X-ray diffraction pattern of quartz. Since the density of quartz is 2·65 g/c.c., the material was estimated to have a porosity of about 1 per cent by volume. Microscopic examination showed that flint was similar to chalcedony. The colour was believed to be the result of light scattering from extremely fine pores.

I have myself examined flint cut from the most homogeneous appearing sections of a boulder of English flint obtained through a commercial source; also chalcedony taken from a geode which was almost filled with a brownish, nearly transparent, dense, homogeneous-appearing silica, kindly furnished by Alvin B. Stiles. For comparison, fused silica of the transparent optical-grade, as well as of the opaque type used for laboratory ware, were tested.

Test bars 0·070 in. square, 0·75–1·5 in. long were cut with a '180 grit' diamond saw of 20 mils thickness on a wafering machine; this gave an almost optically smooth finish. Transverse bending strength was measured by applying the loading at a single point at the centre of a test span 0·75 in. long.

Microstructure was examined by embedding specimens in plastic, polishing, etching with dilute hydrofluoric acid, and examining with a metallurgical microscope at a magnification of 500. For electron micrographs, polished sections, etched with hydrofluoric acid, were replicated in two stages with cellulose acetate and carbon, then shadowed with chromium.

Observed transverse rupture strengths of flint and chalcedony are compared in Table 1 with those of some typical ceramics and natural stone materials. It will be noted that the translucent types of chalcedony and flint are about equally strong and that both are stronger than the ceramics, fused silica and porcelain. They are much stronger than the other natural stone materials that are



A B

Fig. 1. Electron micrographs of replicated surfaces of English flint. *A*, dark brown translucent region; *B*, light brown, opaque region

listed. Flint is almost as strong as modern microcrystalline ceramics made by devitrification of glass.

The structure of the dark translucent flint was compared with that of the light brown opaque type. The opaque material occurred as clearly defined, more or less spherical regions within the matrix of the darker translucent mass of the boulder. The translucent regions appeared darker in colour because light penetrated more deeply and was thus absorbed. The spherical shape of the opaque zones suggested that a process of recrystallization might have occurred as though the masses of crystals were radiating from nuclei. This is sometimes seen in the partial devitrification of silica glass.

X-ray diffraction patterns of English flint showed only the presence of quartz. The diffractometer curves showed marked line-broadening in the case of the stronger, dark-brown, translucent specimens, for which an average crystallite size of 120 mμ was estimated. The weaker, light, opaque flint gave sharp lines with no measurable broadening, indicating that the crystal size was more than 500 mμ.

Optical micrographic examination of polished sections in reflected light indicated that the grain size, after etching with hydrofluoric acid, was of the order of 2–10μ in the light opaque areas, but less than 1μ in the dark translucent areas. Electron micrographs of the surfaces of the dark, translucent (*A*) and light, opaque areas (*B*) are shown in Fig. 1. Although the density of these materials ranged from 2·55 to 2·61 g/c.c., suggesting 1·5–4·0 per cent porosity, no pores could be clearly identified. These electron micrographs confirm that the stronger, more translucent type of material has a finer grain structure.

Table 1. Transverse Rupture Strengths

Material	Type	No. of test bars	Strength (lb./in.²) Average		Maximum
English flint	Dark brown, translucent	7	27,000		30,100
English flint	Light brown, opaque	6	17,100		19,800
Chalcedony	Light, translucent	5	23,500		29,100
Chalcedony	Dark, translucent	6	24,000		29,400
Fused silica	Transparent	4	18,400		21,000
Fused silica	Opaque	8	10,300		12,200
Porcelain (ref. 4)	Electrical grade	–	5,000	Range to	15,000
'Pyroceram' (ref. 4)	Code 9606		32,000		
Common stone materials (ref. 5)					
Limestone			500	to	2,000
Marble			600	to	4,000
Serpentine			1,300	to	11,000
Slate			6,000	to	15,000
Granite			1,380	to	5,500
Sandstone			700	to	2,300

Specimens of dark and light regions of chalcedony were both translucent; this indicates that the colour is related to impurities rather than recrystallization. Electron micrographs of the chalcedony showed a fine grain structure indistinguishable from that of the translucent flint. Also, X-ray diffraction indicated a quartz crystallite size of about 200 mμ. Thus the translucent forms of chalcedony and flint appear to be identical.

It is thus concluded that the high strength of flint is a result of its very fine grain structure. The choice of flint by primitive man was undoubtedly based on its outstanding strength in comparison with other stones, as well as its characteristic cleavage behaviour.

I thank D. P. Ferriss, who carried out the petrographic and electron micrographic portion of this study, and O. E. Schupp, who conducted the X-ray diffraction measurements.

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PHYSICS

A Ready Method for finding Eccentric Dipole Time

Eccentric geomagnetic dipole[1] time at an observatory (*O*) is defined for present purposes as the difference in eccentric dipole longitude (φ') of the Sun (as distinct from the sub-polar point) and the observatory being measured eastwards from the Sun to the observatory, that is:

$$t_{ecc.} = \varphi'_o - \varphi'_{sun} \qquad (1)$$

Elsewhere[2,3] it has been shown by different approaches that $t_{ecc.}$ runs parallel to ordinary geomagnetic time (a centric system) differing by a constant C_o, which is a characteristic of the observatory for the particular epoch of the Earth's magnetic field. Thus, also:

$$t_{ecc.} = \varphi_o - \varphi_{sun} + C_o \quad (2)$$

where φ represents geomagnetic longitude.

$$C_o = \varphi'_o - [\varphi'_o]_{X=Y=Z=0} \quad (3)$$

where[3]:

$$\tan \varphi'_o = \frac{0{\cdot}854\ (\sin\theta_o \cos\varphi_o - X) - 0{\cdot}495\ (\sin\theta_o \sin\varphi_o - Y) - 0{\cdot}160\ (\cos\theta_o - Z)}{-0{\cdot}515\ (\sin\theta_o \cos\varphi_o - X) - 0{\cdot}848\ (\sin\theta_o \sin\varphi_o - Y) - 0{\cdot}126\ (\cos\theta_o - Z)}$$

$X = -0{\cdot}0577$, $Y = 0{\cdot}0320$, $Z = 0{\cdot}0184$ for epoch 1955·0 and (θ_o, φ_o) are geographic co-ordinates of the observatory.

The point of the present communication is to indicate that eccentric dipole time can be found to a useful accuracy for many purposes by a semi-graphical method. Knowing the universal time, the equation of time and the solar declination, the position of the sub-solar point can be found on a map containing both geographic and ordinary geomagnetic grids for the epoch. $\varphi_o - \varphi_{sun}$ is then easily and quickly found. The constant C_o for the observatory, calculated once, is available then for all determinations of $t_{ecc.}$ in that epoch.

Note, in passing, that grids of eccentric dipole co-ordinates cannot be used directly in this manner to find $t_{ecc.}$ because the longitude of the sub-solar point (as given by Greenwich hour angle and declination) is not in the eccentric dipole longitude plane containing the Sun.

Recently Brice and Ungstrup reported[4] the use of a new and different type of time. They define 'auroral' time for a hemisphere by a centric system the axis of which passes through the 'centre' of the auroral zone of that hemisphere. They thus arrive at two different centric systems of time, one for each hemisphere. This dichotomy seems unwieldy and perhaps unnecessary in view of the fact that the eccentric dipole poles[1] are at approximately the centres of the respective auroral zones[5,6] together with the established concept of the eccentric geomagnetic dipole. Eccentric dipole time has the advantages of 'auroral' time combined with the desirable element of magnetic conjugacy, which is known to occur with many high-latitude phenomena. It has also the advantage of global applicability.

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Electrical Conductivity of Ammonium Perchlorate

Zirkind and Freeman[1] have pointed out the increase in the electrical conductivity of ammonium perchlorate crystals on irradiation with 0·637-MeV cæsium-137 γ-rays. In this communication, results are reported from an investigation of the electrical conductivity of ammonium perchlorate solid as a function of temperature and gaseous atmosphere. Data concerning the electrical conductivity of ammonium perchlorate were not found in the literature.

Reagent grade ammonium perchlorate was twice recrystallized from distilled water. The samples were dried overnight at 110° C and compressed into disk-shaped pellets, 0·5 in. in diameter and 0·125 in. in depth. The pellets were annealed overnight under a vacuum of 0·01 mm mercury at 110° C. This treatment was found necessary in order to obtain reproducible results. Silver paint electrodes were used. The ends of the disks were completely silvered. Two leads were connected to the electrodes by means of the silver paint. One lead consisted of a chromel-alumel thermocouple junction, which also served to measure the temperature of the pellet. The other lead is a copper wire. The wired disk is placed in a 'Pyrex' vessel which is heated by a resistance furnace. Resistivity measurements were made with a General Radio 1230 *ADC* amplifier and electrometer.

The electrical conductivity of different samples varies within a factor of 2 under similar experimental conditions and temperature. This variation may, in part, be due to the irreproducibility of the surface contacts and of the mechanical treatment which is known to influence the conductivity of solids[2]. In this work the temperature dependency of the electrical conductivity under vacuum in oxygen and argon was determined. Each complete series of experiments was conducted on a single sample. The conductivity measurements were made over the temperature range of 25° C–125° C. Furnace temperature was raised continuously at the rate of 2° C/min.

The three curves in Fig. 1 show the electrical conductivity of ammonium perchlorate as a function of temperature under vacuum (0·01 mm mercury; in an atmosphere of oxygen (pressure 1·00 mm mercury); and in argon (pressure 1·00 mm mercury). These curves show that argon and oxygen depressed the electrical conductivity of ammonium perchlorate approximately one and two orders of magnitude, respectively. The activation energies determined from curves I, II and III were 1·10, 1·15 and 1·40 eV, respectively. The calculation was based on the relationship:

$$\sigma = \sigma_0 \exp(-E^*/RT)$$

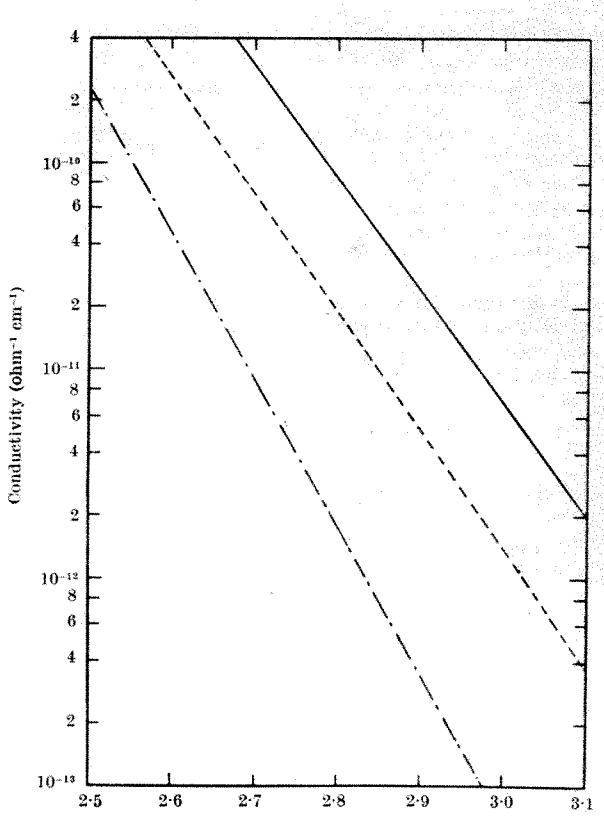


Fig. 1. Temperature dependency of the electrical conductivity of ammonium perchlorate pellets

	Pressure (mm mercury)	Atmosphere
———	0·01	Air
– – – –	1·00	Oxygen
—·—·—	1·00	Argon

where: σ = electrical conductivity; E^* = activation energy; R = Boltzman constant; T = absolute temperature.

It was found that the electrical conductivity results were reproducibly reversible to within ±10 per cent of the original values in going repeatedly from vacuum to argon to oxygen. An activation energy of about 1·1–1·4 eV appears reasonably close to what one may expect for the ionic conductivity of ammonium perchlorate. In the case of the alkali metal halides[3], the activation energy is higher, approximately 2·0 eV as may be expected. This may be an indication that the electrical conductivity of ammonium perchlorate is primarily ionic; however, this cannot be concluded based on the foregoing results. If it is ionic conduction then oxygen and argon are probably trapping cation vacancies. If it were electronic then there would be a transfer of electrons from the solid to the adsorbed gases. Experiments are being conducted to resolve these questions.

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Spectra of Giant Pulses from a Ruby Laser

THE spectral character of the giant pulse[1] emitted by a ruby laser has been investigated to determine the suitability of this source for plasma light scattering experiments. A 'Trion *LS*-4 laser' system consisting of a 10 cm × 1·3 cm × 1·3 cm total internal reflexion ruby with 90° *c*-axis orientation, pumped by means of a 10,000 joule spiral xenon flash-lamp, has been modified to produce giant pulses by the addition of a Kerr cell, a Nicol prism, and a 48 per cent reflectivity dielectric mirror acting as external reflector. Giant pulses which last for less than 40 n sec were produced by switching the Kerr cell with a low inductance thyratron circuit. The Kerr cell was normally gated open for 1 μsec. Throughout the experiment the pumping energy delivered to the flash-lamp was maintained at 6,700 ± 300 joules, which is about 25 per cent above threshold for normal relaxation oscillations in this crystal at the temperature at which it was operated, namely 126 ± 3° K. High-resolution spectroscopy of these pulses was carried out with a variable gap silvered Fabry–Perot interferometer which had a resolving limit of 0·011 cm^{-1} for 1 cm separation of the mirrors. To ensure that only giant pulse light was recorded, a Barr and Stroud framing camera operated at a speed of 10 μsec per frame was used to photograph the interference fringes on Gaevert infra-red sensitive film ('Scientia 52*A*86').

With Fabry–Perot spacings of 2·78 cm and 4·05 cm, giving free spectral ranges of 0·18 cm^{-1} and 0·12 cm^{-1} respectively, we obtained fringe patterns (Fig. 1) which typically fill between 1/2 and 2/3 of an order. They are composed of several lines, usually 4 or 5 although occasionally 3 or 6 have been observed. Relative wave-number spacings and intensities varied from pulse to pulse.

D'Haenens and Asawa[2] have found that the relaxation oscillations of a ruby laser often consist of components which they have identified with axial modes within the crystal cavity. Moreover Hughes[3] has shown that an individual laser spike may contain several modes. We have tried to relate the line structure which we have observed in the giant pulse fringes to possible longitudinal modes in the laser cavity. The condition that a standing wave be set up in the axial mode in a cavity of optical length S is that an integral number, N, of half-wave-lengths can be fitted into S, that is, $2S = N\lambda$. It follows that a shift ΔN in the number of half-wave-lengths gives rise to a shift in wave number, $\Delta\bar{\nu}$, given by $2S\,\Delta\bar{\nu} = \Delta N$.

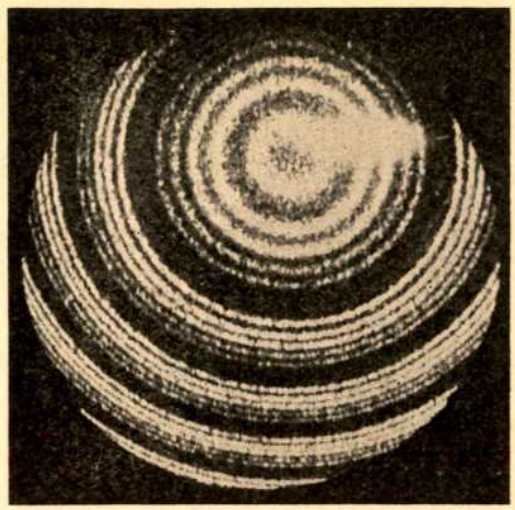


Fig. 1. Fringe pattern of ruby laser giant pulse scattered by a ground-glass screen. Interferometer separation 2·78 cm. Framing camera operated at 10 μsec per frame

We have examined the spacing of the lines contained within a single order and found that the wavenumber separations are frequently small integral multiples of the characteristic $\Delta\bar{\nu}$. To confirm this result the mode separation was varied by changing the optical length of the cavity. As an example we present the results obtained from two pulses both of which resulted in fringes composed of five constituent lines. One of these is illustrated in Fig. 1. In the first case, the optical length of the laser cavity was 77 cm, corresponding to a characteristic $\Delta\bar{\nu} = 0{\cdot}0065$ cm^{-1}; in the second it was 91 cm, corresponding to $\Delta\bar{\nu} = 0{\cdot}0055$ cm^{-1}. Table 1 lists the separations between pairs of lines in the fringes in units of the appropriate $\Delta\bar{\nu}$. The letters *a*, *b*, *c*, *d*, *e* refer to the lines in the fringe in order of increasing wave-length. It will be observed that apart from a set of four pairs in both cases, arising in each instance from a single line, that labelled '*b*', the values of ΔN are very close approximations (measuring error ± 0·03) to integers, and we regard this as evidence that they arise from axial cavity oscillations. Nearly every family of axial modes which we have examined seems to be accompanied by a line, like '*b*' in Table 1, which cannot be fitted into the mode pattern. Occasionally more than one outsider of this sort appeared, and once we observed two distinct sets of modes accompanied by one outsider which was a member of neither.

We have not found a family of more than four modes nor have we found a pair of modes separated by less than 3 $\Delta\bar{\nu}$. The limit of resolution for mode separation was set

Table 1

Exp. 1 $\Delta\bar{\nu}$ = 0·0064 cm^{-1}		Exp. 2 $\Delta\bar{\nu}$ = 0·0055 cm^{-1}	
Line pair	ΔN	Line pair	ΔN
ab	4·30*	*ab*	2·31*
ac	8·01	*ac*	5·04
ad	12·0	*ad*	9·00
ae	15·1	*ae*	14·06
bc	3·71*	*bc*	2·68*
bd	7·74*	*bd*	6·67*
be	10·9*	*be*	11·7*
cd	3·95	*cd*	3·99
ce	7·10	*ce*	9·04
de	3·10	*de*	5·05
	± 0·03		± 0·03

by fringe width, which varied between 0·004 cm^{-1} and 0·018 cm^{-1}, so it is possible that separations of one or two $\Delta\bar{\nu}$ may not have been detected. This, however, would not explain those lines which did not fit into a mode family as their widths were in general not broad enough to contain multiplets. No family of modes extending over a range of wavenumber greater than 18 $\Delta\bar{\nu}$ has been found. That modes separated by higher multiples of $\Delta\bar{\nu}$, but disguised by overlapping of orders in the Fabry–Perot pattern, were not present was confirmed by carrying out a number of trial shots with an interferometer spacing of 2 cm and 0·1 cm. The former spacing would have brought to light members of a family of axial modes over a range of about 40 $\Delta\bar{\nu}$, and the latter, which produced fringes the widths of which were at the limit of instrumental resolution, served to show that over a range of about 800 $\Delta\bar{\nu}$ no more modes were present.

The occurrence of single unrelated lines and more than one family of axial modes in a single giant pulse may be due to different parts of the laser oscillating independently. It is interesting to note that if the fringes are considered as arising from single modes, the observed widths are consistent with mode lifetimes of between 2 and 8 n sec.

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ENGINEERING

Reversed Plastic Flow during the Unloading of a Spherical Indenter

The process of indentation of a metal surface by a hard spherical indenter has been examined in considerable detail by Tabor[1] and others. If the indenter makes contact with the surface over a circular area of radius a under the action of a load W, the mean contact pressure $p_m = W/\pi a^2$. Tabor showed that plastic yielding begins beneath the surface of the metal when p_m reaches the value 1·1 Y (where Y = yield stress of the metal in simple tension). As the load is increased and a marked plastic indentation develops, the mean pressure approaches a value which is independent of the load given by:

$$p_m = cY \qquad (1)$$

where c has a value of approximately 3. This is the state of affairs in a Brinell hardness test.

After the load and indenter are removed the indentation remains, although its curvature is slightly less than that of the indenter itself. The change in curvature is attributed by Tabor to elastic recovery of the material during unloading and his measurements are consistent with this view. If the unloading process is elastic then reloading to the same load should produce no further plastic deformation. Tabor observed no increase in the size of the impression during five repeated applications of the same load. He concluded, therefore, that the deformation after the first application of load was entirely elastic.

Some recent experiments made by Tyler, Burton and Ku[2], in which an impression has been observed to grow in size under the action of a load repeated many times, have led to Tabor's conclusions being re-examined. It is the purpose of this communication to show, on theoretical grounds, that reversed plastic yielding would be expected during the unloading of an indenter, and hence that repeated loading would cause a continuous cycle of plastic deformation.

When the load is first applied the mean contact pressure is given by equation (1), but no exact theory predicts the distribution of this pressure over the contact surface. Tabor[1] quotes the approximate analysis of Ishlinsky to demonstrate that the actual pressure distribution will not differ greatly from the elliptical distribution of Hertz for elastic contact. The unloading process may then be thought to consist of the subsequent application of an equal negative pressure to the contact surface.

At the end of the loading process the material beneath the surface, lying on the axis of symmetry, will be in a state of plastic flow, so that by the Tresca maximum shear criterion of yield:

$$\sigma_z - \sigma_r = Y \qquad (2)$$

where σ_z is the stress (compressive) acting normal to the surface and σ_r is the stress (also compressive) acting parallel to the surface.

To represent unloading we apply a Hertzian distribution of tension to the surface having a maximum intensity:

$$p_0 = \frac{3}{2} p_m = \frac{3}{2} cY \qquad (3)$$

If the unloading process were perfectly elastic this surface tension would result in a maximum principal stress difference at a point beneath the surface on the axis of symmetry the magnitude of which is given by the Hertz theory, that is:

$$(\sigma_z - \sigma_r)_{\max} = 0{\cdot}62 p_0 = 0{\cdot}92 cY \qquad (4)$$

in which σ_z and σ_r are both tensile.

To find the state of stress corresponding to complete unloading we superpose the stresses given by equations (1) and (4), having due regard for sign, with the result:

$$\sigma_z - \sigma_r = (1 - 0{\cdot}93c)Y \qquad (5)$$

Now reversed yielding would be expected if $\sigma_z - \sigma_r$ in this equation exceeds $-Y$, that is, if:

$$c > 2{\cdot}15 \qquad (6)$$

As Tabor has shown by many tests, c has a value lying between 2·7 and 3. We would therefore expect reversed yielding to occur in a small region beneath the surface during unloading from the fully plastic state. This yielded region will be fully contained and will not make a noticeable change in the surface profile, which explains why it was not detected by Tabor's profile measurements.

By the same argument, a second loading to the same maximum load will cause a reversal of yield in the subsurface region. Under cyclic loading, therefore, we would expect a repeated cycle of plastic deformation. This could lead to either a progressive enlargement of the indentation or failure by a fatigue process.

The foregoing argument is based on the assumption that the metal has an equal yield strength in loading and unloading. Actually the Bauschinger effect will make reversed yielding during unloading even more certain.

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METALLURGY

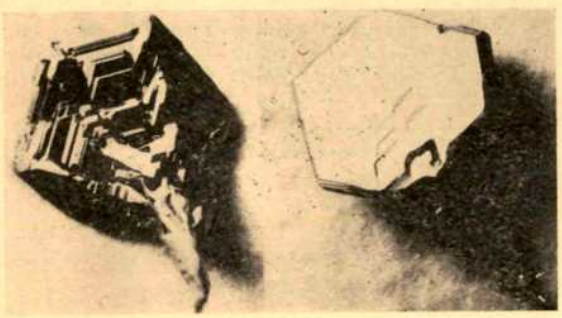
Crystal Growth of Titanium

TITANIUM, grown by the electro-refining method from molten salt at temperatures below the transformation point (870° C), crystallizes in various dendritic shapes, namely, needles and flakes. It crystallizes in various forms based on single crystals or on compound crystals, and each surface is bounded by a definite plane.

So far, crystallographic investigations have been carried out only on crystals of simple shape[1-3] and grown in a fused salt bath at a higher temperature than the transformation point[4]. We have carried out systematic investigations of hexagonal titanium crystals of complicated shapes growing under various conditions of electrolysis.

Crystals were grown in a fused salt bath of alkali halide by electrolysis, using impure titanium (Brinell hardness No. 150–200), as a soluble anode, at temperatures of 850°–680° C and at current densities of 30–300 amp/dm². Under these conditions, flake crystals grew to a size of 1 mm in thickness and about 10 cm² in area, while needles grew as large as 1–2 mm in diameter and a few centimetres in length.

A flake crystal grown from the molten salt is shown in Fig. 1, where development of the basal plane can be seen:



a *b*

Fig. 1. Development of the basal plane in a titanium crystal. *a*, Rapidly growing side with marked steps; *b*, slowly growing side. (× *c*. 2·5)

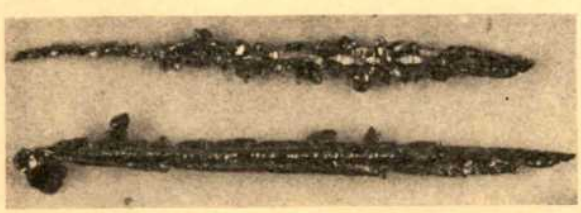


Fig. 2. Titanium crystals grown from molten salt by electrolysis. The crystals grown in needles are parallel to < 10$\bar{1}$1 > direction. (× *c*. 2·5)

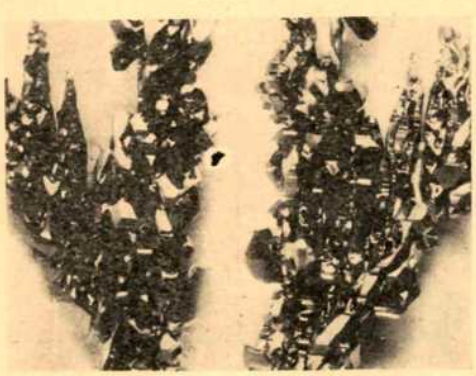


Fig. 3. A dendrite of titanium with two growth axes. (× 2)

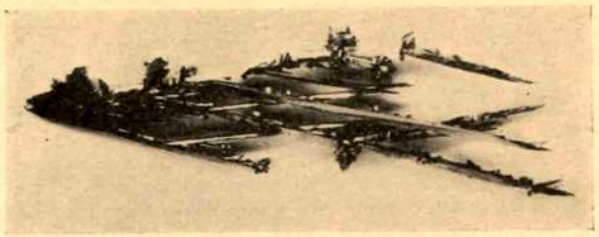


Fig. 4. Dendrites of titanium grown by electro-refining. Each branch is parallel to < 1$\bar{1}$03 >. (× 1)

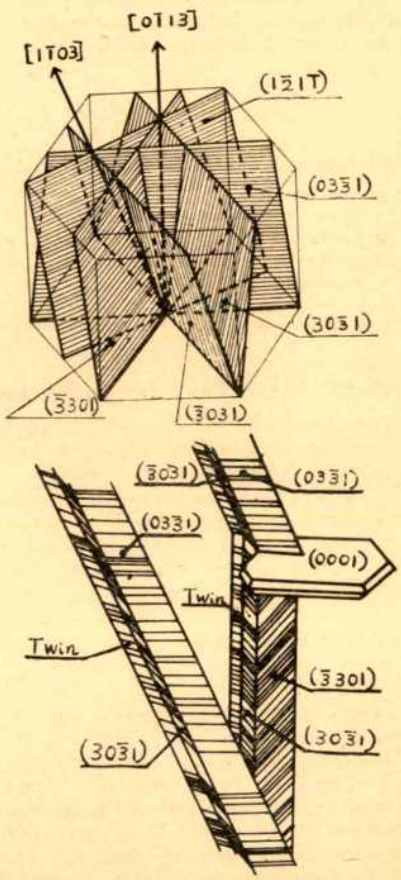


Fig. 5. Schematic representation of a dendrite with < 1$\bar{1}$03 > growth axes. Each branch consists of two thin, twin-related crystals

the step planes of the crystal are {1$\bar{1}$02}, {1$\bar{1}$03} and {1$\bar{1}$04}, while the growth direction is parallel to < 11$\bar{2}$0 >.

In titanium, < 11$\bar{2}$0 > is a close-packed direction, and one would expect that the direction of growth of needle crystals would coincide with this direction; but this was seldom observed. Most needle crystals obtained from the molten salt have a shape similar to that shown in Fig. 2, where it should be noted that the direction of growth is parallel to < 1$\bar{1}$01 >: such a needle crystal is bounded by the ($\bar{1}$101), (01$\bar{1}$1) and ($\bar{1}$012) faces.

The dendrite shown in Fig. 3 consists of needles that grew in the [10$\bar{1}$1] and [2$\bar{1}\bar{1}$2] directions on the ($\bar{1}$012) plane; these are bounded by the ($\bar{1}$101), ($\bar{1}$012), and (0$\bar{1}$11) faces.

In some cases, one obtains a dendritic crystal, as is shown in Fig. 4. These are characterized by pillar-shaped needles (such a compound crystal is shown schematically in Fig. 5).

In general, we found that the crystals grown from the high-purity salt were bounded by the basal planes and planes of the type [10$\bar{1}l$] (here l can be 1, 2, 3 or 4). The

crystals grown from the impure salt were bounded by the basal planes, {10$\bar{1}$1} planes and prismatic planes—such as {30$\bar{3}$1}, {30$\bar{3}$2}, etc. Crystals grew in thin plates when they were deposited from the molten salt at temperatures of 750°–850° C and at low current densities (less than 100 amp/dm²); in such cases their directions of growth were parallel to the basal plane. However, crystals grew in needles when they were deposited from the molten salt at temperature below 750° C with current densities greater than 150 amp/dm²; under such conditions their directions of growth were parallel to $<1\bar{1}01>$, $<1\bar{1}22>$ or $<1\bar{1}03>$.

Growth twins were frequently observed, their twinning planes being {10$\bar{1}$1}, {10$\bar{1}$2}, and {11$\bar{2}$1}. It is unusual to observe the {10$\bar{1}$1} plane as a twinning plane in the case of growth twin, and so far as we know such a situation has not been previously reported for deformation twins[3,5].

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Liquid Metal Heat Transfer in Packed Beds

Investigations of heat transfer in liquid metal irrigated packed beds counter current to gases do not appear to have been, so far, reported in the literature. Yet, the problem is of unquestionable interest in extractive metallurgy, as was shown by Shu-p'ei[1] in his discussions on the iron blast furnace.

The apparent absence of liquid metal heat transfer investigations in packed beds may perhaps be explained by the experimental difficulties of preventing the occurrence of simultaneous mass transfer effects and/or separating the two phenomena analytically from the combined results. It is, however, suggested that useful insight into the foregoing phenomenon could be obtained with ambient, or slightly elevated temperature systems in which the Dufour effect could be justifiably considered unimportant. Mass transfer studies by Warner[2] concerning the absorption of zinc vapour in molten lead flowing through a bed packed with steel Raschig rings have shown the usefulness of this type of investigation. The extension of his work to heat transfer studies in similar systems appeared logical. To this end, in co-operation with Dr. N. A. Warner at the University of New South Wales, I have carried out some heat transfer experiments in beds of different packings irrigated with mercury counter current to various gases. Although many difficulties had to be overcome and some problems still remain to be solved, the results of the studies indicate, *inter alia*, that the transfer is predominantly gas phase controlled and the data could be correlated, with some degree of accuracy, by a Nusselt type expression. The actual correlation featured a liquid hold-up term in addition to the usual moduli which, except for Prandtl number, were modified to account for the unknown heat transfer area involved.

Investigations are at present in progress using a fusible alloy to study the various unsolved problems of heat transfer investigations with mercury. These include the elucidation of the part played by the packing in the heat transfer mechanism and by the various parts of the liquid hold-up, and, the evaluation of the exponent of $Cp\mu/k$, which in the investigations with mercury was assumed to be equal to 0·33.

One of the findings of the work on mercury that is unlikely to be altered appreciably by future results is the apparent heat transfer efficiency of these systems when compared with tubular exchangers or certain fluidized beds and spray columns.

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Twinning at 77° K of 'Flash-annealed' Niobium

Niobium is known to twin at 77° K, and for the purest reported[1] the estimated lower yield stress was ~40 kg mm^{-2} and the maximum tensile stress only a little higher. For specimens of this purity the lower yield stress at room temperature was ~7 kg mm^{-2}.

At about this strain rate the yield stress of niobium free of impurities is predicted[2] to be ~2·5 kg mm^{-2} and this is postulated to be the Peierls–Nabarro force. Considering this model, the yield stress of this 'pure' niobium at 77° K should be ~50 kg mm^{-2}.

Using a 'flash-annealing' technique the proportional limit of niobium foils strained at 1 per cent min^{-1} was lowered to ~3·5 kg mm^{-2}. This material was strained at 77° K (liquid nitrogen bath) and one or more of the following phenomena was found to occur for specimens taken from the same foil. Proportional limit was some-

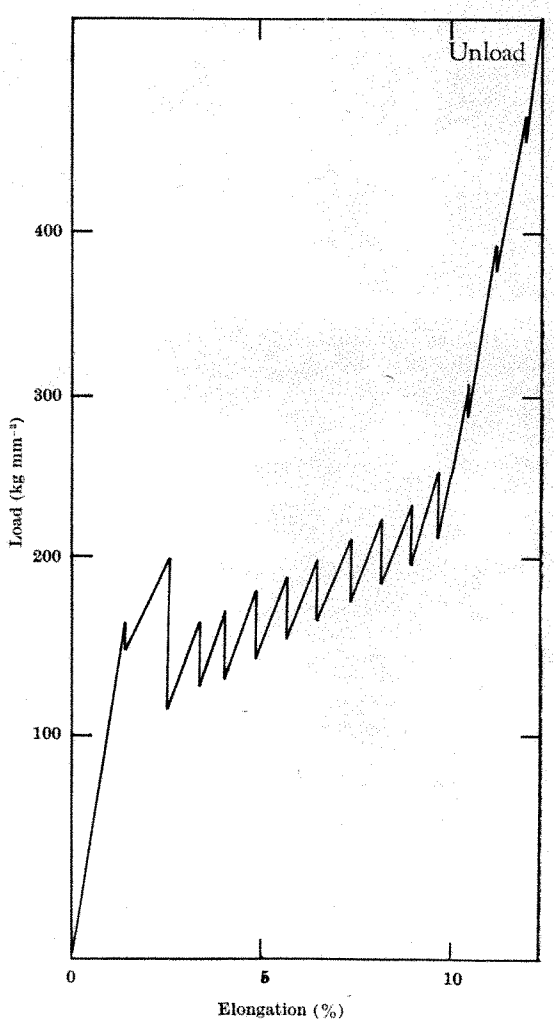


Fig. 1. A load-elongation curve at 77° K of 'flash-annealed' niobium

times found to be ~50 kg mm^{-2}, but twinning occurred before and/or after this stress value. As twinning sometimes commenced at a stress level of ~20 kg mm^{-2}, the magnitude of the Peierls–Nabarro force cannot be as great as originally supposed if a dislocation model for twinning is assumed. Maximum tensile stress was sometimes only a little higher than 50 kg mm^{-2}, but with other specimens profuse twinning continued for appreciable extension. The maximum stress recorded (Fig. 1) was ~500 kg mm^{-2} when the specimen was unloaded, this being the limit of the load cell. Twinning was identified metallographically at all stress values.

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RADIOCHEMISTRY

A Simple Preparation of 5-Iodo-2′-deoxyuridine labelled with Iodine-131 using Iodine Monochloride

Methods so far described for the preparation of 5-iodo-2′-deoxyuridine are based either on the iodination of deoxyuridine by free iodine in the presence of an oxidizing acid or free iodine in an alkaline medium[1–3]. The compound labelled with iodine-131 has been obtained by adapting the iodination in acid[1,4] but the specific activity of the product has not been reported.

The method described here gives consistent yields of 65–70 per cent of 5-iodo-2′-deoxyuridine and iodine-131 uptakes of 55–60 per cent of the iodine-131 used. The method is suitable for preparing mg quantities having a high specific activity.

Carrier-free Na ^{131}I in aqueous solution, obtained from the Radiochemical Centre, Amersham, was placed in a conical 10-ml. stoppered test-tube. The water was evaporated by placing the tube in a water-bath at about 40° and gently blowing a stream of nitrogen over the solution. In order to avoid loss of radioactivity the nitrogen was turned off immediately after the last trace of water had disappeared. For pilot experiments approximately 0·05 μc. was used and in preparations for biological use between 500 μc. and 1 mc. were used.

For each preparation a fresh solution of iodine monochloride[5] in acetic acid, 0·0162 g/ml., was made. For the iodination of 1 mg deoxyuridine, 0·066 ml. of this solution containing 1·5 equivalents was used. The solution was placed in the test-tube which was stoppered and heated in a water bath at 60° for 1 h to effect exchange of ^{131}I and stable iodine. The tube was cooled to room temperature and 1 mg deoxyuridine (grade II obtained from Sigma Chemical Co.) was added. The tube was stoppered and heated in the water bath for a further 1½ h at 60° with occasional gentle shaking. If the bath temperature exceeded 65° there was a tendency for some of the iodine chloride solution to evaporate from the reaction mixture and condense on the glass stopper, giving rise to erratic results. After cooling to room temperature 1 ml. of water was added to the reaction mixture. The resulting solution was adjusted to about *p*H 10 by careful addition of N sodium hydroxide using phenolphthalein as an internal indicator. The product was purified by placing the alkaline solution on a column of 'Dowex resin *A.G.*1' (formate form; × 2; 200–400 mesh, manufactured by the Bio-Rad. Corp.), 2 cm long and 1 cm in diameter[3].

The column was washed with 15 ml. 0·01 N sodium hydroxide, which removed a small amount of iodine-131 containing impurity and eluted with 0·1 N formic acid in 2 ml. fractions. Unchanged deoxyuridine came off in the first 6 ml. of eluate. The next 20 ml. of eluate contained 95 per cent of the radioactivity which could be eluted with 0·1 N formic acid. The radioactivity was measured in a well-type scintillation counter.

The phenolphthalein and unreacted ^{131}I-iodide were retained by the resin. The yield of 5-iodo-2′-deoxyuridine was determined by measuring the ultra-violet absorption of the solution at 288 mμ in a Unicam model 500 spectrophotometer.

The purity of the product was checked by comparing it with authentic material (California Corp. for Biochemical Research) by descending chromatography in ethyl acetate saturated with phosphate buffer (0·05 M, *p*H 6·0)[3].

The radioactivity on the chromatogram was counted in an automatic chromatogram scanner. The main peak was followed by a small peak which contained 10 per cent of the total activity.

I thank Dr. R. Pitt-Rivers for helpful discussions.

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CHEMISTRY

Occurrence in Egg Yolk of Plasmalogenic Triglycerides

Evidence has recently been obtained for the existence of a new class of glycerides containing a vinyl ether linkage. Such glycerides were extracted from the digestive glands of *Asterias forbesi*, the common starfish[1,2], and from the milk fat, beef tallow and ox heart[3]. According to Kiyasu and Kennedy, such neutral plasmalogens may be named plasmalogenic diglycerides and plasmalogenic triglycerides respectively[4].

The present report describes the isolation from egg yolk of a plasmalogenic triglyceride to which the tentative structure I could be assigned:

$$\begin{array}{l} CH_2\text{—}O\text{—}CH{=}CH\text{—}(CH_2)_{15}\text{—}CH_3 \\ | \\ CH\text{—}O\text{—}CO\text{—}R_1 \text{ (or } R_2) \\ | \\ CH_2\text{—}O\text{—}CO\text{—}R_2 \text{ (or } R_1) \end{array} \qquad \text{(I)}$$

$R_1 = CH_3\text{—}(CH_2)_{14}$, $R_2 = CH_3\text{—}(CH_2)_{16}$

In two different runs, dry hen eggs and fresh egg yolks were extracted successively with acetone, diethyl ether and boiling ethanol. The acetone extracts contained about 96 per cent of the total extractive material and were discarded. The combined ether extracts furnished a colourless, crystalline solid which was recrystallized from 95 per cent ethanol. Working up the mother liquor by crystallization, chromatography of the resulting substance over activated alumina, followed by recrystallization from acetone, gave colourless prisms, m.p. 34°–39°. The yield of the thus obtained substance I was about 100 mg from 4 kg dry eggs.

The following chemical and physical data were decisive for the establishment of the structure I. The lipide contained no phosphorus. Carbon–hydrogen analysis, calculated for $C_{55}H_{106}O_5$: C, 77·9; H, 12·6 per cent; found C, 77·3; H, 12·2 per cent. Catalytic hydrogenation in the presence of Adams platinum catalyst raised the m.p. to 54°–57°. A spot test for aldehydogenic substances with 2,4-dinitrophenylhydrazine[5] was positive. The infra-red

absorption spectrum taken in nujol showed a characteristic strong peak at 1,100 cm^{-1} attributable to 'C–O–C' deforming, a strong ester carbonyl absorption at 1,740 cm^{-1}, and a medium absorption at 1,650 cm^{-1} which is attributable to 'C=C' stretching. There was no band in the hydroxyl region near 3,600 cm^{-1}. The combined alkaline and acid hydrolysis gave a mixture of fatty acids and aldehydes. They were converted into both methyl esters and dimethyl acetals and subjected to vapour-phase chromatography. The gas-chromatographic analysis was carried out on an Aerograph Wilkens *A*–90 *C* instrument. A silicone 5-ft. column was operated at 225° with hydrogen at a flow rate of about 30 ml./min. The records indicated the presence of hexadecanoic acid, octadecanoic acid and octadecan-1-al.

The present evidence, taken in conjunction with the results reported previously[1–3], gives the structure I for the lipide from egg yolk. On the basis of the investigations reported so far, it is likely that this new class of lipids is widely distributed in Nature.

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Electrical Conductivity of Solutions of Aluminium (III), Gallium (III) and Indium (III) Iodides in Alkyl Iodides

Several investigations have been devoted to the electrical conductivity of aluminium halides in alkyl halide solutions[1–5]. The conductance of these solutions has been attributed to the formation of some complexes or addition compounds composed of both interacting molecules. No investigations on analogous systems with gallium and indium halides have hitherto been performed. In order to gain more information of the nature of interaction of MI_3 with RI, where M is aluminium, gallium or indium and R is an alkyl radical, the conductances of the solutions of aluminium, gallium and indium triiodides in anhydrous methyl, ethyl and propyl iodides were studied. It should be noted that particular attention has been directed to a thorough purification and drying of the reagents, the electrical conductivity being very sensitive to even small concentrations of various impurities.

The determined values for seven investigated solutions, the molar ratio MI_3 : RI being always $4{\cdot}2 \times 10^{-3}$, are presented in Table 1. From the experimental results it can be readily seen that MI_3 are weak electrolytes in alkyl iodides, and that the complex compounds in these solutions, if actually present, have a very weak polar character.

Since the electrical conductivity of the systems under investigation increases with time (rapidly during the first 20–40 min, then slowly during a long period of time), the specific conductivity values given in Table 1 were obtained by using the extrapolation method (for $t = 0$) described by Fairbrother and Scott[5]. These authors have attributed the increase of the conductivity of $AlBr_3$—C_2H_5Br solution to a certain chemical reaction leading to the formation of hydrogen bromide, which could be volatilized off with the ethyl bromide and detected. In order to test this hypothesis as regards AlI_3—C_2H_5I solution we have investigated the chemical composition of the vapours in equilibrium with the solution, using a mass spectrometric technique. However, the proportion of the peak heights of ethyl iodide and hydrogen iodide undergo only very slow changes during more than 70 h. Thus, the rapid rise in conductivity of AlI_3—C_2H_5I system cannot be explained in the way proposed by Fairbrother and Scott.

Table 1. Specific Conductivity of MI_3—RI Solutions (M = Al, Ga, In, R = Methyl, Ethyl, Propyl)

Solution	Specific conductivity × 10^{-7} ohm^{-1} cm^{-2}
AlI_3—CH_3I	5·4
AlI_3—C_2H_5I	6·2
AlI_3—C_3H_7I	3·1
GaI_3—C_2H_5I	5·1
GaI_3—C_3H_7I	2·3
InI_3—C_2H_5I	7·0
InI_3—C_3H_7I	6·0

A more detailed description of the present work will be published shortly.

We thank Prof. I. Zlotowski, head of the Department of Nuclear Chemistry, University of Warsaw, for a number of discussions and suggestions.

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Effect of Electron Donors on the Isotopic Exchange between Aluminium (III), Gallium (III) and Indium (III) Iodides and Alkyl Iodides

Very few investigations have been devoted to the isotopic exchange reactions of halogens in AlX_3—RX solutions[1], where X is a halogen atom and R is an alkyl radical. No work on analogous systems with gallium and indium halides has hitherto been published. In the work recorded here, the isotopic exchange reactions of iodine between aluminium, gallium and indium triiodides and methyl, ethyl and propyl iodides have been studied. It should be noted that the reagents were purified and dried with the greatest possible care. The experiments have been carried out in 'all glass' vacuum apparatus, at temperature $20 \pm 1°$ C, the molar ratio MI_3 : RI varying from 4×10^{-3} to 6×10^{-3}. The metal iodides were labelled with iodine-131.

The exchange rate was notable in each of the nine investigated systems. In the $Al^{131}I_3$—CH_3I solution (the fastest exchange) the equilibrium is reached within less than one minute, while in the $In^{131}I_3$—C_3H_7I solution (the slowest exchange) the fraction exchange is equal to 0·11 at the end of the first minute. The exchange rate decreases with the increase of the atomic number of the metal and the size of the radical.

The addition of small amounts of an electron donor substance (for example, ethyl alcohol, ether, etc.) to the alkyl iodide causes strong 'exchange blocking'. For example, if per each mole of aluminium triiodide 4 moles of ethanol had been added to ethyl iodide, the fraction exchange reached 0·50 in 5 min, while without the addition of ethanol the exchange equilibrium was attained in 3 min. Moreover, within the next 25 min the fraction exchange remained practically constant. The 'exchange blocking' was also observed after adding small amounts of water, *n*-butyl amine or ether; in the first two cases a precipitate is produced, making the quantitative determinations impossible.

The fraction of the radioiodine atoms which do not participate in the exchange when the electron donor is

present in the solution ('retained activity') increases regularly with the value of the molar ratio [electron donor]: [AlI_3].

The 'exchange blocking' may be attributed to the well-known tendency of the trihalogenides of the aluminium group metals, which are distinct electron acceptors, to the formation of $D \rightarrow MX_3$ complexes, where D is the electron donor molecule[2]. The effect can be explained if one assumes that the exchange of iodine between the alkyl iodide and the $D \rightarrow MI_3$ complex is much slower than the exchange between alkyl iodide and metal iodide. Further investigations are needed to test this assumption.

We thank Prof. I. Zlotowski, head of the Department of Nuclear Chemistry, University of Warsaw, for many valuable comments.

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Preparation of Aluminium Arsenide by a Vapour Phase Transport Reaction

Little work has been reported on the preparation of the semi-conducting compound aluminium arsenide, mainly because of the practical difficulties involved. Preparation from the melt is difficult because of the high melting point of the compound (about 1,700° C) and of the extreme reactivity of aluminium at this temperature. A few workers have prepared small crystals from the melt, and Stambaugh has produced polycrystalline ingots. This work is reviewed in ref. 1. The best of this material has an impurity carrier density of the order of $10^{19}/cm^3$ and is p-type.

We have prepared aluminium arsenide by an open-tube vapour phase transport method in which aluminium is transported in the presence of arsenic vapour in a stream of hydrogen. Aluminium is transported by the reversible reaction:

$$2\,Al + AlCl_3 \rightleftharpoons 3\,AlCl$$

in the temperature region 1,150°–1,000° C, and aluminium arsenide is formed either as free-growing crystals or as single crystal epitaxial layers on gallium arsenide substrates.

The material is transparent and orange brown in colour. It is not stable in moist air, quickly developing a 'bloom' on the surface, and slowly crumbling away, but good protection is afforded by dry hydrogen.

Epitaxial layers up to 200μ thick on substrates of 1 cm^2 area have been grown at rates of 0·4 μ/min. The substrate can be entirely removed by lapping and polishing with diamond paste under kerosene to leave a thin wafer of aluminium arsenide, to which electrical contacts may be made by soldering on gold wires with indium. Hall-effect measurements made on such wafers show them to be n-type with carrier densities of about $6 \times 10^{17}/cm^3$ and mobilities of about 100 cm^2/V-sec; compensation is suspected.

Spectrographic analysis has shown the major impurity to be silicon and improvements to the apparatus to eliminate sources of contamination are expected to yield material with a significantly lower carrier density.

Aluminium phosphide has also been prepared by a similar technique as an oriented growth on silicon substrates and as thin pale yellow needles. The conditions appear to be more critical than for the arsenide and growth rates have been very low.

We thank Mr. R. Greaves for his assistance and the management of these Laboratories for permission to publish this communication.

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Characteristics of Benzylic Carbanions

The formation of a benzylic carbanion from 2-phenylethanethiol in the presence of potassium hydroxide at 200° ultimately yields polystyrene (53 per cent) by a β-elimination of sulphide ion[1]. Recently, we have observed that the carbanions formed from benzylsulphide and related species in potassium *tert*-butoxide/dimethylformamide (DMF) at 80° undergo a 1,3-re-arrangement and subsequent β-elimination of the sulphur moiety to yield stilbene[2] in the same manner as above. As a logical extension of these studies, we have examined the effect of the hetero atom on rearrangement-elimination reactions of this type. The results obtained are of theoretical rather than synthetic interest.

All decomposition studies were carried out in a 1 molar solution of potassium *tert*-butoxide in dimethylformamide at 80° for 20 h. The molar ratio of potassium *tert*-butoxide/reactant was three. The presence or absence of specific products was determined by infra-red, ultra-violet, and gas chromatography (temperature programmed on a 2-ft. silicone rubber column between 75 and 250°) comparison to authentic compounds. Under these conditions, dibenzyl ether gave only 3 per cent yield of stilbene despite the visual evidence of carbanion formation (deep red colour). Further, 1,2-diphenylethanol, 1-phenylethanol, and 2-benzyl-2-propanol did not form any measurable quantity of olefinic products. The latter can be attributed to an equilibrium reaction between *tert*-butoxide ion and the alcohol which leads to alkoxide ions and *tert*-butyl alcohol and to deactivation of the *tert*-butoxide ion by formation of alcohol-KO*t*Bu aggregates. In the former case, β-elimination would require formation:

$$t\,BuO^{\ominus} + RCH_2OH \rightleftharpoons RCH_2O^{\ominus} + t\,BuOH$$

of an oxide ion ($O^{(=)}$) which is highly unfavourable. Dibenzylamine behaved in a manner similar to dibenzylether and did not form any stilbene after 20 h. In contrast, 1,2-diphenylethyl-*N*-methyl amine afforded stilbene in 42 per cent yield and presumably methylamine. As previously noted, dibenzyl-sulphide readily undergoes a rearrangement-elimination to stilbene (45 per cent) and hydrogen sulphide in DMF.

When the foregoing results are considered all together one is led to several general conclusions under these reaction conditions. Elimination of sulphide ion proceeds much more readily than elimination of an oxide ion. β-elimination occurs in 1,2-diphenylethyl-*N*-methyl amine because of preferential proton abstraction from the 2-benzyl carbon atom and not from the amino group. Finally, the rearrangement of benzyl sulphide is facilitated not only by *d*-orbital stabilization of the initially formed carbanion (I) but also by the electron donor and accepting properties of:



I II

the sulphur atom[3] in the transition state for re-arrangement (II).

Although benzylic type ethers do not readily undergo rearrangement-elimination in DMF, it has been found that aralkyl ethers (ArOR) do β-eliminate to form olefines if a more active dipolar solvent is used[4]. In a 0·62 molar solution of potassium *tert*-butoxide/dimethylsulphoxide (DMSO) β-elimination of *n*-butyl phenyl ether and *n*-propyl phenyl ether occurred at 55°. For these experiments, 2,2-dimethylbutane was added as an internal standard and the olefinic products were identified by gas chromatography (21 ft. Dow Corning silicone oil on 'Chromasorb'). In the case of the *n*-propyl compound, a 24·5 per cent yield of propylene was observed after 169 h of reaction. The *n*-butyl derivative gave a 17·6 per cent yield of an equilibrium mixture of but-1-ene and *cis*- and *trans*-but-2-ene in the same reaction time. However, the leaving group in these ethers is a resonance stabilized phenoxide ion. Attempts to activate dialkyl ethers under these conditions was, as anticipated, not successful.

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Preservation of Thin-layer Chromatograms

The preservation of chromatograms is one aspect of thin-layer chromatography which cannot be translated directly from the paper chromatographic technique. It is obviously impracticable to store the glass plates themselves and the answer to the problem lies in removing the completed thin-layer chromatogram from its glass support. Techniques have been described which rely on spraying the thin layer with suitable polymer dispersion, which hardens on drying to coat the layer with a clear film. The coating, plus the layer, is then removed mechanically from the glass plate. The method which is described here is an alternative means of preserving thin layer chromatograms which obviates the need for any spraying of the completed chromatogram and which is, in consequence, particularly useful for thin layers of a brittle nature.

In this method the chromatogram is removed from the glass plate by means of a self-adhesive plastic film. Films composed of cellulose acetate and PVC have been used in this connexion. ('Transpasene' and 'Transpaseal', manufactured by Dispro, Ltd., were used in this investigation.) The PVC film was more flexible and less porous than the cellulose acetate film and because of this was more useful for the preservation of chromatograms, which have a tendency to fade.

The completed thin layer chromatogram is placed face upwards on a flat surface. A sheet of plastic film is cut to a size which allows about 1-in. overlap around the edges of the glass plate. The backing paper is removed from the film and one edge of the film is turned under the glass plate and fixed in position. The film is then carefully smoothed over the thin layer working from the fixed edge. When in position over the layer, pressure is applied uniformly to the surface of the film using the edge of a ruler or a glass rod until there is a uniform dispersion of the particles of the adsorbent over the adhesive surface.

In this way the surface layer of the adsorbent particles, including the coloured zones, are transferred to the plastic film, which can then be peeled from the plate.

As a final step the plastic film is backed by a sheet of white card or paper of the same size as the original glass plate.

It has been found that using this procedure even amino-acid chromatograms, which fade readily, can be preserved in the absence of light for periods of several weeks provided the transfer of the thin layer to the plastic film is carried out immediately after completion of the chromatogram.

I thank the Directors of W. and R. Balston, Ltd., for permission to publish this work.

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BIOPHYSICS

Preparation of Sieves with Microscopic Pore Diameters

Sieves with pore diameters in the micron range are finding increasingly frequent applications. While a number of commercially available articles fulfil criteria of pore size uniformity, these pathways are generally tortuous, with great individual variation in the exact hole pattern. A simple technique for preparation of such filters has therefore been developed.

A bundle of nylon monofilaments (kindly supplied by E. I. du Pont de Nemours and Co., Inc.) were oriented parallel to the long axis of a gelatin capsule mould, and embedded in butyl methacrylate polymerized at 60° C for 24 h. These blocks were then cut perpendicular to the fibres with a sliding microtome, and the sections floated in 37 per cent hydrochloric acid, dissolving the nylon.

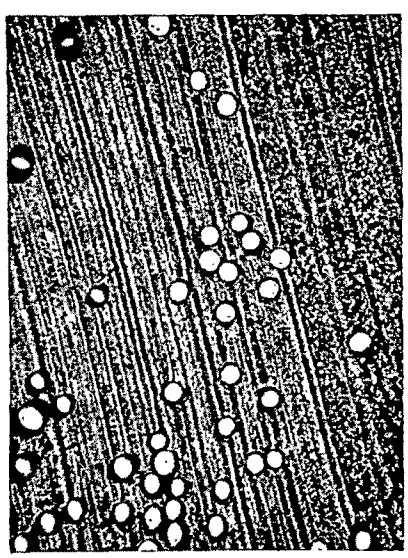


Fig. 1

The illustrated sieve in Fig. 1 has pores approximately 22μ diameter in a 40μ thick matrix. Orifice diameter/depth ratios as high as 1/10 are attainable with prolonged acid treatment, while the other specifications depend on choice of fibre, the mould used, and the microtomy technique. Serial sections are virtually identical, while the parallel orientation of the holes can be improved by keeping the fibres under tension during the embedding procedure.

Other fibre-embedding media combinations are now being investigated.

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BIOCHEMISTRY

Origin of Norepinephrine in the Heart

NOREPINEPHRINE is present in various organs of the body and in the blood plasma[1]. The adrenal medulla is not necessary for the maintenance of tissue stores of this catecholamine[1], and it is likely that synthesis of norepinephrine occurs in the sympathetic nervous tissue throughout the body. Norepinephrine in the plasma originates from discharge of the neurohumour from the sympathetic nerve endings. The circulating catecholamine is excreted, metabolically inactivated (predominantly by *O*-methylation), or rebound in the tissues[2,3]. A portion of the norepinephrine present in each organ is derived from the circulating pool of catecholamine. The ability of the heart to take up, concentrate, and store intravenously administered norepinephrine has been amply demonstrated[4–6]. It has recently been shown that the isolated perfused heart is capable of synthesis of norepinephrine from tyrosine, at a sufficiently rapid rate to account for a considerable portion of the norepinephrine in this organ[7]. Thus norepinephrine stores in the heart could be maintained in part by extraction of the catecholamine from the circulation and in part by synthesis. We have used an isotope dilution technique to demonstrate that in the normal rat about 60 per cent of the norepinephrine present in the heart is synthesized in this organ, while the remainder is derived from the circulating pool of norepinephrine.

Male Sprague-Dawley rats, weighing 250–300 g, were placed in small restraining cages which allowed collection of urine during intravenous infusion through the tail vein. L-norepinephrine-8-^{14}C (130,000 c.p.m./μg) was infused slowly (0·896 μg/h) for 2, 6, 10, 24, 36, or 48 h. Groups of five to eight rats were used for each infusion period. Urine samples were collected during the 2-h interval before the end of the infusion. At the end of the infusion the animals were killed by a blow on the head and the hearts removed, weighed, and homogenized in 10 volumes of cold 0·4 N perchloric acid. The catecholamines were isolated from the clear supernatant of the tissue homogenates and from urine by absorption alumina and elution with 0·4 N perchloric acid[8]. Norepinephrine and epinephrine in the eluates were determined by a modification of the trihydroxyindole method[9]. Radioactivity present in 0·5 ml. of these eluates was estimated by liquid scintillation spectrometry[10]. The specific activity of the norepinephrine was calculated from the radioactivity and norepinephrine present in the alumina eluates. The ratio of the specific activity of norepinephrine isolated from the heart to that of the urinary catecholamine was determined.

Table 1. SPECIFIC ACTIVITY OF URINARY NOREPINEPHRINE-^{14}C

Time (h)	2	6	10	24	36	48
Specific activity (c.p.m./mμg)	32·0	60·0	62·0	63·6	52·6	59·4

L-Norepinephrine-8-^{14}C (130 c.p.m./mμg) was infused at a constant rate (0·896 μg/h). The specific activities shown are the means for the norepinephrine isolated from the urine of 5–8 animals at each time, collected during the 2-h interval prior to the indicated time.

After 6 h, the specific activity of the urinary norepinephrine was constant at about 60 c.p.m. ^{14}C/mμg norepinephrine (Table 1). This is considerably below the specific activity of the infused norepinephrine, indicating that the amount of norepinephrine being administered was largely diluted by endogenous norepinephrine and did not excessively increase the plasma catecholamine concentration. During the steady state that was established, the specific activity of the urinary norepinephrine may be assumed to be about the same as the plasma norepinephrine from which it is derived. If all the norepinephrine in the heart were derived from the plasma, then the specific activity of the catecholamine in this organ would be expected to increase until it became the same as the specific activity of the plasma (or urinary) norepinephrine.

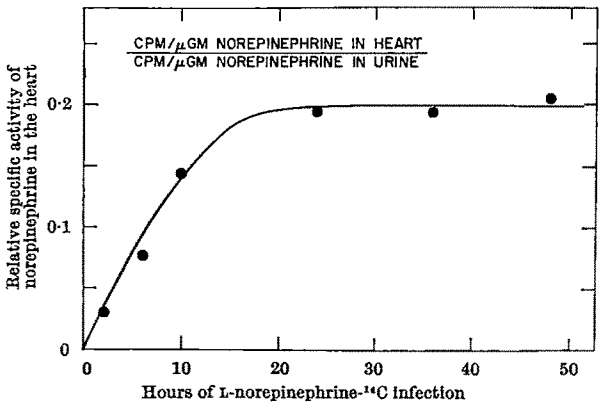


Fig. 1. Ratio of specific activity of norepinephrine in the heart and urine during a constant infusion of L-norepinephrine-8-^{14}C. Each point is the mean of this ratio for each of five to eight rats

If a portion of the norepinephrine in the heart were not derived from the plasma, but from synthesis, then the specific activity of the catecholamine in the heart would always be lower than the specific activity in the plasma (and urine). In the steady state, the ratio of the specific activity of the heart and plasma norepinephrine would be equal to the fraction of myocardial norepinephrine derived from the circulation. Fig. 1 shows the increase in the ratio of the specific activities of the myocardial and urinary norepinephrine. After one day of a constant slow infusion of L-norepinephrine-^{14}C the specific activity of the catecholamine in the heart remains at about 20 per cent of the specific activity of the urinary (and presumably plasma) norepinephrine. There was no increase in the myocardial norepinephrine concentration.

It can be concluded from these results that the myocardial stores of norepinephrine are maintained by both extraction of norepinephrine and by synthesis of catecholamine in the heart. About 80 per cent of the norepinephrine in the heart is synthesized in this organ (presumably within the sympathetic nervous tissue), while 20 per cent is derived from the circulating pool of norepinephrine.

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A Mucoid isolated from Bovine Red Cells exhibiting Strong *Pneumococcus* Type XIV Cross-reactivity

IT is well known that all human and some animal red cells are agglutinated by anti-Type XIV pneumococcus serum[1–3]. The chemical nature of this phenomenon has been elucidated by Watkins and Morgan[4] by means of hæmagglutination inhibition tests and has been interpreted as being due to the presence of *O*-β-D-galactopyranosyl-(1->4)-*N*-acetyl-D-glucosamine in terminal non-reducing position. This structure is considered to be

an essential part of the human ABH— and Lewis blood group precursor substance, concluded from studies on the secreted material[5].

In the course of investigating the bovine erythrocyte mucoids, first described in 1958 by Klenk and Uhlenbruck[6], we have recently reported them to show blood group activity in the bovine system and to possess phytagglutinin receptors for the purified lectin from *Solanum tuberosum*[7,8]. Further investigations have led us now to the result that the neuraminic acid-free bovine erythrocyte mucoids also exhibit strong cross-reactivity with anti-Type XIV *Pneumococcus* serum. This was proved by precipitation tests, agar-gel diffusion and hæmagglutination inhibition tests. Such a finding was not unexpected, since the carbohydrate moiety of bovine erythrocyte mucoids is known to consist mainly of D-galactose and *N*-acetyl-D-glucosamine, besides *N*-glycolyl- and *N*-acetyl neuraminic acid[6,7].

According to the hypothesis of Bird[9], the receptors for anti-*Pneumococcus* Type XIV horse serum and for the lectin of *Ricinus communis* (as well as those of *Abrus precatorius*[10]) are very similar and probably identical in part. Therefore, it is noteworthy that we have found these bovine erythrocyte mucoids to react in nearly the same way also with the *Ricinus communis* lectin. Our experiments in fact confirm Bird's results.

The cross-reactivity of several other mucoids, such as x_1-seromucoid, secreted ABH-substances and mucoids from human red cells with the *Pneumococcus* Type XIV specific reagents will be presented in a detailed report, including chemical data on the multiple bovine erythrocyte mucoid receptors. Furthermore, most of these mucoids have been found to react also strongly with lectins from *Solanum tuberosum*, *Datura stramonii* and *Viscum album*, but in a slightly different manner. These observations are still under investigation.

We thank Dr. J. L. Hendry, New York State Department of Health, for supplying anti-*Pneumococcus* Type XIV horse serum, and Prof. W. T. J. Morgan, Lister Institute of Preventive Medicine (London), for testing some of our substances.

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Sedimentation Constant–Molecular Weight Relation of Ribosomes

Roughly speaking, RNA of high molecular weight could be regarded as a polymer composed of nearly the same units as in the synthetic high polymer, if the minor differences in the structure of its base components are assumed to be negligible and hence the relation such as $[\eta]=KM^{\alpha}$ would be expected between its intrinsic viscosity $[\eta]$ and its molecular weight, M, which has been well known for the synthetic high polymers since the pioneer works of Staudinger. Indeed, Kurland[1] proved it on the ribosomal RNA, reporting that the value of α is nearly 0·5. Summarizing the data reported hitherto, Iso[2] also pointed out the following relation:

$$S_0=1{\cdot}4\times10^{-2}M^{0{\cdot}54} \qquad (1)$$

is applicable to the RNA of the high molecular weight, where S_0 is the sedimentation constant in Svedberg units and M the molecular weight. The value of the exponent in these formations is very close to those calculated theoretically on the model of spherical molecules of matted chains without free drain, a fact which provides us with an indirect support for the view of Hall and Doty[3] that RNA molecules could be regarded as an ellipsoid of revolution hydrodynamically.

These relations are available to estimate M of RNA from the measurements of S_0 or $[\eta]$ only and seem very useful from the practical point of view. But such a relationship has not yet been established for the ribosomal RNP. Using some data reported[4–9], therefore, we tried to find out an experimental formula available to the approximate estimation of its molecular weight as in RNA.

As shown in Fig. 1, a linear relationship between log S_0 and log M was found regardless of the source from which RNP was extracted. This linear relation can be expressed as follows:

$$S_0=0{\cdot}43\times10^{-2}M^{0{\cdot}65} \qquad (2)$$

As to the $[\eta]$, Ts'o stated already that it is independent of M. In fact, as shown in Fig. 2, its dependency on M is obscure, the measured points being scattered. As a rough approximation, the solid straight line was drawn in the figure, which is given by:

$$[\eta]=0{\cdot}28\times10^{-2}\,M^{0{\cdot}06} \qquad (3)$$

Both relations (2) and (3) suggest $S_0\alpha M^{2/3}$ and the independence of $[\eta]$ on M. If so, the ribosomal RNP molecules could be approximated, from the hydrodynamical point of view, by a compact, solid sphere.

Assuming that RNP is a compactly packed spherical molecule with a radius of R and a compactness of c, its M is given by $(4\pi/3)R^3\times c$ and hence we have:

$$[\eta]=\frac{5}{2}\cdot\frac{4\pi}{3}R^3\frac{N_A}{M}=2{\cdot}5\,N_A/c \qquad (4)$$

where N_A is the Avogadro number. Putting $c=14/(4\text{Å})^3$ in (4), we have $[\eta]=0{\cdot}068$, a value very close to those reported by Tissières *et al*[5]. When the frictional coefficient of RNP, ξ_0, is given by $6\pi\eta_0R$ (η_0 = the viscosity of the solvent), we have:

$$S_0=\frac{M(1-\rho\upsilon)}{N_A\xi_0}=\left(\frac{4\pi}{3}\right)^{1/3}\frac{M^{2/3}\,C^{1/3}\,(1-\rho\upsilon)}{6\pi\eta_0N_A} \qquad (5)$$

where υ is the partial specific volume of RNP, ρ the density of the media. Introducing the above value of c into equation (5), we obtain:

$$S_0=0{\cdot}69\times10^{-2}\,(1-\rho\upsilon)\,M^{2/3} \qquad (6)$$

Taking into account $\rho\sim1{\cdot}0$ and $\upsilon\sim0{\cdot}55$–$0{\cdot}60$, this relation is represented by the dashed line in Fig. 1, which is close to that obtained empirically.

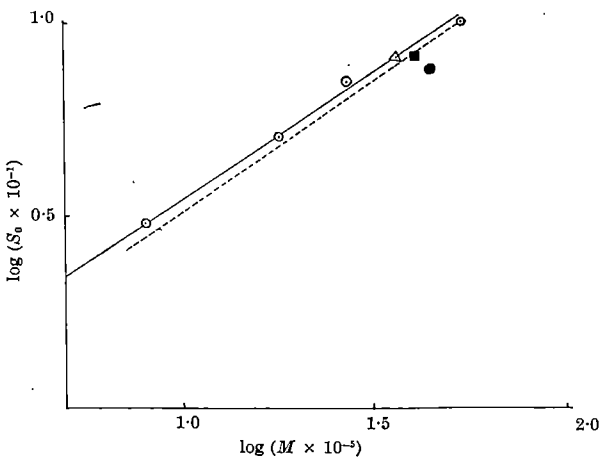


Fig. 1. ⊙, *E. coli* RNP (refs. 5 and 6); △, calf liver RNP (ref. 3); ■, yeast RNP (ref. 7); ●, pea seedling (refs. 4 and 8)

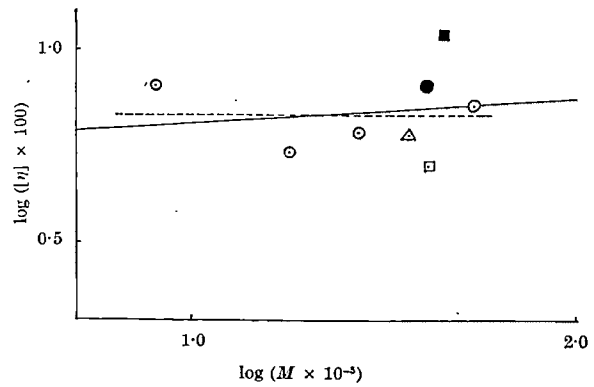


Fig. 2. ⊙, *E. coli* (refs. 5 and 6); □, yeast (ref. 7); △, calf liver (ref. 3); ■, pea seedling (refs. 4 and 8); ●, reticulocyte of rabbit (ref. 9)

The value of c assumed here means that, in the ribosome, an atom or an atomic group with the atomic weight of 14 (such as N or —CH_2—) is on an average contained in the volume of (4 Å)[3]. If the ribosomal particle were really a closely packed solid molecule as supposed from the electronmicroscopic observations, such a value of c would not seem so extraordinary or unreasonable. Thus it might be concluded that RNA should be compactly folded up in the ribosome, which is, in contrast to the isolated RNA, hydrodynamically approximated by a solid sphere, and the isolated RNA by a rigid spherical one composed of matted Gaussian chains or those similar to them, but without free drain. Moreover, the relations (2) or (6) would be available for estimating the approximate value of molecular weight of a ribosomal RNP by its ultracentrifugal data only as (1) with the isolated RNA.

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Maltose in Red Clover (*Trifolium pratense*) Leaves

Traces of maltose in leaf extracts are generally attributed to amylase action on starch during processing of the samples and are avoided if enzymes are inactivated immediately after sampling. During investigations of diurnal fluctuations in red clover (*Trifolium pratense*) leaf carbohydrates, however, we have obtained the following evidence that free maltose is present in leaves collected at night.

Leaf samples (25 g wet wt.) collected at intervals over 24 h, during periods of sunny, summer weather (daybreak, 5–5.30 a.m.; darkness, 7.30–8 p.m.), were plunged immediately into boiling ethanol and processed to soluble sugar extracts[1]. Chromatograms of the extracts showed an easily detected reducing component, corresponding to maltose, in the night samples; this component was not detected in the day samples or in similar extracts of day or night samples of stems and petioles. The reducing disaccharide (20 mg), isolated by fractionation on charcoal-'Celite' of the extract from 100 g (wet wt.) of leaves collected at 1 a.m., was identified as maltose as follows. On chromatograms developed with a variety of solvents it had the same R_G as maltose and was clearly different from other diglucoses including cellobiose, while with diphenylamine-aniline it gave the blue colour typical of a 1–4 linked reducing aldohexose disaccharide[2]. The sugar also had $\alpha_D + 131°$ (maltose monohydrate, $\alpha_D + 130{\cdot}4°$), yielded glucose only on acid hydrolysis and gave an osazone with the characteristic crystalline form of maltosazone. The maltose-level in the leaves was measured by quantitative paper chromatography of the leaf extracts[3], using aniline hydrogen phosphate reagent, after first hydrolysing sucrose with an invertase which did not attack maltose. Similar results were obtained each time samples were collected; in a typical run the maltose (dry wt. basis) rose steadily from 30 mg per cent at 8 p.m. to 120 mg per cent at 5 a.m., after which it declined to an undetectable level by 7 a.m.

Enzyme extracts, prepared by grinding clover leaves in citrate buffer (0·1 M, *p*H 6·0), centrifuging and dialysing, were incubated with amylose and maltose respectively at various *p*H values and the digests analysed at intervals, by paper chromatography, for liberated sugars. Amylose digests showed a rapid release of maltose (*p*H optimum, 4·0–6·5) followed by a slower release of glucose, while the maltose digests showed a slow release of glucose (*p*H optimum 5·5–6·5). It is possible, therefore, that the maltose found in the leaves results from the different rates of activity of these two enzymes provided they are involved in the degradation of chloroplast starch at night[4]. Disappearance of maltose during the early hours of daylight may be due to continuing maltase action or to the use of the maltose as a primer in subsequent starch resynthesis.

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Carbohydrases of the Rumen Oligotrich *Eremoplastron bovis*

From small-scale, pure cultures of several rumen oligotrichs, including *Eremoplastron bovis* (*Eudiplodinium neglectum*, Dogiel[1]), Hungate[2] prepared extracts containing cellulase and cellobiase. The extracts were not tested for other carbohydrases and appear to represent the only direct evidence for cellulolytic enzymes in rumen protozoa, apart from a recent observation of cellodextrinase in the extract from mixed *Entodinium* species[3]. Cultures of several oligotrich species were recently established[4] in this laboratory, enabling us to prepare 2–3 g (wet wt.) of cells of *Eremoplastron bovis* for a more detailed examination of its carbohydrases.

The *E. bovis* cells were collected from a clonal culture[4], freed from external bacteria, ground with Ballotini beads, diluted with water and centrifuged to yield extract *A* (10 ml.). The debris was extracted successively with citrate and phosphate buffers (0·1 M, *p*H 6·0 and 7·0 respectively) to yield extracts *B* and *C*. Extracts were dialysed, at 2° against water or buffer, and tested for carbohydrases in digests[3] which were incubated under toluene for 48 h and analysed at intervals for liberated sugars by paper chromatography. Extract *A* rapidly hydrolysed starch, xylans, xylodextrins and cellodextrins, hydrolysed maltose, xylobiose, cellobiose, laminaribiose,

sucrose and melibiose more slowly and was without action on various β-glucosides and β-galactosides, pectin and finely ground cellulose. Extracts *B* and *C* showed only weak amylase and xylanase activity.

As both amylose and β-limit dextrin were hydrolysed, at the same rate, to a similar mixture of end products (maltose plus smaller amounts of glucose and maltotriose) the amylase is probably an α-amylase. Xylans were hydrolysed by rapid removal of any arabinose followed by a slower, random hydrolysis of the xylan chain. Xylotriaose-pentose and wheat flour arabino-xylan were rapidly hydrolysed while insoluble xylan and clover hemicellulose were hydrolysed more slowly. Hydrolysis of cellodextrins was by the endwise removal of cellobiose. Cellotriaose was scarcely hydrolysed, but there was a rapid release of cellobiose from cello-tetraose and -pentaose and a slower release from long-chain, water-insoluble, cellodextrins[5]; in this latter case there was no sign of higher oligosaccharide intermediates.

The presence of amylase and xylanase in the extracts suggests that *E. bovis* can degrade plant polysaccharides other than cellulose. Although our extracts did not hydrolyse cellulose, including a sample of pebble-milled cellulose kindly supplied by Prof. Hungate, the presence in the extracts of cellodextrinase activity suggests that the organisms are able to degrade cellulose and that extraction of the entire complex of cellulolytic enzymes is more difficult.

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Isolation of Alkaloids using Ultrasonic Energy

In recent years ultrasonic energy has been used in the extraction of various materials from plants, animals, and micro-organisms for biochemical analysis[1]. The application of ultrasound has proved advantageous for the isolation of enzymes, nucleic acids, and other heat-labile substances from cells and tissues. Moreover, ultrasonic energy has been used widely for various industrial applications to facilitate the extraction of commercially important substances[2]. The increasing use of ultrasonic instruments in extraction processes is based on the observations that: (1) disruption of cells is rapid; (2) the extraction process is accelerated; (3) the yield of extract usually is increased; (4) the use of ultrasonic energy permits the extraction of certain materials otherwise not readily obtainable.

While considerable attention has been devoted to explaining the mechanisms by which ultrasonic energy promotes the disruption of cells and aids in the extraction process, there is no generally accepted theory to account for the observed effects[3]. Many of the chemical, physical, and biological effects produced during ultrasonic treatment have been attributed to the activity accompanying gaseous cavitation, that is, the process of formation and collapse of cavities produced in an ultrasonically treated liquid due to successive rarefaction and compression. Though it is known that mechanical, thermal, chemical, and electrical energy is produced during cavitation[4], the part which each of these moieties of energy plays in cell disruption and subsequent extraction is not yet clear[3]. Nevertheless, in the specific reports where ultrasonic energy has been applied during the extraction process the results have been beneficial, and in many cases an increase in the amount of the extractable material was obtained or the extraction was completed in a much shorter time[5].

Our interest in increasing the yield of alkaloids obtained from *Datura stramonium* L. by various nutritional, cultural, and extracting procedures[6,7] led us to investigate the effectiveness of ultrasonic treatment when applied during the isolation of alkaloids. Previous workers have indicated the feasibility of this approach in the isolation of medicinal constituents from plants. But the technical refinements which we have introduced in the experiments briefly reported here have permitted us to evaluate this method further.

For the present work an extractor was designed which made it possible to carry out continuous extraction and at the same time expose the mixture of ground plant material and solvent to ultrasonic energy[7]. Utilizing this extractor it was possible to analyse separately the effect of ultrasonic treatment on the two processes, maceration and extraction, ordinarily involved in the isolation of alkaloids. The term extraction is used here to describe that process during the isolation of alkaloids in which the plant material is exposed repeatedly to fresh supplies of solvent. The term maceration implies swelling and soaking of the material without continuous recycling of the solvent. Frequencies of 20- or 40-kc/s ultrasound were obtained using two commercial ultrasonic generators and cleaning tanks. (Bendix Aviation Corp., model No. *UC–4X8*, 20-kc/s magnetostrictive unit, and Circo Ultrasonic Corp., Model No. *125T*, 40-kc/s piezoelectric unit.)

To determine the influence of ultrasonic energy when applied during the maceration process, each of a series of 10 g samples of ground *D. stramonium* leaf was placed in 300-ml. polyethylene containers and covered with a macerating solvent of 20 parts of ether, 12 parts of alcohol, and 8 parts of ammonia by volume. The containers were then immersed in the water of the ultrasonic tank and exposed for varying lengths of time to ultrasonic energy. To prevent vaporization of the solvent during this treatment, water-cooled reflux condensers were attached to the polyethylene containers and the temperature of the water in the ultrasonic tank was maintained continually at 24° C. After treatment, the samples were continually extracted with solvent for 3 h in Soxhlet extractors. All alkaloid determinations were made according to the U.S.P. assay procedure for *Belladonna* leaf[8]. Results of typical experiments illustrating the beneficial effect of 20- and 40-kc/s ultrasonic treatment during short maceration periods are shown in Table 1.

Table 1. Alkaloid Yield (mg/10 g Sample of *D. stramonium*) obtained after Short-term Ultrasonic Maceration of the Plant Material followed by 3 h of Conventional Extraction

Figures in parenthesis indicate percentage increase over control in recovery of alkaloids. Each determination represents the average value of not less than 3 assays

Treatment	Duration of ultrasonic maceration ½ h	1 h
Control	27·5	29·0
20 kc/s	30·0 (9%)	27·0 (0%)
40 kc/s	30·5 (10%)	31·5 (9%)

Samples of the ground plant to be used for ultrasonic extraction were treated in the same manner as described except that these samples were macerated in the conventional manner, without ultrasonic treatment. After a designated maceration period, each polyethylene container with macerating solvent was attached in turn to the new extractor, replacing the glass extraction flask, and placed in the water of the ultrasonic tank. In this manner it was possible to maintain the plant material in an ultrasonic environment throughout the duration of continuous extraction.

Our experiments indicate that short-term (2–4-h) conventional maceration followed by a 2-h period of ultrasonic extraction with the 20-kc/s generator results in significant increases in total alkaloids recovered when compared with

Table 2. Alkaloid Yield (mg/10 g Sample of *D. stramonium*) obtained after Conventional Maceration of the Plant Material followed by 2 h of Ultrasonic Extraction

Figures in parenthesis indicate percentage increase over control in recovery of alkaloids. Each determination represents the average value of not less than 3 assays.

Treatment	Duration of conventional maceration 2 h	4 h	8 h	24 h
Control	29·0	30·5	34·0	40·0
20 kc/s	32·0 (10%)	33·5 (10%)	36·6 (4%)	41·2 (3%)

control samples (Table 2). Similar experiments utilizing the 40-kc/s generator have demonstrated that increasing the frequency of ultrasound used during an extraction period of 1–2 h produces an increased yield of 15 per cent in total alkaloids recovered when compared to control samples.

The mechanical and thermal effects accompanying cavitation appear to be important in facilitating extraction by the use of ultrasound. Hydraulic shocks and intense stirring occurring at the cavitational points aid in increasing the interfacial surface between the solvent and the substrate by dispersing adhered and clumped particles, thereby making extraction more efficient. Localized stirring or 'acoustic streaming'[9] increases the diffusion rate of the desired material from the substrate and, consequently, decreases the diffusion gradient. Cavitation also causes adsorbed air on poorly wetted surfaces to be liberated, in this way increasing the area exposed to the solvent. Moreover, if cavitation is intense enough, disruption of the substrate particles occurs, increasing further the interfacial surface.

These results demonstrate that the application of ultrasound to the isolation of medicinal constituents from plants is advantageous in shortening considerably both the length of the maceration and the length of the extraction period. That this reduction in time is accompanied by efficient extraction without degradation of the active constituents or other unfavourable effects has been reported previously[10]. However, to our knowledge there has been no previous attempt to combine ultrasonic treatment with continuous solvent extraction. This technique should provide the means for further study to determine whether the same ultrasonic mechanism causes the increased yield of alkaloids observed during maceration and extraction. Our evidence suggests that during the maceration process the activity of ultrasound is to liberate alkaloids from bound sites within the cell and hasten their rate of diffusion into the solvent. During the extraction process it appears that ultrasound accelerates the physical separation of the alkaloids from the ground plant material.

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PHYSIOLOGY

Sodium and Water Transport across Isolated Intestine of a Marine Teleost

The special osmo-regulatory mechanisms of the marine teleosts were originally outlined by Homer Smith[1]; he postulated that these animals maintain a constant blood concentration lower than that of the external sea-water by continual drinking of sea-water and simultaneous excretion of ions mainly through the gills. In addition to this and the work of Keys[2] on the common eel, several workers, Mullins[3] and House[4], have recently confirmed this theory of osmotic regulation by ion transport experiments *in vivo*. However, there have been no reported *in vitro* transport studies of the epithelial membranes involved in the regulation processes. The purpose of the investigation reported here was to determine the magnitude and direction of net transport of sodium and water, if any, across the small intestine of the marine teleost, *Cottus scorpius*.

Since there was no available information on the ionic concentrations in the blood of *Cottus*, we used *Lophius* Ringer (modified from Young[5]), which contained 205 mM NaCl, 8 mM KCl, 1·6 mM $CaCl_2$, 1 mM $MgCl_2$, 2·3 mM $NaHCO_3$, 0·5 mM KH_2PO_4 and 2·8 mM/l. glucose. The *p*H was 7·2. This Ringer was considered to be satisfactory because the tissue respired continuously in it for periods of at least 6 h. Respiration was followed in a Warburg apparatus, and the mean rate of oxygen consumption of six pieces of gut (3 animals) over a total of 35 h was $0·4 \pm 0·2$ µl. oxygen/mg dry weight/h at 25° C. In all the experiments recorded here the tissue was bathed on both sides by Ringer solution.

Net water transport was measured by the change of weight of normal and everted sacs, filled with Ringer, during 1 h incubation periods in Ringer, and also by a gravimetric chamber technique. The latter technique (to be described elsewhere) permitted simultaneous measurement of potential differences and short circuit current. In both these techniques the tissue was always equilibrated with Ringer for 1 h before commencing the hourly weight measurements. Observations of the net water flux (mucosal to serosal) across 22 pieces of small intestine from 16 fish gave a mean $\pm S.D.$ of $4·9 \pm 3·0$ µl./cm²/h (35 measurements). In four pieces of tissue exhibiting a net transport of water the movement was halted by the addition of 1 mM potassium cyanide to the serosal medium. This confirms the view that there is a net uptake of water across the intestine when no bulk osmotic driving force exists across the tissue and that this transport is dependent, at least indirectly, on the metabolism of the tissue.

The potential difference measured by silver–silver chloride electrodes for 9 pieces of small intestine was $0·0 \pm 0·5$ mV (mean $\pm$ *S.D.*). Since there exists no measurable potential difference across the tissue the magnitude of the fluxes of any ion in both directions determines whether there is active transport of the ion or not.

The serosal to mucosal sodium flux, ${}_SJ_M$, was found by immersing normal filled sacs in a continuously aerated ^{22}Na-loaded Ringer and monitoring the sacs in a well-type scintillation counter at known times after immersion. Before the counting procedure the sacs were washed by four rapid successive immersions in three separate volumes of Ringer; this washing technique was found to be tolerably efficient. Values of ${}_SJ_M$ (µequiv./cm²/h) were calculated from the familiar equations for a two-compartment system. The sacs were then transferred to a large volume of aerated Ringer, and values of mucosal to serosal sodium flux, ${}_MJ_S$, were found from the decrease in specific activity of the sacs with time. A few representative values of ${}_SJ_M$ and ${}_MJ_S$ in the same sacs are shown in Table 1.

It is clear from the results that there is an active transport of sodium, ${}_MJ_S^{act.}$, from mucosal to serosal surfaces of the isolated intestine of *Cottus*. However, as the

Table 1

Sac No.	Sodium fluxes (μequiv./cm²/h) $_{S}J_{M}$	$_{M}J_{S}$	$_{M}J_{S}^{net}$
1	13	30	17
3	15	21	6
6	13	23	10
9	15	25	10
Mean ± *S.D.* (9 sacs)	14 ± 3	28 ± 5	12 ± 4

potential difference measured was zero there must be either net anion transport in the direction of active sodium movement or net cation transport from serosal to mucosal surfaces. The interesting question of whether there is active anion transport or not remains to be investigated.

The work recorded here tends to support previous *in vivo* measurements on ion absorption by marine teleosts, as it appears that sodium and perhaps chloride are transported actively by the intestine. Schoeffeniels[6] has shown that there are permeability differences between separate regions of the turtle intestine, and similar studies on marine teleosts would lead to a better understanding of ionic regulation in this group.

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Transient Responses of Purkinje Fibres to Non-uniform Currents

Comparisons between experimentally recorded responses of cardiac muscle to applied currents[1–3] and responses predicted by a modified form of the Hodgkin–Huxley nerve equations[4] have led to conflicting conclusions about the applicability of these equations to the cardiac cell membrane[2,4,5]. The existence of a negative membrane conductance in the equations means that strong enough inward currents applied uniformly to the membrane during the plateau of the action potential should initiate an all-or-nothing repolarization response. Fig. 1 shows computed responses to uniform currents.

However, the experimental records at present available

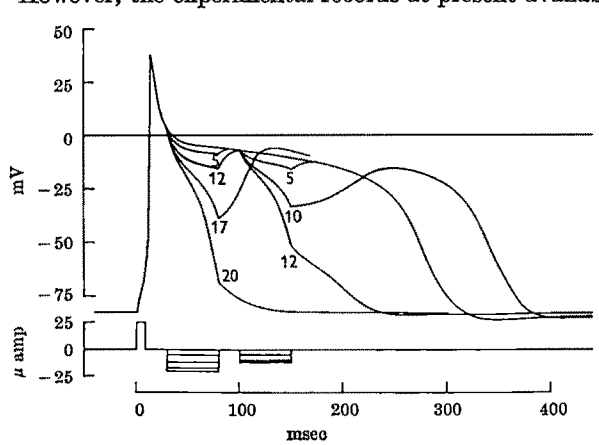


Fig. 1. Computed responses of Purkinje fibre to uniform currents. Current strengths and durations are indicated by current plot at bottom of illustration and by the figures (in μamp/cm²) on the potential curves (Noble, D., ref. 6)

were obtained by applying currents non-uniformly from one point in cable-like preparations[3] or sheet-like preparations[2]. Fig. 2 shows the effects of currents applied at one point in cable-like preparations (mammalian Purkinje fibres). It can be seen that there are large differences between these responses and those shown in Fig. 1. The voltage threshold is beyond the resting potential and little or no inflexion is observed until the current pulse is terminated. The larger sub-threshold responses are followed by a break re-excitation.

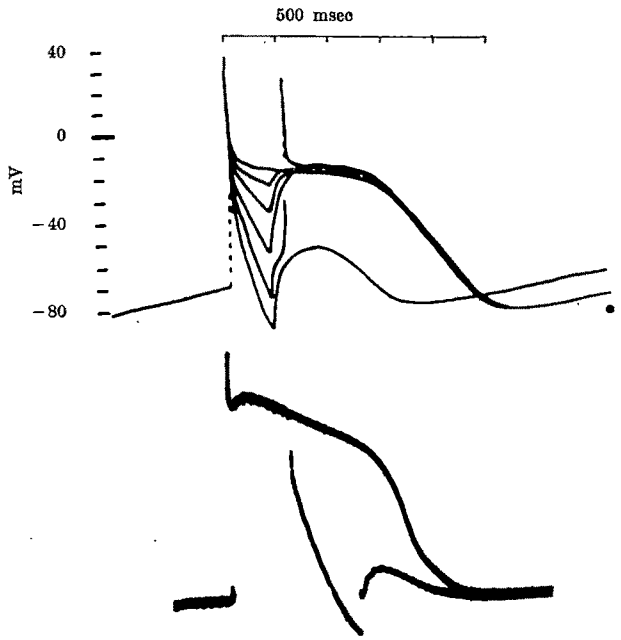


Fig. 2. Experimentally recorded responses of Purkinje fibres to currents applied at one point. Top, from Weidmann, S. (ref. 3); bottom, from Hoffman, B. F., and Cranefield, P. F. (ref. 1)

Recent computations of the steady-state behaviour of the equations for non-uniform polarization have shown that the experimental results do not necessarily conflict with the theory[6]. We have now computed transient responses to currents applied at one point in a cable-like preparation. The equation for this situation is:

$$\frac{a}{2R_i}\frac{\partial^2 E_m}{\partial x^2} = C_m\frac{\partial E_m}{\partial t} + g_{Na}(E_m - E_{Na}) + g_K(E_m - E_K)$$

where a = radius of fibre (38μ), R_i = intracellular fluid resistance (105 ohms/cm), C_m = membrane capacity (12 μF/cm²), E_m = membrane potential (mV), E_{Na} = sodium equilibrium potential (40 mV), E_K = potassium equilibrium potential (−100 mV), g_{Na} = sodium conductance (mmho/cm²), g_K = potassium conductance (mmho/cm²), x = distance along fibre (cm) and t = time (msec). The equations for g_{Na} and g_K are those used to reproduce the Purkinje fibre action potential[4].

Fig. 3 shows computed responses at $x = 0$ to square wave currents of various amplitudes applied between 100 and 200 msec after the initiation of an action potential. It can be seen that these responses resemble the experimental records very much more closely than those in Fig. 1. The voltage threshold for initiating the all-or-nothing termination of the plateau is beyond the resting potential and is considerably more negative than the computed steady-state threshold at 200 msec[6]. Currents which re-polarize the membrane beyond about −60 mV and which fail to terminate the plateau are followed by a break re-excitation and, as in experimental records, the spike is preceded by a delay. This effect was not reproducible in the computed responses to uniform currents (Fig. 1) since the membrane could not be repolarized sufficiently to reactivate the sodium-carrying system without terminating the plateau.

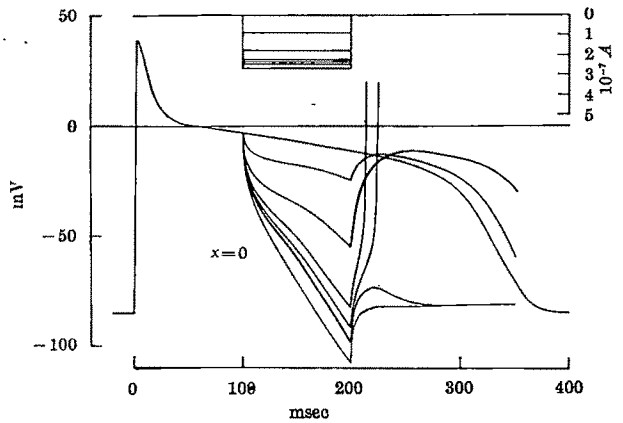


Fig. 3. Computed responses of Purkinje fibre to currents applied at one point between 100 msec and 200 msec after initiation of an action potential. Fibre-length, 6·25 mm; fibre-diameter, 75μ; resting space constant $\simeq$ 2 mm. The heights of the spikes following the strongest sub-threshold currents are uncertain

These results show that the theory is capable of reproducing some of the features of the transient responses to non-uniform currents.

We thank Prof. A. F. Huxley for allowing us to use his formula for numerical integration. This was used together with the Crank–Nicolson method[7,8] modified to apply to non-linear partial differential equations. The numerical computation was done on the Ferranti *Atlas* computer at the University of Manchester.

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Substance *P* Content and Effect on Capillary Permeability of Extract of Various Parts of Human Brain

Chemically pure substance *P*, apart from causing vasodilatation and stimulation of smooth muscle[1], also increases capillary permeability[2]. We have now tested extracts of four parts of human brain, containing different amounts of substance *P*, for their effectiveness in increasing capillary permeability. The effects on the guinea pig ileum of acid-boiled extracts of substantia nigra, basal ganglia, cortex and cerebellum were compared with the effect of a standard preparation of substance *P* made from bovine intestine (for methods, see ref. 3). The results are listed in Table 1, col. 3. For estimating the effects of the brain extracts on capillary permeability, other vasoactive substances (acetyl choline, adrenergic amines, histamine, serotonin, ATP, ADP and AMP) had to be removed so far as possible; this was done by successive precipitations with ammonium sulphate and acetic acid-ether. The resulting 'purified extracts' were tested for activity in increasing capillary permeability of rabbit skin, using a modification of the method of Frimmer and Müller[4].

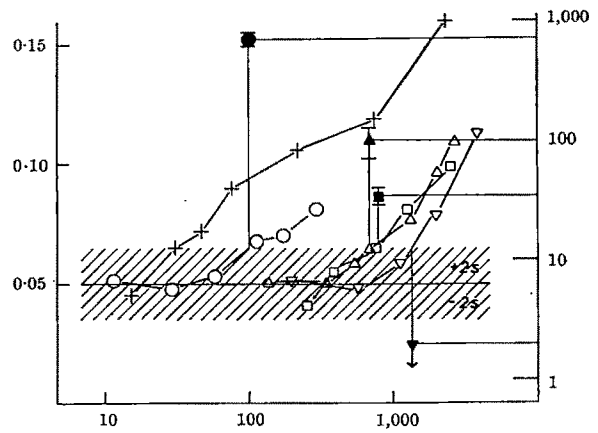


Fig. 1. +, Substance *P* standard (13·2 units/mg, made from bovine intestine); ○, substantia nigra; △, basal ganglia; □, cortex; ▽, cerebellum. Abscissa, μg of purified extract injected intradermally. Left ordinate, extinction of dye solution extracted from blued skin areas. Full symbols show the relation between the amounts of purified extracts producing +2s increase of capillary permeability and the amount of substance *P* (right ordinate (μ/g)) in the respective brain area

The amounts of 'purified extracts' of the different parts of the brain which caused a significant increase (+2s) in capillary permeability differed considerably (Table 1, col. 7); for example, 0·1 mg of extract of substantia nigra containing 719 units substance *P*/g, compared with 1·3 mg of extract of cerebellum containing less than 2 units substance *P*/g. With regard to the corresponding amounts of tissue the differences were still more pronounced (Table 1, col. 9); e.g., extract corresponding to 41·5 mg of substantia nigra produced the same increase in capillary permeability as extract corresponding to nearly 17 times more cerebellum.

'Purified extracts' with equal activity in increasing permeability contained different amounts of substance *P*; for example, 18·8 units for substantia nigra and less than 0·1 unit for cerebellum (Table 1, col. 8). This can be explained by assuming that even the 'purified extracts' contained, apart from substance *P*, other material capable of increasing capillary permeability. This became apparent especially when larger quantities of the 'purified extracts' had to be injected.

Fig. 1 shows the dose-response curves of the four 'purified extracts' and their permeability-increasing activities. The +2s increase in capillary permeability is compared with the substance *P* content of the same brain area: there is a clear correlation between them.

Table 1

1	2	3	4	5	6	7	8	9
	Dried extract mg/g tissue	Substance *P* units/g tissue	Purified extract mg/g tissue	Units of substance *P*/mg purified extract	Recovery of substance *P* in purified extracts (%)	Increase in capillary permeability of (+2s) produced by: μg purified extract	= Corresponding units of substance *P*	= Corresponding mg of wet tissue
Substantia nigra	58	719 ± 82	2·4	188·0	60·8	100·0	18·8	41·5
Basal ganglia	53	102 ± 33	2·4	19·0	51·1	680·0	12·9	282·0
Cortex	60	34 ± 6	2·6	9·0	60·7	800·0	7·2	308·0
Cerebellum	60	2·0	1·9	< 0·1		1·300	< 0·1	685·0

This indicates that less than 50 mg substantia nigra contain enough substance *P* to produce quite a pronounced increase in capillary permeability.

It is possible, therefore, that substance *P* enables those parts of the brain which are rich in it to have particularly high rates of exchange, either potential or permanent, between the capillaries and the surrounding tissue.

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Reduction by Chlorpromazine of Pylorus-ligation Ulcers in the Rat

Recent research indicates that chlorpromazine is an effective inhibitor of gastric secretion[1-3] and reduces starvation ulcers in mice[4]. This investigation was undertaken to determine quantitatively the extent of ulceration in the fore-stomach and glandular stomach of the pylorus-ligated rat after treatment with chlorpromazine.

Twenty-eight adult, male and female albino rats (Holtzman strain) were involved in the investigation. Each rat was separately caged and water was accessible *ad libitum* to all rats for a 24-h pre-operative fasting period. The Shay rat procedure[5] of duodenal ligation was performed under ether anæsthesia. Three subcutaneous injections (inguinal region) were given post-operatively at 8-h intervals to all rats. Treatments consisted of the following injections: 5·0 mg chlorpromazine/kg bodyweight (Thorazine, Smith, Kline, and French Lab., about 0·06 c.c.); 2·5 mg chlorpromazine/kg (approx. 0·03 c.c.); or 0·06 c.c. water (control animals). The number of rats per treatment is indicated in Table 1. Drinking water was removed subsequent to the surgical procedures. Twenty-four h after pyloric ligation the stomachs were removed, opened along the lesser curvature, and both the fore-stomach and glandular region of each stomach were evaluated for gastric pathology by the ulcer index developed by Pfeiffer and Gass[6], which has a minimum rating of 1 (normal stomach) and a maximum rating of 15 (highly ulcerated stomach).

Table 1. Ulcer Reduction by Chlorpromazine in the Pylorus-ligated Rat

Treatment*	No. of animals	Mean weight at surgery (g)	Mean ulcer index ± *S.E.* (fore-stomach)	Mean ulcer index ± *S.E.* (glandular region)
Water (control) 0·06 c.c.	10 (5 ♂, 5 ♀)	337	10·8 ± 1·5	7·2 ± 0·2
Chlorpromazine 2·5 mg/kg 0·03 c.c.	8 (6 ♂, 2 ♀)	362	4·2 ± 2·0	3·2 ± 1·0
Chlorpromazine 5·0 mg/kg 0·06 c.c.	10 (4 ♂, 6 ♀)	336	2·9 ± 1·4	2·9 ± 1·0

* All injections subcutaneous (inguinal region) t.i.d. at 8-h intervals for 24 h.

The results of the investigation are illustrated in Table 1. The mean fore-stomach ulcer index was higher than the mean glandular stomach ulcer index except in the 5·0 mg/kg group where the indices were equal. Greater ulceration of the fore-stomach of pylorus-ligated rats is a phenomenon commonly reported by earlier investigators[6]. Ulceration of both regions of the stomach was graded from severe ulceration in the control group to greatly minimized ulceration in the 5·0 mg/kg group. The protective action of the higher dose of chlorpromazine was only slightly greater than that of the lower dose.

It can be concluded that chlorpromazine minimizes ulceration in the Shay rat. This reduction of ulceration is similar to the protective action afforded by bilateral vagotomy in the pylorus-ligated rat[7]. The present investigation quantitatively confirms past findings that chlorpromazine reduces ulcers in the Shay rat[8,9], supposedly by inhibition of gastric secretion[1-3]. By means of an ulcer index, the protective effect of chlorpromazine on both the fore-stomach and the glandular stomach has been evaluated.

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ANATOMY

Centrifugal Fibres in the Lateral Olfactory Tract

For the interpretation of results of electrophysiological studies of the responses in the olfactory bulb to stimulation of the lateral olfactory tract it is a matter of some importance to know whether this tract consists solely of efferent fibres from the bulb or whether it also contains centrifugal fibres to the bulb[1-3]. Such centrifugal fibres were described by Cajal[4], who, although uncertain of their origin, clearly distinguished them from those in the anterior commissure (which, coming from the opposite olfactory bulb, should be considered as commissural). The commissural fibres have been demonstrated experimentally by several workers; but with one exception[5] the presence of centrifugal fibres in the lateral olfactory tract has been either denied or overlooked. However, as all reports agree that after interruption of both the anterior limb of the anterior commissure and the lateral olfactory tract the degeneration is heavier in the ipsilateral than in the contralateral olfactory bulb[5-7], it is clear that a proportion of the fibres to the bulb must be arising in the ipsilateral hemisphere, but it is not known to what extent these run in the lateral olfactory tract or in the anterior commissure. In order to determine if centrifugal fibres run in the lateral olfactory tract it is necessary to place lesions which are strictly limited to the tract.

Following such lesions in the rat heavy fibre degeneration has been found, in Nauta preparations, passing forwards in and along the deep aspect of the tract to the ipsilateral olfactory bulb. In the main bulb severe fibre fragmentation is seen in the periventricular and granule cell layers; but in addition a considerable number of degenerating fibres pass beyond the mitral cell layer to the external plexiform layer, many reaching as far as the glomeruli. Although no degeneration is seen within the glomeruli themselves, the terminal degeneration on the

periglomerular cells clearly outlines them. In the granule cell layer of the accessory olfactory bulb there is some fibre fragmentation, but no degeneration could be traced with certainty beyond the mitral cells; there was no degeneration in either the main or the accessory olfactory bulbs of the opposite side. Following a 'leucotomy'-type lesion just caudal to the frontal pole with complete interruption of both the anterior limb of the anterior commissure and the lateral olfactory tract[5] the fibre degeneration in the periventricular and granule cell layers of the ipsilateral olfactory bulb is very much denser than after section of the tract alone, but the amount of degeneration seen in the external plexiform layer and around the glomeruli is no greater. In the contralateral olfactory bulb there is moderately severe fibre degeneration in the periventricular and granule cell layers, but in the external plexiform layer only a very occasional degenerating fibre is seen, and there is no degeneration around the glomeruli. In the granule cell layers of the accessory olfactory bulb of both sides a little fibre degeneration is found.

The exact site of origin of the centrifugal fibres which run in the lateral olfactory tract must await further research, but from the material available the amygdala and the adjacent part of the pyriform cortex, together with the hypothalamus and the preoptic areas caudal to the olfactory tubercle, can be excluded. On the other hand, all experiments in which the olfactory tubercle has been damaged have resulted in degeneration of the coarse centrifugal fibres running with the lateral olfactory tract; but as lesions of the rostral part of the pyriform cortex inevitably involve the tract itself a contribution from this area cannot be eliminated. It would be of interest to determine in future experiments whether the olfactory tubercle is the major source of centrifugal fibres, and also whether these fibres arise from the antero-lateral part of the tubercle which receives direct afferents from the olfactory bulb, or from the larger posteromedial part which is related to the olfactory bulb indirectly by way of the pyriform cortex. The conclusive demonstration of the olfactory tubercle as the principal site of origin of the centrifugal fibres to the bulb would mean that in the olfactory system there is provision for the reflex control of activity in the bulb (comparable to that of the γ efferent system in the spinal cord[3] and the feed-back loop in the avian visual pathway[2]) and, at the same time, for a possible influence by the brain-stem reticular formation by way of the ascending fibres in the medial forebrain bundle. The finding that the centrifugal fibres in the lateral olfactory tract terminate in relation to the periglomerular cells as well as with the granule cells indicates that the centrifugal control of olfaction may operate at the level of both the first- and second-order neurones, as well as interacting with the commissural fibres from the olfactory bulb of the opposite side.

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HÆMATOLOGY

Occurrence of Hæmoglobin H in Leukæmia

Appreciable proportions of fœtal hæmoglobin (Hb–F; $\alpha_2^A\gamma_2^F$) have been found[1,2] in occasional cases of childhood leukæmia. This hæmoglobin has also been detected in some adult leukæmias[3]. Among the adult cases of erythroleukæmia examined by us[4], three were exceptional in showing red-cell inclusion bodies after supravital staining, associated with the presence of a minor hæmoglobin component with electrophoretic properties very similar to those of Hb–H (Hb–β_4^A).

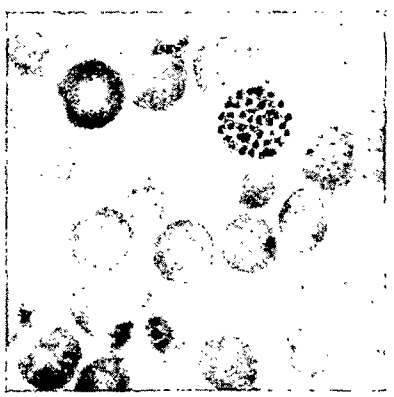


Fig. 1. Photomicrograph of blood film, spread after incubation with brilliant cresyl blue supravital stain, showing some red cells containing inclusion bodies (× 1,750)

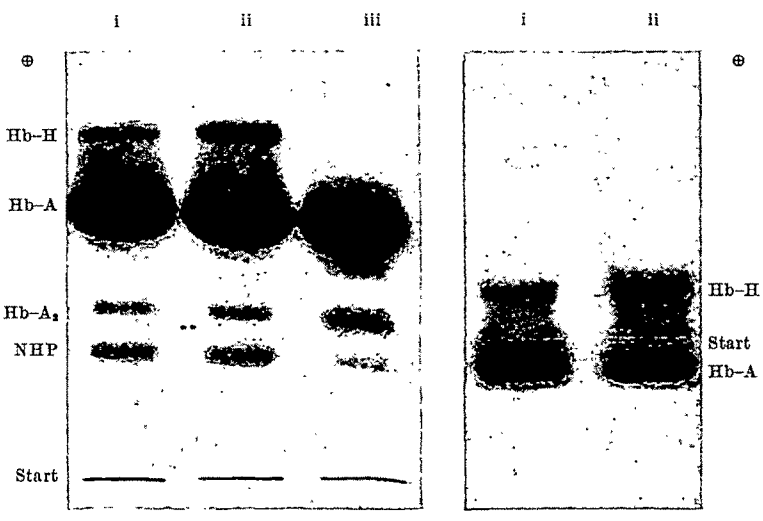
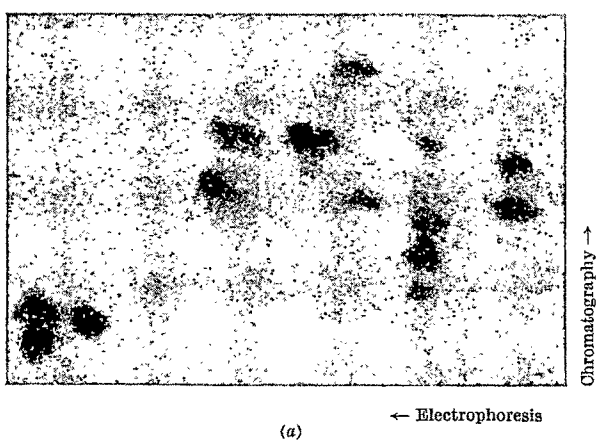


Fig. 2. Starch-gel electrophoretic analyses of hæmolysates. (*a*) *p*H 8·6 *tris*/borate discontinuous buffer system: (i) patient, (ii) Hb–H trait, (iii) normal adult (NHP denotes non-hæm protein); (*b*) *p*H 6·9 phosphate buffer: (i) patient, (ii) Hb–H trait. Stained with naphthalene black

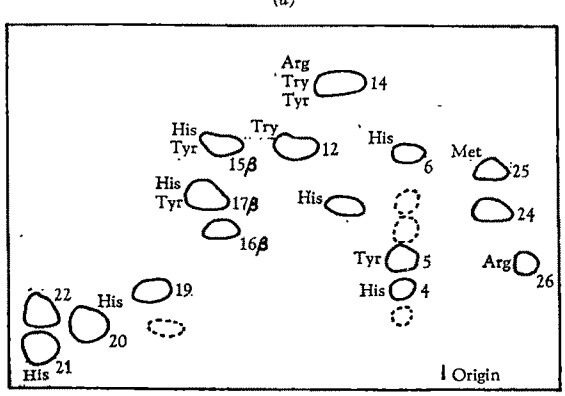
A case of atypical chronic myeloid leukæmia in a 56-year old male of known British descent has now been found which exhibits similar red-cell inclusion bodies (Fig. 1), associated with the presence of about 10 per cent of a minor hæmoglobin, with no detectable proportions of Hb–F and Hb–Bart's (Hb–γ_4^F) and no alteration in the level of the normal minor hæmoglobin Hb–A_2($\alpha_2^A\delta_2^{A_2}$). The abnormal hæmoglobin has now been identified as Hb–H by direct comparison (Fig. 2) with authentic Hb–H by starch-gel electrophoresis at *p*H 8·6 and *p*H 6·9; the latter *p*H, using a phosphate buffer, is particularly suitable[5] for the detection of Hb–H and Hb–Bart's which have anodic mobilities under these conditions, in contrast to Hb–A



Fig. 3. Separation of abnormal hæmoglobin by starch-block electrophoresis of patient's hæmolysate; *p*H 8·6, barbitone buffer, ionic strength 0·05



(*a*)



(*b*)

Fig. 4. Photograph and tracing of 'finger-print' of abnormal hæmoglobin. Electrophoresis in pyridine–acetic acid buffer, *p*H 6·4, 75 min at 40 V/cm; ascending chromatography with pyridine/iso-amyl alcohol/water (35 : 35 : 27) overnight; stained with ninhydrin

which moves toward the cathode. The abnormal component was readily separated from Hb–A by electrophoresis on starch block at *p*H 8·6 (Fig. 3), and examined by the 'finger-print' method, after removal of the hæm, using a modification of the procedures of Ingram[6] and Baglioni[7]. The locations of the arginine, methionine, histidine, tryptophan and tyrosine residues in the separated peptide spots were found by using the specific staining reagents described by Smith[8]. All the peptides, namely, Nos. 3, 9, 10, 11, 13, 15α, 16α, 17α, 18 and 23 arising solely from the α^A chain (numbering system of Baglioni[7]), were absent. The remaining peptides corresponded in both position and specific staining reactions to those expected for a normal β^A chain (Fig. 4).

The parents of the patient were not available for study, but his daughter was hæmatologically normal with no indications of abnormal hæmoglobins or of thalassæmia. There was no history suggestive of any hæmatological abnormality in the patient prior to the onset of the present leukæmic illness. The formation of Hb–H may well be an acquired process associated with the leukæmic state, as seemed possible in the cases of erythroleukæmia studied previously[4].

A possible explanation for the presence of Hb–H is that the leukæmic state sometimes leads to an imbalance of hæmoglobin chain synthesis, resulting in a relative excess of β^A chains which combine to form Hb–H, as in the case of α-thalassæmia[5]. The absence of Hb–F and Hb–Bart's in this subject precludes the compensatory production of γ^F chains, though this appears to be a feature of some childhood leukæmias[1,2]. A disturbance of the balance of hæmoglobin chain synthesis of this nature does not necessarily imply the operation of genetic factors, such as those discussed by Shuster *et al.*[2], though this possibility cannot be excluded at the present time.

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PHARMACOLOGY

Thyroid-adrenocortical Antagonism and Histamine Metabolism

Bjurö, Westling and Wetterqvist[1] have recently suggested that there is a direct antagonism between the actions of the adreno-corticosteroids and those of the thyroid hormones on histamine metabolism in rats. Their results were obtained, chiefly, by estimating the urinary output of histamine. We have been investigating this problem over the past two years[2,3] and conclude that this action of the thyroid hormones is mediated indirectly through their effects on the level of adrenocorticosteroids in the body. Thus only the glucocorticosteroids exert a direct effect on histamine metabolism, their blood concentrations determining the level of histamine sensitivity and of other aspects of histamine metabolism.

We have administered thyroxine sodium (1 or 2 mg/kg daily) to groups of rat and mice and then tested them for sensitivity to histamine. In all experiments, a temporary increase in sensitivity to histamine was noted, coupled with elevated tissue and urinary histamine. Continued treatment with thyroxine beyond 14 daily doses in rats, however, tended to reduce the increased sensitivity to

histamine, and adrenal cortical hypertrophy resulted. A similar temporary increase in sensitivity to histamine was observed in mice but thyroxine treatment beyond 8 days caused a return to normal sensitivity and a marked adrenal hypertrophy. On the other hand, hypothyroid animals showed a depressed sensitivity to histamine which again was only temporary, and continued treatment with antithyroid drugs tended to reduce the depressed sensitivity to histamine and adrenal cortical atrophy resulted. It appears, therefore, that a prolonged change in thyroid hormone-levels leads to a compensatory change in adrenocortical secretion which may be responsible for the return of histamine sensitivity to control-levels. Further experiments showed that simultaneous administration of a glucocorticosteroid prevented the thyroxine-induced increase in histamine sensitivity; instead, a histamine-resistant animal was produced. When hypothyroid animals were adrenalectomized, a marked increase in histamine sensitivity was found instead of a decrease usually observed in hypothyroid animals. Thus, irrespective of the thyroid state, the level of circulating corticosteroid in the body determines the level of histamine sensitivity, and this is represented diagrammatically in Fig. 1 where the sensitivity to histamine and histamine releasers in various hormonal states is indicated.

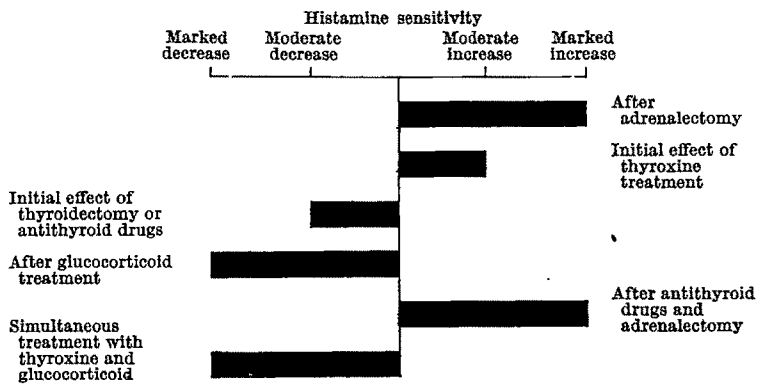


Fig. 1. Sensitivity of rats and mice to injections of histamine and of histamine releasers in various hormonal states

The rate of disappearance of corticosteroid from the plasma has already been shown to be accelerated in thyrotoxicosis[4], the steroids being metabolized at a faster rate[5], and we suggest that the histamine-sensitizing effects of thyroxine treatment are brought about by a reduction in tissue levels of corticosteroid. The internal compensatory mechanism stated here consists of a marked increase in endogenous glucocorticosteroid secretion from the adrenal cortex, after stimulation from the pituitary gland, becoming effective after prolonged thyroxine treatment. The initial effect of a hypothyroid state can be explained on a similar basis: there is a reduction in the rate of steroid metabolism and an accumulation of glucocorticosteroid in the tissues, leading to a depressed histamine sensitivity. Adrenal cortical atrophy follows and histamine sensitivity returns to control-levels. Thus there is antagonism between the adrenal cortex and the thyroid secretion, but the adrenal cortex appears to be the more important factor as regards a direct action on histamine metabolism.

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PATHOLOGY

Rapid Liver Tumour Induction by Concerted Action of *N*-Hydroxy-2-acetamidofluorene and Hormonal Stimulation

Liver is not usually considered a target organ of hormonal factors known at present. Nevertheless, in certain animal species, the operation of such agents is clearly evident. Thus, liver cancer induction by certain carcinogenic azo dyes occurs more readily in female than in male mice. With certain other azo dyes[1], and with 2-acetamidofluorene[2] and its probable proximate agent *N*-hydroxy-2-acetamidofluorene[3], male rats are considerably more susceptible than females. Liver carcinogenesis is enhanced by the joint administration of anabolic hormones, testosterone or cortisone, and 2-acetamidofluorene, its diacetyl derivative[4] or its *N*-hydroxy derivative[5]. Castration of female rats and joint administration of testosterone and 2-diacetamidofluorene gave rise to liver tumours to almost the same extent as in male rats[6]. Whether these hormonal factors act directly or by way of alteration of the total hormonal balance involving the pituitary, gonads, adrenals and target organ is, at present, not known. Surgical removal of the adrenals or of the pituitary gland delays or abolishes carcinogenesis in the liver by azo dye or the carcinogenic fluorene derivatives (see ref. 7). Participation of hormonal factors derived from the pituitary in the carcinogenic process in the liver might therefore be surmised. However, direct evidence on this point has so far not been adduced. Bielschowsky[8], Laws[9], and also Glinos *et al.*[10], have attempted to devise experiments to establish a multiple stage mechanism for liver carcinogenesis, but their observations were either negative or at best suggestive for the operation of such a process.

Furth *et al.*[11], making use of the functional pituitary tumours produced by them, have demonstrated the essential promoting action of hormones elaborated by the transplantable tumour in the induction of primary mammary gland tumours in rats or mice treated with a single dose of a carcinogenic hydrocarbon, of radiation or of a viral preparation. We have now applied this type of approach to the successful demonstration of a concerted action between pituitary hormonal factors and low doses of *N*-hydroxy-2-acetamidofluorene in regard to liver cancer induction in male and female rats.

Weanling, four-week-old male and female Fischer strain (*F*344/*N*) rats were divided into four groups (Table 1). Group 1 consisted of untreated controls fed a semi-synthetic 18 per cent casein diet with adequate micro- and macro-nutrients (diet *A*). Group 2 was fed a diet containing 0·016 per cent *N*-hydroxy-2-acetamidofluorene in an otherwise identical diet (diet *K*). Animals in Group 3 were fed control diet *A* and received a subcutaneous implant of the Furth mammotropic tumour *MtT*/*F*4 in the back. Group 4 was administered carcinogenic diet *K* and received implants of the mammotropic tumour. This transplantable pituitary tumour served as a source of ACTH, growth hormone and prolactin[12].

The implanted mammotropic tumours in Groups 3 and 4 were first palpable in about 6 weeks and grew progressively to a large size in the subsequent 7 weeks. Animals in all experimental groups were killed at the end of 13 weeks.

The livers of all control animals, Group 1, were perfectly normal. The expected response was found in Group 2. The male rats had livers weighing twice as much as compared with the control animals. Six out of the 7 were grossly nodular. Microscopically, small nodules of hepa-

Table 1. Effect of *N*-Hydroxy-2-acetamidofluorene and Pituitary Mammotropic Tumour singly and together on Liver in Male and Female Fischer Rats

Group	No. rats	Sex,	Normal	Gross enlarged	Liver picture* Nodular	Hypertrophy	Microscopic nodule of hyperplasia	Carcinoma
1 Control	9	*M*	9	—	—	—	—	—
	10	*F*	10	—	—	—	—	—
2 *N-OH-AAF*	7	*M*	—	1	6	—	7	—
	8	*F*	—	4	4	—	8	—
3 *MtT/F4* implants	13	*M*	—	13	—	13	—	—
	14	*F*	—	14	—	14	—	—
4 *N-OH-AAF* + *MtT/F4* implants	12	*M*	2†	3	7	—	2	10
	14	*F*	—	3	11	—	2	12

* After 13 weeks of treatment.

† Nodule of hyperplasia in these two males after microscopic examination.

toma along with cirrhotic changes were seen in the livers of all the males and to almost the same extent in the females. In four of the females, however, the hyperplastic nodules were only seen at the microscopic level. In the *MtT* control Group 3, the livers of both male and female animals were enlarged about equally as was evidenced by the hypertrophic aspect at the microscopic level. Furthermore, as reported[13,14], the kidneys of both sexes were 4–5 times larger than those of the control groups. In Group 4, namely, the animals receiving carcinogen and mammotropic tumour, the livers were enlarged in all and most of them were nodular. Compared with the findings in Group 2 on carcinogen alone, the lesions of both male and female animals were similar and extensive. Hepatocellular carcinoma—either trabecular or adenomatoid type—were seen in livers in these groups. Renal hyperplasia was much less extensive in Group 4 than in Group 3.

Thus the combined action of the hormonal stimulus and chemical carcinogen gave rise to advanced lesions in the liver in a short time, even though only moderate amounts of carcinogen were used in this experimental series. These investigations demonstrate clearly the participation of pituitary factors in the genesis of liver tumours. Considering the similarity in response of males and females, it would appear that the pressure of the hormonal stimulation is so strong as to overcome sex-linked differences.

By means of this system and carcinogenic hydrocarbons, Furth *et al.*[11] observed mammary cancer development in female rats (see ref. 15). In contrast, there was no sign of mammary cancer in our experimental series. The mammary glands in both male and female rats were filled with milk cysts and were obviously functional. Possibly, the animals have not survived long enough or insufficient carcinogen was present at the level of the mammary gland to inhibit normal functioning of the gland. Some years ago, Cantarow, Paschkis *et al.*[16] proposed the idea that carcinogenesis proceeds best in a hyperplastic but non-functional gland, but does not occur in a hyperplastic and functioning organ.

The results obtained warrant an extension to a study of the applicability of the joint action of hormones and chemicals to liver carcinogens of different potency. In view of the higher sensitivity of this system, it is conceivable that compounds with weak carcinogenic activity can be detected more readily by this means. In any event, the rapidity with which the tumours arise might make this system suitable as a screening technique for carcinogenicity. Furthermore, the pituitary tumour used in the present instance generated a number of hormonal factors[12,17]. It will be of interest to determine whether individual hormones produced by this or other transplantable pituitary tumours as well as additional implants of the pituitary gland itself can accelerate liver carcinogenesis. The present investigations are suggestive in respect to a promoting effect of pituitary factors in liver carcinogenesis. Dissection of the individual sequences involved may throw some light on the possible operation of a sequential multi-stage effect in liver carcinogenesis, as it is recognized with respect to skin carcinogenesis[18].

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Carcinogenicity of Preheated Fats

Roffo[1] claimed to induce gastric adenocarcinoma in rats after feeding fats preheated to 350° C for 30 min. However, several authors[2–4] repeating Roffo's experiments have failed to find glandular tumours in the stomach. Later, British investigators have examined the possible carcinogenic action of preheated cottonseed oil *per os* in mice and hamsters, but again without success[5]. In view of the repeated failure of the experiments when giving the fat orally, we attempted to induce glandular neoplasms by injecting fat—preheated to 360°–365° C for 30 min—into the stomach wall of the guinea pig, a rodent with a purely glandular stomach. The series reported here is an extension of a preliminary observation reported previously[6].

Sixty-five female guinea pigs with an average initial body-weight of 470 g were used. The fat to be injected was obtained from the peritoneal cavity—perirenal and mesenteric fat—of guinea pigs. The adipose tissue was minced with scissors in a Petri dish, placed in an open Erlenmayer flask, heated to 360°–365° C for 30 min, filtered (filter paper) to eliminate carbonaceous residue and cooled. The fat used in control animals—receiving non-heated fat—was extracted by ether. Both kinds of fats were warmed to a liquid state (22°–25° C) for injection. Each animal received an injection of 0·05 c.c. of preheated fat into the ventral wall of the fundus and the same amount into the wall of the antrum, except the animals killed at 150 days' post-injection, which received a single dose of 0·10 c.c. into the wall of the antrum only. Usually the fat was

Table 1. Effects of Intramural Injection of 0·05 c.c. of Preheated Fat on the Fundus and on the Antrum of the Guinea Pig

Group	Treatment	No. of animals	Days after injection	No. of animals bearing gastric lesions	Gastric lesions (per cent)	Gastric lesions*
I	Non-heated fat	9	17–18	0	0	—
II	Non-heated fat	7	90	0	0	—
III†	Non-heated fat	6	150	0	0	—
IV	Non-heated fat	6	240	0	0	—
V	Preheated fat	9	16–26	5 (24–26 days)	55·5	Foci of atypical glands in mucosa (2); glands infiltrating submucosa (3)
VI	Preheated fat	11	90	3	27·2	Glands infiltrating submucosa (3)
VII†	Preheated fat	6	150	6	100·0	Focal hyperplasia of mucosa (4); glands infiltrating submucosa (3); infiltration of muscularis propria (1); diverticulum (3)
VIII	Preheated fat	10	240	4	40·0	Focal hyperplasia of mucosa (2); glands infiltrating submucosa (2); diverticulum (1)

* Numbers in parentheses indicate number of lesions.

† In this group, a single dose of 0·10 c.c. in the wall of the antrum was injected.

deposited into the submucosa or the muscularis propria. Twenty-nine animals were injected with non-heated fat and 36 with preheated fat. One animal which received non-heated fat and died from an infectious disease at 60 days' post-injection (showing no gastric lesion) was not included in Table 1. For the schedule of death see Table 1.

The gastric lesions, observed microscopically, are summarized in Table 1. In the present series only local effects in the stomach were observed. None of the 28 control animals showed gastric lesions. On the contrary, in 18 out of 36 animals receiving preheated fat gastric lesions were found. These consisted mainly of focal hyperplasia of mucosal epithelium, diverticula, and glandular infiltration of submucosa through perforation of muscularis mucosæ sometimes at two separate points. In some cases these lesions were found in association with one another. In one case the lesion consisted of infiltration of muscularis propria by epithelial foci exhibiting hyperchromatic anaplastic cells. In addition, control and experimental animals showed various inflammatory and degenerative changes at the site of injection. The difference in incidence of lesions between control and experimental groups is highly significant ($P < 0{\cdot}001$). With respect to the carcinogenicity of non-heated fat, there was not an adequate number of control animals to prove conclusively that it has no carcinogenic activity. Furthermore, because non-heated fat was extracted by ether, in order to avoid heating, it is possible that ether extraction eliminated some ether-insoluble substance with a weak carcinogenicity. The diverticula and infiltrating lesions induced in the present series show striking morphological and histogenetic similarities to the precancerous lesions induced by 3-methylcholanthrene deposited on the gastric serosa[7], implanted[8], or injected[9] into the stomach wall of guinea pigs. We have observed under all these conditions infiltrating lesions originating from basal glands, sometimes perforating the muscularis mucosæ at multiple points in association with diverticula. Furthermore, the case of invasion of muscularis propria suggests the possibility that, when permitting preheated fat to be in contact with glandular cells for longer periods of time, a malignant change may occur. However, we have not classified the present epithelial lesions as precancerous because we have not at this time information on their progression.

Finally, it is necessary to emphasize that a great part of the disagreement among experimental results on the carcinogenicity of naturally occurring lipoid substances is due mainly to the different: (*a*) chemical composition of the material tested, and, furthermore, the variable composition of similar commercial samples, for example, lards and oils; (*b*) temperatures reached during heating; (*c*) duration of the heating process; (*d*) species and strains of rodents used, and (*e*) duration of the experiments (for references see Zaldívar[10] and Arffmann[11]).

I thank Prof. G. W. Casarett for diagnosis of some microscope slides and helpful criticism on this work.

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RADIOBIOLOGY

Induction of Mutations and Killing of Cells in Irradiated Spermatogonia of *Drosophila*

In experiments where *Drosophila melanogaster* spermatogonia were irradiated in the 20-h old embryo with doses of 0 r., 55 r., 110 r., 160 r. and 310 r. given at 25 r./min, and the induction of sex-linked recessive lethals observed, a non-linear dose effect curve was found[1]. This curve had a rather flat maximum for doses of 110 r. and 160 r. (about 0·15 per cent lethals above controls), whereas 310 r. gave no increase in lethal frequency above the control value. These results were based on slightly more than 100,000 *X*-chromosomes tested (Fig. 1).

It was presumed that differential killing of cells which carried mutations was the mechanism involved. In an attempt to explain the results quantitatively, a mathematical model for the irradiated population of spermatogonia was formulated. This model is based on the assumptions that: (*a*) there is a cyclic variation in cell radiosensitivity as regards induction of mutations as well as regards cell killing[2,3]; (*b*) only damage inflicted in the sensitive phase need be considered in relation to the above results. If *Drosophila* spermatogonia are assumed to have a radiogenetic sensitivity of $2{\cdot}0 \times 10^{-5}$ sex-linked lethal mutations per r. (about the same as sperm), and in the sensitive phase a survival curve with $LD_{50} = 135$ r. and extrapolation number = 3·0, and that 0·1 of the spermatogonial population is in the sensitive phase at the time of treatment, the theoretical dose effect curve for recovered mutations which can be calculated from the

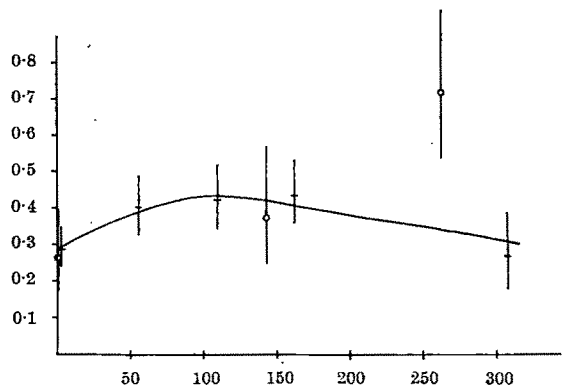


Fig. 1. Frequencies of sex-linked recessive lethals (per cent) after treatment of spermatogonia with doses given at 25 r./min (+) or protracted over 8 h (○). Full line shows theoretical curve for scorable lethals based on hypothetical values for cell sensitivity and cell survival. Vertical lines indicate 95 per cent expectation interval (ref.10)

model fits the experimental results in a rather satisfactory way[4] (Fig. 1).

On the further assumptions that sub-lethal radiation damage is repaired completely from one cell generation to the next[5], whereas 'genetic' damage is not, the hypothesis led to the conclusion that if a given dose were protracted over several cell generations, the observable genetic damage should be higher than that found after acute treatments. This conclusion has now been tested with doses of 144 r. and 267 r.

The same experimental procedure was used as before. Spermatogonia were irradiated in wild-type embryos/young larvæ. The irradiated ♂♂ were afterwards mated singly to Basq ♀♀. Only one heterozygous ♀ among the offspring of each such mating was then tested for the presence of a lethal-bearing *X*-chromosome, whereby the problems connected with clusters of mutations were avoided. Irradiations were performed by means of a 5-c. ^{137}Cs-unit. Doses were given over 8 h, $16 \pm 0{\cdot}5$ to $24 \pm 0{\cdot}5$ h after egg laying. 144 r. and 267 r. were given simultaneously to different batches of eggs collected from the same group of layer ♀♀. Eight irradiations were performed during the autumn of 1962. The totals are given in Table 1 and Fig. 1. The results seem to support the hypothesis of differential killing as an explanation for the results after acute irradiation of spermatogonia. The quantitative conformity with the hypothetical value for the radiogenetic sensitivity is also fairly good for a first attempt: $1{\cdot}7 \times 10^{-5}$ mutations per r. after 267 r., as compared with the hypothetical $2{\cdot}0 \times 10^{-5}$ mutations per r.

Table 1. Frequencies of Sex-linked Recessive Lethals after Protracted Treatments of Spermatogonia. Upper and Lower 95 per cent Expectation Limits calculated according to Fisher and Yates (ref. 10)

	0 r.	144 r./8	267 r./8
Chromosomes tested	6,934	4,835	5,788
Lethals found	18	18	41
Lethal frequency (%)	0·26 (0·39 / 0·17)	0·37 (0·56 / 0·25)	0·71 (0·94 / 0·53)

The results show that protracted irradiations under some conditions may have a higher observable genetic effect than have acute treatments. This is a dose rate effect in the opposite direction of that found by Russell *et al.*[6] in the mouse and Oster *et al.*[7] in *Drosophila* ♀♀ and sought by several authors in other materials[8]. The present effect is similar to that found by Tazima *et al.* after irradiation of silkworm larvæ 3–10 days old, and opposite that found after treatment of earlier stages[9]. It is believed that consideration of the dynamics of the irradiated cell populations in the different systems may provide clues to an understanding of the divergent findings.

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BIOLOGY

Accumulation of Amino-acids in Plant Cell Tissue Cultures

It was recently reported that when plant cell tissue cultures derived from sycamore cambial callus (*Acer pseudoplantanus L*) were grown with limited access to air, there was a marked reduction in the amount of leucoanthocyanins produced although there was little effect on aldohexose concentration[1]. It has now been found that, in limited air, cell cultures from callus of sycamore, bean (*Phaseolus vulgaris, L*), rose (*Rosa* sp., var. Dr. van Fleet), and *Haplopapus gracilis* grown on agar slopes as before[1], all show a marked increase in the amount of free amino-acids compared with tissue grown with free access to air. Again little or no change was found in the concentration of sugars (Table 1), nor, in the case of the sycamore callus, in the total perchloracetic acid-soluble ribonucleic acid. Analyses of the changes with time in the relative proportions of carbon dioxide and oxygen, using a Fisher gas analyser, in the culture bottles showed that, as expected, there was a build-up of carbon dioxide to ~20 per cent and a decrease of oxygen to ~0·5 per cent in the sealed bottles.

Table 1. Amino-acids and Aldohexoses in Cultures grown (*a*) with and (*b*) without Free Access of Air

Culture	Amino-acids (*a*)	Amino-acids (*b*)	Sugars (*a*)	Sugars (*b*)
Bean	8·5	40·2	67	60
Sycamore	4·2	25·1	109	103
Rose	19·4	47·0	7·3	5·8
Haplopapus	7·2	60	10·3	10·8

All values expressed as μmoles/g callus in terms of β-phenylalanine and glucose respectively.

To determine whether the observed increase in total amino-acid concentration was due either to increased carbon dioxide or decreased oxygen, callus tissue was grown in bottles kept in vacuum desiccators filled with artificial gas mixtures containing different proportions of carbon dioxide and oxygen (Table 2). The desiccators were evacuated and filled with the appropriate gas mixtures every other day. The results indicate that both increased carbon dioxide and decreased oxygen lead to an accumulation of amino-acids. The effect of growth in restricted air increasing the concentration of amino-acids

Table 2. Total Amino-acids in Cultures grown in Gas Mixtures containing Different Proportions of Carbon Dioxide and Oxygen

Gas mixture: Carbon dioxide (%)	Gas mixture: Oxygen (%)	Total amino-acids: Bean	Total amino-acids: Sycamore
0	20	6·5	2·5
10	20	22·9	—
20	20	19·5	—
5	15	6·5	2·5
10	10	13·8	4·7
15	5	24·6	9·5
20	1	28·2	14·2
0	1	27·7	—

Values for total amino-acids expressed as μmoles/g callus in terms of β-phenylalanine.

also occurred when 0·1 per cent yeast extract was added to the growth medium, but not when a rich source of amino-acids (1 per cent acid-hydrolysed casein) was used. This would indicate that the growth factors present in yeast extract have no effect on the accumulation process.

Paper chromatographic analyses of the accumulated amino-acids showed that there were marked quantitative differences in the relative proportions of each, the most striking being a large increase in γ-aminobutyric acid, an effect which was accentuated when the tissue was grown in the presence of yeast extract. Other workers[2] have reported a similar accumulation of γ-aminobutyric in other organisms. The relationship between this amino-acid and related metabolites in cell tissue culture is now being examined.

We thank Dr. N. Sutherland for supplying the isolates of *Haplopapus gracilis* and *Rosa* var. Dr. van Fleet, and Dr. D. H. Northcote for the isolate of *Acer pseudoplantanus L.* One of us (R. L. W.) thanks the Director, Tea Research Institute of Ceylon, for permission to publish this communication.

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Intermittent Germination of *Capsella*

Previously, I have directed attention to the prevalence of intermittent germination as a feature of the most successful of our annual garden weeds and suggested that human cultivation activities have probably effected an artificial selection of strains that has accentuated this feature[1]. Attention was there directed to the need for sowing freshly ripened seeds from individual plants to avoid the concealment of this feature, through overlap of the germination flushes from seeds of different individuals or seeds stored for varying periods. These conditions, however, frequently impose a severe limitation on the number of seeds available for any one test and it might therefore be suggested that if a considerably augmented number of seeds were simultaneously ripened the intermittence would be appreciably diminished or might actually disappear.

Last summer I was fortunate in finding an exceptionally large plant of the *Capsella simplex* type from which, on July 16, I was able to obtain no less than 2,327 ripe seeds which were sown immediately on a pan of soil maintained thenceforth in a moist state, in an unheated glasshouse so as to avoid possible interference by birds. The conditions were thus similar to those in Nature except that the maximum daytime temperatures were higher than those in the open. As germinations occurred the seedlings were removed daily with forceps and their numbers recorded, when also their distribution was observed, and it may be stated at the outset that the individual seedlings of the different flushes appeared to be more or less evenly dispersed and no 'edge effects' were manifest.

The results, together with the maximum and minimum temperatures, are presented in the accompanying graph (Fig. 1). It will be seen that the great majority of the germination flushes occurred, as might have been anticipated, when there was a rise of temperature, but it must be emphasized that such temperature changes may occur without any corresponding germination flush, as can be seen, for example, about September 12, November 22 and February 15. It is very obvious, from a comparison of the germination fluctuations with the temperature changes, that the increasing warmth triggers off the germination of those seeds, and only those, that are in a state for such stimulation.

Two major aggregations of daily germinations are seen to have obtained, namely in the autumn and spring respectively, but even at the periods when there was no actual interruption of the daily germinations there is a marked irregularity in their numbers which, from its multimodal character, probably represents concealed intermittence.

Despite the large number of seeds involved, of which more than 77 per cent proved viable, the intermittent

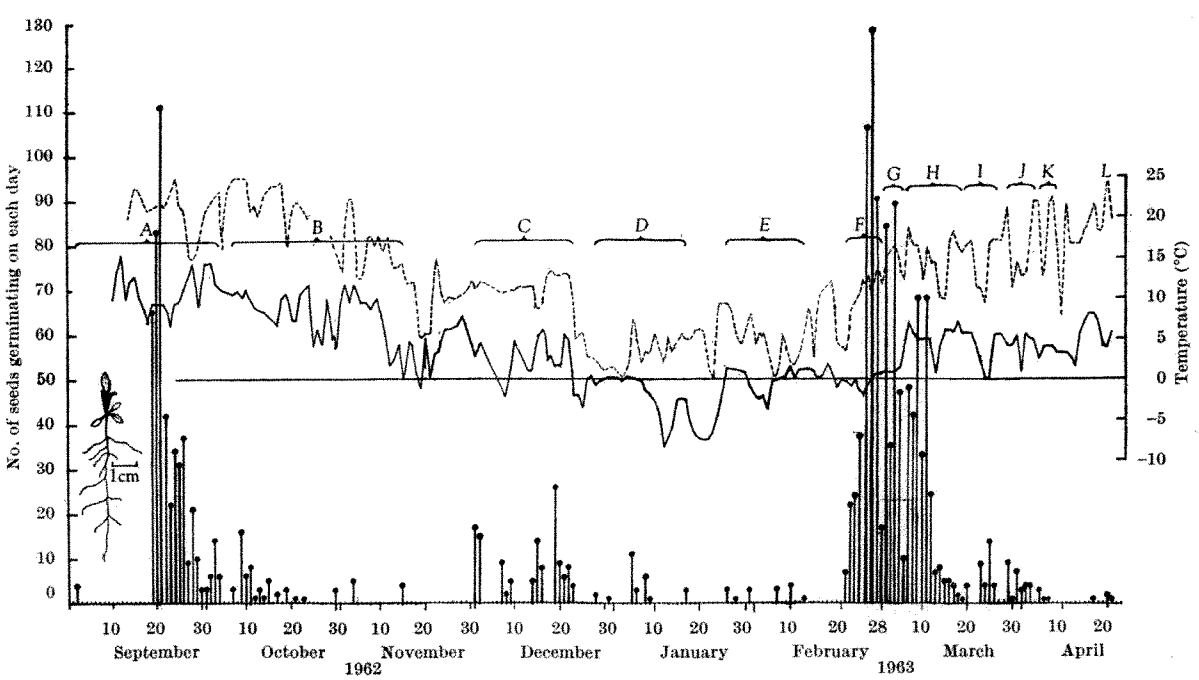


Fig. 1. Daily germinations from 2,327 ripe seeds obtained from a plant of the *Capsella simplex* type sown on July 16, 1962. Period *A*, 501 seeds obtained in 33 days (21·5 per cent); *B*, 62 from 36th to 74th day (2·6 per cent); *C*, 129 from 90th to 112th day (5·5 per cent) *D*, 27 from 117th to 137th day (1·1 per cent); *E*, 15 from 146th to 163rd day (0·6 per cent); *F*, 432 (18·6 per cent); *G*, 265 (11·4 per cent); *H*, 307 (13·1 per cent); *I*, 35 (1·5 per cent); *J*, 28 (1·2 per cent); *K*, 5 (0·2 per cent); *L*, 4 (0·17 per cent). This represents a total of 1,810 seeds during period of 230 days (77·7 per cent). – – –, Maximum day temperatures; ——, minimum night temperatures

character of the germinations is quite conspicuous. From the first germinations on September 2 to the last in the following April, they are spread over a period of no less than 215 days. If we ignore the fluctuating numbers and only regard periods when there were uninterrupted daily germinations, these number no less than thirty, forming about ten groups of active germination. Germinations occurred on 96 of the 215 days over which they were spread. It should, however, be stressed that biologically a succession of flushes of germinations probably have a similar biological significance whether interrupted or not unless the actual discontinuity be so marked, as in periodic types of germination, as to preclude the build-up of pests or predators.

Incidentally, it may be noted that during the very severe weather of January the seedlings were as brittle as glass, but some that had been transplanted when removed showed no sign of injury after they had thawed.

We could reasonably expect that the factor, or more probably factors, responsible for the differing natural dormancy of contemporaneous seeds would exhibit degrees of intensity from being very marked to perhaps only slight and that this variation would be normal and continuous. The discontinuity that we actually observe might be imparted by the erratic occurrence of the meteorological conditions that bring about the marked temperature changes which observations have shown stimulate germination of these seeds that have attained a condition to respond. For *Capsella*, such an interpretation conforms to the observed facts that while all the germination flushes occur in relation to abrupt changes of temperature such changes may nevertheless occur without any accompanying germination response, presumably because no seeds were contemporaneously in an appropriate physiological state.

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Electron Paramagnetic Resonance in Non-irradiated Bone

The generation of electrical potentials by bone subjected to mechanical stress has been recently reported by two groups of investigators[1,2]. Bassett and Becker suggested a semiconduction mechanism as being the source of the phenomenon, while Shamos *et al.* favoured a classical piezo-electric mechanism similar to that earlier described by Fukada and Yasuda[3]. The semiconduction theory has recently been reported on in more detail[4] and evidence presented for the formation of multiple *pn* junctions from the apatite crystal (*p* type) collagen fibre (*n* type) in the bone matrix. Similar inorganic *pn* junctions have been shown to be exceedingly stress sensitive[5,6].

Since the semiconduction theory would require the presence of free charge carriers while the piezo-electric theory would have no such requirement, the application of electron paramagnetic resonance techniques to this problem seemed to be of interest. If semiconducting materials with adequate concentration of donors are present in bone, resonance signals should be observable without previous irradiation, and should be increased by light and thermal activation and possibly by mechanical stress. Electron paramagnetic resonance signals have previously been reported in electron irradiated bone[7] and in X-ray irradiated teeth[8] and bone[9]; no signals have been reported in non-irradiated material.

Cortical bone samples, human tibia and bull-frog tibia, were obtained by surgical dissection from extremities rendered bloodless by tourniquet or vessel ligation. The periosteum and inner cancellous bone were removed and

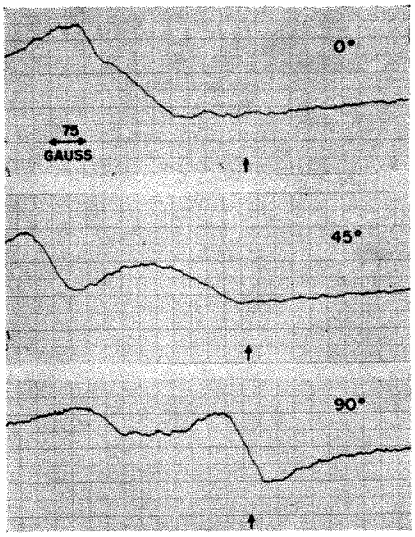


Fig. 1. Electron paramagnetic resonance spectra obtained from amphibian whole cortical bone. The orientation of the sample long axis relative to the *H* field is indicated in the upper right on each tracing. The arrow indicates *g* 2·0 value

cortical fragments approximating 3 mm × 3 mm × 5 mm were obtained by osteotome. Further dissection was done with quartz knife and probe under 20 times magnification. All surfaces were shaved free of any possible metallic contamination and sections with visible vascular capillaries were discarded. Final specimens were approximately 0·5 mm wide and 3 mm long, the original long axis being represented by the larger dimension. Only dense mature cortical bone was utilized. Samples were mounted on quartz filaments with a small amount of 'Cenco' red wax and suspended in 5-mm diameter quartz tubes, in such a manner that the angular relationship between the long axis of the bone sample and the main magnetic field (*H*) could be altered. Control tracings were made in each case by removing the sample, retaining the original mounting wax, quartz filament and tube. All electron paramagnetic resonance spectra were obtained at room temperature, the first derivative curve being recorded. A Varian *X* band spectrometer with 100-kc/s field modulation was used in conjunction with a 6-in diameter Varian electromagnet with ring shim pole caps and gap diameter of 2·58 in.

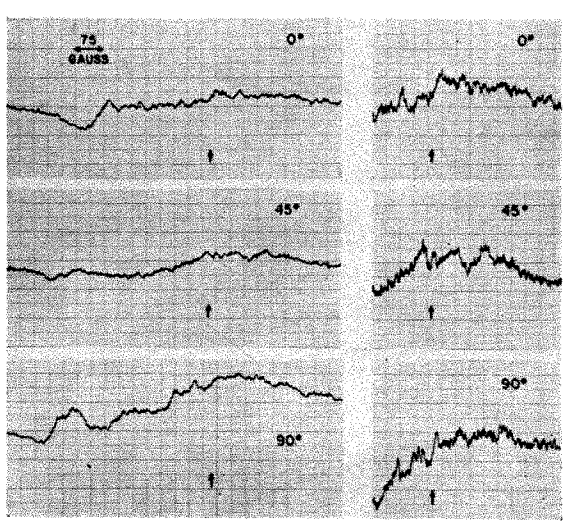


Fig. 2. Electron paramagnetic resonance spectra obtained from human whole cortical bone. Sample long axis orientation and *g* 2·0 value are indicated in the same fashion as in Fig. 1. The tracings at the left were obtained with similar amplification to those of Fig. 1. The recordings to the right were obtained with an amplification of three

Resonance signals with g values dependent on the angular relationship of sample long axis to H field were observed in all samples of bone from each species. In amphibian bone (Fig. 1) a large well-defined resonance about 200 gauss wide was noted in the vicinity of g 2·3, with a shift in g value with change in angular orientation. In the 90° position a well-defined, almost symmetrical resonance appeared at g 2·0 which was much more anisotropic, appearing to merge with the g 2·3 peak in the 0° position. In human bone (Fig. 2) a similar well-defined resonance was found in the g 2·3 vicinity, being more prominent in the 0° and 90° orientation than in the 45° with again moderate g value anisotropy. A very broad resonance signal appears, most prominently at 90° orientation, reaching a peak at approximately g 1·8. Higher amplification scans of the g 2·0 area demonstrated a small superimposed resonance, about 35 gauss wide with a g value of 2·0 in the 90° position. This signal is light sensitive, increasing in amplitude with irradiation from a wide band (2000–10000 Å) source.

The similarity of the two major resonances obtained from non-irradiated bones of two different vertebrates as well as the evident anisotropic characteristics of the signal indicate that they are produced by actual components of the bone rather than surface contamination. At this time neither resonance can be definitely ascribed to a free charge carrier population such as is present in doped semiconductors[10]. Identification of the substances involved requires further separation and purification procedures.

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A Graphical Method for comparing the Ratio of the Number of Cells to the Volume of Medium in Tissue Culture Containers of Various Sizes

IN the course of virus yield studies it is often necessary to convert a tissue culture system from one size container to another in such a way that the ratio of the number of cells to the volume of medium remains constant. An easy way of determining the various medium volumes which will give this constant ratio is to plot the relevant data graphically.

From previous work carried out in this Department, a value for the number of cells of a given tissue culture per unit area of confluent cell sheet was available. This value was obtained from six hundred counts of cells grown on cover-glasses. The confluent cell sheets were stained and a total of forty areas were counted on each cover-glass using stage and ocular micrometers, ten cover-glasses being counted for each batch of cells. The experiment was repeated six times using a different batch of cells each time. The particular tissue cultures used in this experiment were primary monolayers of embryonic chick fibroblasts and the mean value obtained was 1,150 cells/mm² of cell sheet.

Knowing this value, it was then necessary to measure the total area of the cell sheet in each type of flat bottle under consideration. As the contours of the bottles made

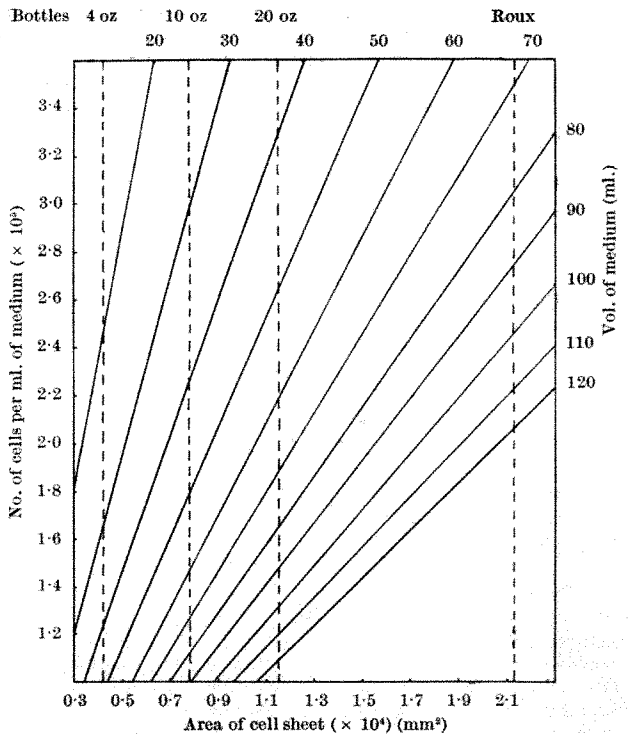


Fig. 1. No. of cells/ml. of medium plotted against area of cell sheet calculated for chick embryonic fibroblasts of density 1,150 cells/mm²

the exact shape of the cell sheets irregular it was found that the best way to measure the area was first to mark the edge of the cell sheet on the outside of the bottle. This outline was then traced on to thin paper and from there copied on to millimetre squared paper. A count of the squares enclosed by this outline gave the area of the cell sheet in square millimetres directly. Twelve experiments were made in this way to obtain a mean value.

The ratio of number of cells to volume of medium (expressed as cells/ml.) can be calculated from the following equation:

$$R = \frac{AN}{V}$$

where R = cells/ml.; A = area of cell sheet (mm²); N = cells/mm² = 1,150 (as determined); V = volume of medium (ml.).

Hence if R is plotted against A (for different sizes of bottles) then a straight line will be obtained with a slope of N/V. As N is a constant for a given tissue, then the slope will be inversely proportional to V and as the volume increases so the slope of the line decreases. Therefore, if graphs of R against A are plotted for various values of V then a series of straight lines with different slopes will be obtained.

Fig. 1 gives the values for the cell sheet areas corresponding to four sizes of bottle (4 oz., 10 oz. and 20 oz. medical flats and Roux) which are marked by the dotted lines. The point where the dotted line intersects a continuous line marks the value of R at that volume of medium represented by the continuous line.

This method can be used to translate a system from one type of bottle into the equivalent for another type. A 20-oz. bottle is represented by the dotted line on the graph at $1{\cdot}15 \times 10^4$ mm² on the A axis. If this bottle contains 60 ml. of medium then the equivalent in other sizes of bottle is found by placing a ruler across this line parallel to the A axis and where it intersects the 60 ml. line. Where the ruler crosses the 10 oz. line the nearest volume line is for 40 ml. (A line for 41 ml. would be more accurate if drawn.) Similarly for a Roux bottle the nearest volume line is 110 ml.

It can be concluded that a 10-oz. bottle with 40 ml., a 20-oz. bottle with 60 ml. and a Roux bottle with 110 ml. of medium all have approximately the same cell to volume of medium ratio. Hence different cell systems can be rapidly converted from one size of bottle to another while still maintaining the relationship between the number of cells and the volume of medium.

We thank the Directors of Pfizer, Ltd., for permission to publish this letter.

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Effect of Electrostatic Fields on Cell Mitosis

From quantum mechanical calculations on the electronic structure of DNA[1–3] a mechanism was proposed for the onset of DNA-duplication[3,4]. It has been assumed that the mobile π-electrons of the macromolecule, which, according to Eley and Spivey[5], cause the semi-conductivity of DNA, can be polarized by the effect of local electrostatic fields present inside the cell. As a result of polarization free electron charges may appear at the end of the DNA double-helix; the repulsion between the charged nucleotide bases at the ends of the molecules may lead to the breaking of the bonds between them and thus induce the unwinding of the double-helix[3,5]. The unwinding of the helix being, according to the Watson–Crick duplication mechanism[6], the starting point of DNA duplication, it seemed reasonable to suppose that the appearance of free charges at the ends of the molecule might induce DNA multiplication. It was therefore decided to examine the behaviour of cell cultures exposed to the effect of strong electrostatic fields[4].

In the present experiments chick-embryo heart fibroblast cultures, cultivated in plasma coagulum with 20 per cent chick-embryo extract and prepared using the hanging-drop method[7], have been exposed to the effect of an electrostatic field. The latter was produced by a d.c. voltage source (designed and constructed by the Central Research Institute for Physics, Budapest) to supply a potential difference of 14–25 kV. A voltage of 15 kV was led into the parallel plates of a capacitor with reinforced insulation. The plates were 30 cm × 14 cm in size and 1 cm thick; the distance between them was 1 cm during the experiments. (The actual field strength in the cells is until now unknown in our experiments.)

The cultures contained in depression slides 0·4–0·5 cm thick were placed between the two plates of the capacitor and kept there for 8 and 4 h respectively. In the 8-h series the cultures were exposed to the electrostatic field 18–20; in the 4-h exposures, 1 h after explantation. Control specimens, their number corresponding to the cultures under investigation, were kept in the same laboratory at identical temperatures and for identical lengths of time. After exposure to the electrostatic field, the cultures along with the controls were placed for 18 h into a thermostat kept at 37° C. Thereafter, the material was fixed in Carnoy-fluid and stained by Giemsa's method.

The mitotic indices of the cultures which underwent treatment and those of the control specimens as well were determined by direct counting. Table 1 shows the results of both experimental series.

Table 1. Mitotic Indices of the Cultures exposed to the Effect of an Electrostatic Field, and of the Control Cultures

	8 h						4 h			
Series	1	2	3	4	5	6	1	2	3	4
N	12	14	4	20	6	12	20	12	16	16
$\overline{X}$	2·50	2·70	2·00	2·00	5·70	7·24	2·79	3·81	1·68	2·53
$\overline{X}_c$	1·00	1·70	1·30	0·90	3·80	5·62	2·73	2·62	1·61	2·36
S_x	0·19	0·25	0·06	0·11	0·60	1·51	0·32	0·59	0·37	0·41
S_c	0·16	0·13	0·14	0·10	0·19	1·15	0·29	0·24	0·28	0·44
SD	5·95	3·56	4·58	7·36	3·01	0·85	0·14	1·87	0·15	0·69

Here N is the number of the cultures, $\overline{X}$ and $\overline{X}_c$ respectively correspond to the algebraic mean value of the mitotic indices of the exposed, control cultures, S_x and S_c are the standard deviation of the respective mitotic indices, and finally:

$$SD = \frac{\overline{X} - \overline{X}_c}{\sqrt{S_x^2 + S_c^2}}$$

is the index of significance. According to the usual biological practice, experiments where $SD > 2{\cdot}00$ is obtained are considered as having a significant difference[8].

It appears from Table 1 that the cultures exposed to the electrostatic field have in each case a larger mitotic number than the control specimens. 8-h exposures gave significant differences between the mitotic indices for all cultures but one. (The SD values vary from 3 to 7·4.) In the 4-h treatments, on the other hand, these differences were found to be insignificant (SD values varying from 0·1 to 1·9).

This preliminary investigation does not, of course, provide definite evidence for our hypothesis regarding multiplication of DNA. It may, however, be stated that the results obtained are at least not in contradiction with it.

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Incidence, Size and Shape of Meta-cortex Fibres in Wool

The presence of a basophilic, sulphur-deficient core in some wools has been reported[1,2], and the core has been called the meta-cortex to differentiate it from the ortho- and para-portions of the cortex. Recently, solvent-cleaned samples of several types of wool have been stained by being boiled for 15 min in methylene blue (0·05 g/l.) at pH 6·5, thoroughly rinsed, and afterwards cross-sectioned using a Hardy microtome. The fibre cross-sections were examined for the presence of a medulla, or of a stained meta-cortex, and their major and minor axes were measured using a projection microscope at a magnification of 500. For each sample approximately 500 fibres were examined. The percentages in each class are set out in Table 1.

Naturally, the samples cannot be taken by themselves as characteristic of the whole breed or type—in an

Table 1. Percentage of Medullated and Cored Fibres

Type	Ortho/para only	Meta-cortex fibres	Medullated fibres
South Australian merino 60's	97	3	—
Montevideo 60's	100	—	—
Australian 58's	100	—	—
Southdown 60's	84	16	—
Shropshire	95	5	—
Corriedale	98	2	—
New Zealand 56's	92	8	—
Romney 48's	91	2	7
Romney lamb	94	1·5	4·5
Wensleydale	79	21	(may include some medullated)
Lincoln	89	11	,,
Swaledale	85	15	,,
Cheviot	40	32	28

earlier investigation a much higher proportion of another Corriedale sample contained meta-cortex. However, the differences between the merino types, the Downs, the English Longwools and the mountain types are fairly clearly marked.

The shapes of the cross-sections, as expressed by the ratios of major and minor axes, also show some distinctions, as will be seen from Table 2.

Table 2. Ratio of Major/Minor Axes

Type	Ortho/para only	Meta-cortex fibres	Medullated fibres
South Australian merino 60's	1·21	1·17	—
Montevideo 60's	1·24	—	—
Australian 58's	1·29	—	—
Southdown 60's	1·30	1·53	—
Shropshire	1·33	1·44	—
Corriedale	1·27	1·12	—
New Zealand 56's	1·25	1·28	—
Romney 48's	1·25	1·31	1·41
Romney lamb	1·24	1·29	1·44
Wensleydale	1·20	1·54	—
Lincoln	1·18	1·18	—
Swaledale	1·24	1·29	not measured
Cheviot	1·30	1·48	1·63

In most cases the fibres containing a meta-cortex were more elliptical than those without. The high axial ratio of the Down types is notable, some Southdown fibres being markedly concave on one side. The remarkably circular cross-sections of most Longwool fibres are, of course, well known.

The products of the mean semi-axes are shown in Table 3 for the various types.

Table 3. Products of Mean Semi-Axes (μ^2)

Type	Ortho/para only	Meta-cortex fibres	Medullated fibres
South Australian merino 60's	172	194	—
Montevideo 60's	154	—	—
Australian 58's	210	—	—
Southdown 60's	256	342	—
Shropshire	316	440	—
Corriedale	194	263	—
New Zealand 56's	199	282	—
Romney 48's	418	608	687
Romney lamb	303	528	613
Wensleydale	330	420	—
Lincoln	462	752	—
Swaledale	253	536	—
Cheviot	310	496	652

The average area of cross-section of the cored fibres is, in every type, greater than the average of the uncored, and both the major and minor semi-axes are likewise bigger for the cored fibres than for the others. However, there does not appear to be a maximum value of size, above which the fibre must be cored or medullated, since the areas of cross-section of the Romney and Lincoln ortho/para fibres are greater than those of the meta-cortex fibres in the samples of Southdown, Corriedale and New Zealand 56's. The size and number of the fibres with a meta-cortex suggest that they might represent an appreciable fraction of the fibres grown in primary follicles, and an investigation of this might be desirable.

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CYTOLOGY

An Effect of Thyroxin on the Fine Structure of the Rat Liver Cell

We have recently observed a striking change in the fine structure of the liver cells of rats given large doses of thyroxin. Wistar rats (Alderley Park strain 1) were given thyroxin in their diet at levels of 0·001 or 0·005 per cent. Control animals were given unmedicated diet. After one, two or three weeks on the diet, groups of three test and three control animals were killed, small fragments of liver were fixed in buffered 1 per cent osmium tetroxide, and processed through 'Araldite'. Thin sections were cut and stained with lead citrate[1], and examined in the electron microscope.

The changes observed were fully developed in animals killed at one week and did not differ in animals killed later. They consist of a moderate increase in the numbers of

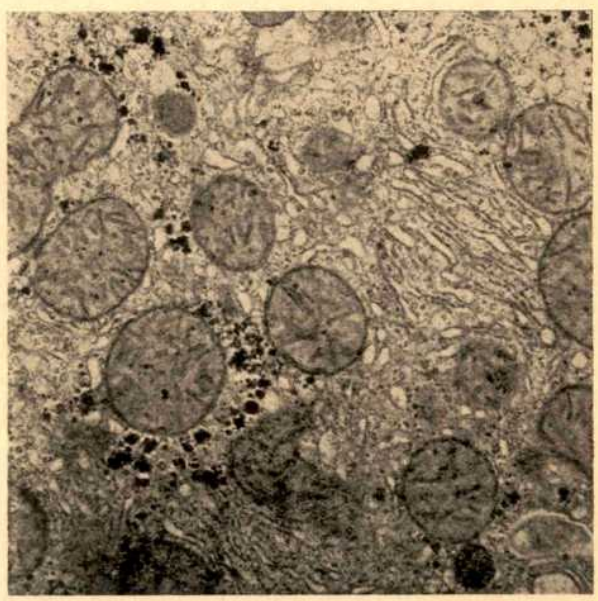


Fig. 1. Control rat. Group of mitochondria. (Lead citrate stain. × *c.* 9,000)

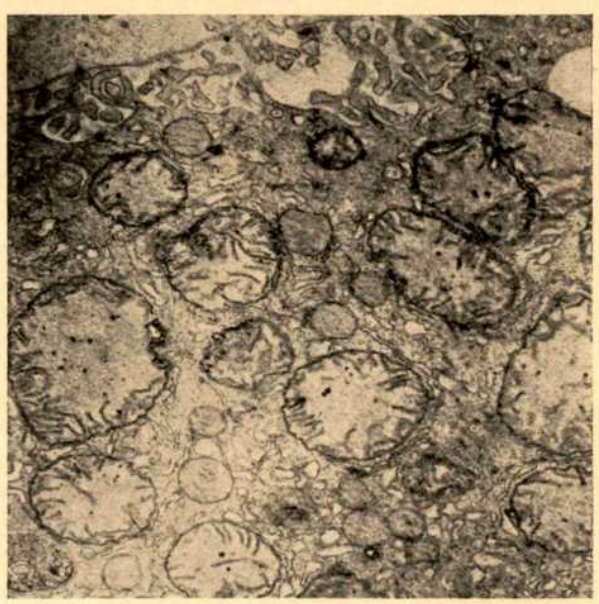


Fig. 2. Rat after 1 week thyroxin treatment. Group of mitochondria near sinusoidal margin. Note swelling and increase in number of cristæ mitochondriales. (Intra-mitochondrial granules are also more prominent than in the control.) (Lead citrate stain. × *c.* 9,000)

mitochondria, some swelling of these organelles, and a very marked increase in the number of cristæ mitochondriales found in each organelle (Figs. 1 and 2). In addition, a number of large mitochondria of bizarre shapes were observed.

Changes in the mitochondria of liver cells of animals given thyroxin have been observed by Schulz, Löw and Sjöstrand[2]. They noted swelling and vacuolation of the mitochondria only. However, since the mitochondria are the site of respiratory enzymes, and since thyroxin increases cell respiration, the observations reported here provide a morphological basis for the known biochemical action of the hormone.

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MICROBIOLOGY

Specificity of the Solute Requirement by Marine Bacteria on Primary Isolation from Sea-water

The partial replacement of sodium chloride in a culture medium for a marine bacterium by sucrose or potassium chloride was recently described[1]. The non-specific solute requirement was shown to control the rate of growth and under the conditions of experimentation was not concerned with osmotic fragility. Some previous workers found relatively little replacement of sodium salts by other solutes and concluded that the organisms being studied had little or no osmotic requirement[2,3]. In contrast, the solute requirements of the marine luminescent bacterium *Photobacterium fischeri*, both for growth and metabolism, were found to be non-specific and were considered to be primarily osmotic[4,5]. The various findings suggested the existence of species variation with respect to this property among marine bacteria. As a direct approach to a better understanding of this situation, samples of sea-water were plated directly on to media having either sodium chloride, potassium chloride, or sucrose as the principal solute. The purpose was to determine the proportion of cells in a natural inoculum which would allow a partial replacement of sodium chloride in the growth medium.

The basal medium contained 0·2 per cent trypticase ('BBL'), 0·001 M magnesium chloride, 0·005 M potassium sulphate, 0·0005 per cent ferrous sulphate, 1 per cent Oxoid 'Ionagar No. 2'. The medium was adjusted to *p*H 7 with a few drops of 2 M *tris* (hydroxymethyl) amino methane. Those media containing sucrose were supplemented with 0·2 per cent ammonium succinate to buffer the acidity possibly produced by some organisms. The solutes added are indicated in Table 1. Potassium chloride and sucrose were tested both in the presence and absence of a sub-optimal amount of sodium chloride to evaluate how frequently sodium ion could be demonstrated to be essential in a specific way to the growth of marine bacterial species. Three samples were taken from the Atlantic Ocean at Matanzas, Florida; for a comparison, one sample was taken from a small fresh-water pond. The plates were inoculated in triplicate with 0·1-ml. amounts; the inoculum was spread over the surface of the agar with a sterile bent glass rod. A one-hundred fold dilution of the pond water was used to give a comparable number of colonies. The cultures were incubated at 32° C and were observed at daily intervals through 72 h.

Table 1. Effect of Solutes on Colony Numbers obtained with Marine and Freshwater Samples

Addition to basal medium	Marine samples			Pond water
	A	*B*	*C*	
NaCl, 0·35 M	62	73	138	1
NaCl, 0·025 M	8	7	14	91
KCl, 0·35 M	5	9	25	2
KCl, 0·325 M; NaCl, 0·025 M	23	33	76	2
Sucrose, 0·5 M	15	14	35	5
Sucrose, 0·5 M; NaCl, 0·025 M	28	41	67	1
No addition	6	2	8	82

The values are the average number of colonies per plate; 0·1-ml. amounts of the marine samples were plated in triplicate; 0·1-ml. amounts of a 100-fold dilution of the pond water were plated in duplicate. The basal medium was 0·2 per cent trypticase, 0·001 M $MgCl_2$, 0·005 M K_2SO_4 and 0·0003 per cent $FeSO_4 \cdot 7H_2O$. The media were solidified with 1·0 per cent Oxoid 'Ionagar No. 2'.

The highest colony counts obtained from the marine samples occurred on the medium containing 0·35 M sodium chloride; this contrasted with the counts from the fresh-water sample where the highest counts were obtained on the media containing the lowest amounts of salt (Table 1). The minimal level of sodium chloride required by the majority of the marine bacteria was greater than 0·025 M, which was ineffective by itself; however, approximately half the maximal number of colonies grew in media containing, in addition to 0·025 M sodium chloride, either 0·32 M potassium chloride or 0·5 M sucrose. The latter solutes were much less effective in the absence of sodium chloride, indicating that for the majority of the organisms in the samples the sodium chloride could not be completely replaced. As compared with the basal medium, a larger number of colonies grew in that to which only sucrose was added; because of the presence of a complex nutrient like trypticase, this observation cannot be considered as evidence of growth in the absence of sodium. Replicate plates made using the original plates as templates indicated that most of the organisms which responded to partial replacement of sodium ions by potassium chloride responded in a similar way to sucrose; a few exceptions were observed which would accept only one or the other but not both.

The results support the view that variability occurs among marine bacteria with respect to the relative specificity of the requirement for sodium chloride and that approximately half the organisms studied would grow in media in which a substantial replacement of sodium chloride by sucrose or potassium chloride had been made.

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Effect of *Mycobacterium tuberculosis H37Rv* grown *in vivo* (*LH37Rv*) on Ehrlich Carcinoma Ascites Cells

Previous reports from this laboratory have shown that cells of *Mycobacterium tuberculosis H37Rv* separated from lungs of mice infected with this strain are coated with nicotinamide adenine nucleosidase (NADase) of the host[1]. Discussing the possible role of NADase adsorbed by the tubercle bacilli in the pathogenesis of tuberculosis, we have assumed that, on phagocytosis of tubercle bacilli, the nicotinamide adenine dinucleotide splitting enzyme may impair the electron transport system of the phagocyte, thus bringing about the death of the latter[2]. It should be recalled that a similar hypothesis was propounded by Bernheimer *et al.*[3], who found a remarkable correlation between the capacity of streptococci to produce extracellular NADase and leukotoxicity. The authors suggested that the leukotoxicity might well be due to the destruction of NAD of the phagocytizing cell by streptococcal

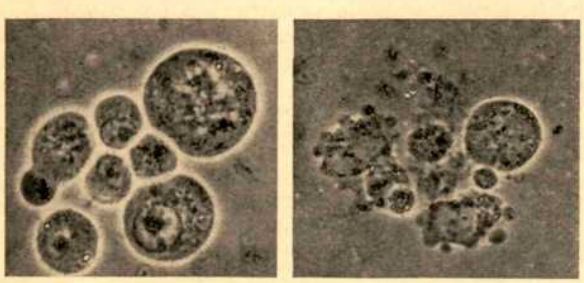
NADase. Since there is an indication in the literature that Ehrlich carcinoma ascites cells are capable of phagocytosis[4], it seemed of interest to examine the effect of tubercle bacilli grown *in vivo*, coated with NADase, on Ehrlich carcinoma ascites cells and to compare it with that of the tubercle bacilli grown *in vitro*. This communication is a preliminary report of these investigations.

Tubercle bacilli grown *in vivo* (*LH37Rv*) were obtained as previously described[5]. The *in vitro* grown bacteria, *M. tuberculosis H37Rv*, were obtained from 10–15 day cultures in albumin medium from which 'Tween-80' was omitted. The bacilli were collected by centrifugation, washed and homogenized in a 'Teflon' grinder. *H37Rv* and *LH37Rv* cells were suspended in 0·1 M phosphate buffer, *p*H 7·2 to which bovine albumin fraction V (Armour Pharmaceutical Co.) was added to 0·6 per cent final concentration. Proliferation of contaminants was prevented by addition of dihydrostreptomycin sulphate (100 μg/ml.) and penicillin (100 units/ml.). During the experiments, the bacterial suspensions were stored in the cold (2° C). The experiments were conducted on groups of 5–8 Swiss albino mice weighing 20–22 g. These were injected intra-abdominally with $1{\cdot}5 \times 10^6$ Ehrlich carcinoma ascites cells in 0·2 ml. of 0·1 M phosphate buffer, *p*H 7·2. Twenty-four hours afterwards the groups of mice were injected intra-abdominally with 0·2 ml. of 0·2 mg dry weight of tubercle bacilli grown *in vivo* and *in vitro*. In some experiments, the mice were given a second injection of tubercle bacilli, three to four days after inoculation with ascites cells. In order to prevent proliferation of the injected tubercle bacilli, dihydrostreptomycin sulphate (2 mg/mouse) was administered every second day subcutaneously. The control mice were also given the suspending medium and antibiotic treatment. Seven to eight days after the beginning of the experiment the mice were killed and the tumour cells collected from the peritoneal cavity by careful washing with a balanced salt solution containing heparin (3 units/ml.). The cells were counted in a counting chamber and examined by phase contrast microscopy.

Table 1. Effect of Tubercle Bacilli grown *in vivo* and *in vitro* on Ehrlich Carcinoma Ascites Cells (EAC)

Mouse No.	No. of EAC ($\times 10^6$) recovered from the peritoneal cavities of mice injected with				
	Tubercle bacilli grown *in vivo* (*LH37Rv*)		Tubercle bacilli grown *in vitro* (*H37Rv*)		Control
	One injection	Two injections	One injection	Two injections	
1	1·12	0·06	0·95	0·63	4·10
2	0·29	0·02	3·35	2·90	2·50
3	0·08	0·16	3·09	0·06	1·60
4	0·02	1·00	3·00	0·70	3·70
5	0·72	0·06	0·69	2·10	2·80
6		0·17		1·50	1·30
Mean	0·45	0·25	2·20	1·30	2·66
Reduction in the No. of EAC (%)	83·1	90·5	17·4	51·3	

The results of a typical experiment are presented in Table 1. It can be seen from the table that injection of tubercle bacilli grown *in vivo* into mice bearing Ehrlich carcinoma ascites cells led to a marked reduction in the tumour cells, amounting to 83 per cent after a single injection and to 91 per cent after two injections. Microscopic examination of the remaining cells showed a high percentage of disintegrated cells. Vacuolation of the cellular cytoplasm was clearly defined and the nuclear membrane dissolved (Fig. 1). On the other hand, injection of tubercle bacilli grown *in vitro* had a considerably smaller effect on the tumour cells. The reduction in the number of tumour cells after one injection amounted to 17 per cent and after two injections to 51 per cent.



a *b*

Fig. 1. (*a*) Ascites cells from a control mouse; (*b*) ascites cells from a mouse treated with *LH37Rv*

Although the results of these experiments are in keeping with our hypothesis on the role of NADase in phagocytosis, we have not yet a direct confirmatory evidence for the tubercle bacilli entry into the ascites tumour cells. The mechanism by which the tubercle bacilli grown *in vivo* exert their effect on the Ehrlich carcinoma ascites cells is now being examined.

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VIROLOGY

Murine Infection with Reovirus Type 3 and the Runting Syndrome

The purpose of this communication is to direct attention to the similarity, in clinical and pathological findings, of infant mice infected with reovirus type 3 and infant mice which develop as runts following the inoculation of immunologically competent lymphoid cells.

An examination of the literature on runt disease reveals a clinical and pathological picture of great variability. A general pattern of agreement is emerging and it is apparent that variations may be ascribed to cell dosage, route of inoculation, time of inoculation, source of lymphoid cells, species of animal, and the genetic constitution of host and donor. In mice the following features have been stressed: retardation of growth, an ataxic gait, diarrhœa, hunched posture, facial œdema, oily hair effect, jaundice, and alopecia[1]. Up to 90 per cent of mice may show focal, necrotic, coagulative liver lesions[1]. Some investigators report that the thymus may appear normal in size and appearance[2]. Although hæmolytic anæmia with a positive Coombs's test, leucopænia and splenomegaly have frequently been observed, these are not an invariable accompaniment of runting[2].

The clinical and pathological features of reovirus type[3] infection of mice have been described in some detail[3–5] and closely resemble the runting syndrome. The clinical syndrome of reovirus infection has only been demonstrated in infant mice less than five days of age and usually comprises retardation of growth, oily hair effect, diarrhœa, alopecia, jaundice, an ataxic mincing gait, and hunched posture. Although all mice show focal necrotic, coagulative liver lesions, the thymus appears normal[5]. There is, also, following intraperitoneal inoculation, a peritoneal ascites containing large numbers of primitive lymphoid cells.

These similarities may be considered alongside the observations that (1) polyoma virus may cause runting and anæmia[6]; (2) mouse hepatitis viruses have been associated with runting[7]; (3) cytomegalic inclusion

disease virus infection of infants may result in sequelæ which have been confused with erythroblastosis fœtalis[8]; (4) reovirus infections of humans appear to be mainly in infants; (5) the reovirus-induced hepatic lesions closely resemble those seen in immunological liver injury.

It would thus appear that further exploration of virus infection of the immunologically immature should be made to determine whether such infection will permit the establishment of a clone of cells which can react against the host. Thoughts have already been expressed[9] as to the possible mechanisms involved following the induction of various runting syndromes in mice.

In addition, the possibility that the lymphoid cells may be a source of mouse viruses cannot be entirely excluded.

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Incorporation of 5-Iodo-2′-deoxyuridine into the Deoxyribonucleic Acid of Vaccinia Virus

The *in vitro* development of animal viruses containing deoxyribonucleic acid, such as vaccinia or herpes simplex, is inhibited by the thymidine analogue 5-iodo-2′-deoxyuridine (IUdR), whereas RNA-containing viruses are not affected[1]. This reagent has also been used successfully for the *in vivo* treatment of herpes[2] and vaccinia[3], and on tumours induced in hamsters by human adenovirus[4]. The mechanism of viral inhibition by IUdR is under investigation in these laboratories. Previous investigations[5] suggest that one possible mechanism may be that replacement of thymidine by IUdR in viral DNA renders the virus non-infective. It has been shown that IUdR has no direct inactivating effect on vaccinia virus particles *in vitro* (unpublished results). This communication presents evidence for the incorporation of IUdR into the DNA of vaccinia virus.

IUdR labelled with iodine-125 was synthesized by the method described previously[6] and had a specific activity of $3{\cdot}8 \times 10^5$ c.p.m./μmole. All radioactive measurements were made using a well-type γ scintillation counter.

Labelled vaccinia virus was prepared as follows. The chorioallantoic membranes of 12-day chick embryos were dropped with 0·1 ml. of antibiotic saline (0·9 per cent sodium chloride, M/100 phosphate buffer, *p*H 7·2; 1,000 units of penicillin and 0·1 mg of streptomycin/ml.). Four mg of IUdR-I^{125}, suspended in 5 per cent gelatin at a concentration of 10 mg/ml., were dropped on to each chorioallantois. The eggs were rocked on various axes to spread the reagent as evenly as possible. Previous experiment had shown that this amount of IUdR reduced the average number of viral pocks by 50–80 per cent, but had no effect on the viability of the embryos. Approximately 40 min later, each egg was inoculated with 1,000 pock-forming units of the fully egg-adapted Levaditi strain of vaccinia virus. At the same time, control virus was inoculated into eggs that had received antibiotic saline instead of IUdR. The chorioallantoic membranes were removed from treated and control eggs after incubation for 40 h at 37°, and washed in large volumes of antibiotic saline. It was observed that membranes treated with IUdR showed an average of approximately 100 very small, faint pocks, whereas control virus membranes were uniformly covered with typical confluent hæmorrhagic pocks.

The virus was extracted and purified from IUdR-treated, and from control membranes, by a modification of the fluorocarbon method described by Epstein[7]. The final supernatant fluids were strongly opalescent, and examination in the electron microscope (tungsten shadowing) revealed virus particles only. In several experiments a small proportion of flat ovoid structures, of similar dimensions to normal vaccinia particles, was seen in IUdR-treated virus preparations. These structures appeared devoid of internal nucleoid. The concentration of purified virus particles from IUdR-treated membranes was approximately 10 per cent of that from an equal number of control membranes. The infectivity titres of the purified IUdR and control virus samples were $3{\cdot}5 \times 10^6$ and $4{\cdot}3 \times 10^7$ pock-forming units/ml., respectively. Both samples gave rise to typical vaccinia pocks.

The purified IUdR-treated virus was then washed three times by centrifugation with large volumes of antibiotic saline to remove the relatively large amounts of RNA that are present after fluorocarbon treatment. The final washing contained no detectable radioactivity or material absorbing at 260 mμ. A small proportion (0·01 per cent) of the radioactivity found in the unwashed virus preparation was associated with the final viral pellet, and this proportion remained constant on further washing. The washed pellet was resuspended in absolute ethanol for several days at 4°, deposited by centrifugation, extracted with 1 : 1 ethanol-ether, and dried *in vacuo*. The viral pellet was then resuspended in 'Marmur' citrate-saline containing 2 per cent 2-mercaptoethanol and stirred for 20 h at 4°. The suspension was stirred for 6 h at 40° with pronase, and the DNA extraction continued as described previously[8]. The first two extracts contained 98 per cent of the original radioactivity, whereas only 2 per cent remained in the deposited viral 'debris'. Previous experience has shown that 90–95 per cent of vaccinia DNA is released in these extracts[8].

The two DNA extracts were pooled and purified further by density-gradient centrifugation. Sufficient cæsium chloride was added to bring the density to 1·70 g/ml., and the mixture centrifuged for 48 h at 38,000 r.p.m. in the Spinco *SW* 39 head. Fractions were collected by dripping from the bottom of the tube, and the radioactivity of each sample (10 drops) was determined. In a total of five separate runs, the radioactivity was banded at density levels of 1·71–1·73 g/ml. These bands were considerably broader than were those obtained with normal vaccinia DNA, 90 per cent of which bands sharply at a density of 1·71 g/ml.[9]. It is possible that incorporation of the base analogue in varying amounts causes density heterogeneity in the viral DNA. It was observed that counts of the γ-radiation derived from iodine-125 are reduced by about 80 per cent in solutions of cæsium chloride of this density.

Fractions obtained from the density-gradient centrifugation that showed high radioactivity were pooled, dialysed to remove cæsium chloride, and the presence of DNA was confirmed by a positive indole reaction. The ratio of the ultra-violet absorption (260/280 mμ) of this preparation of DNA was 1·747. The specific activity of the DNA was $5{\cdot}4 \times 10^4$ c.p.m./μmole of mono-nucleotide.

That IUdR was indeed a component of the DNA polymer was established by the following procedure. The radioactive DNA plus added non-radioactive DNA were hydrolysed to mono-nucleotides[10] and separated by ion exchange chromatography[11]. The eluates were examined for ultra-violet absorption at 260 mμ in a Beckman spectrophotometer, and for γ-radiation in a well-type scintillation counter. The peak of the radioactivity was found in an area known to be associated with 5-iodo-2′-deoxyuridylic acid and overlapping with deoxyguanylic

acid. The mixture of these two nucleotides was adsorbed on a column of 'Dowex-1' (chloride form), eluted with hydrochloric acid gradient, lyophilized, dissolved in water, and subjected to paper chromatography (Whatman No. 1; solvent, *n*-butanol : acetic acid : water, 5 : 2 : 3). The R_F of the radioactive area coincided with a known preparation of 5-iodo-2′-deoxyuridylic acid (R_F 0·16) and was separated from deoxyguanylic acid (R_F 0·05). Thus, it was established that the radioactivity derived from IUdR-^{125}I was present in the DNA-polymer and in nucleotide form.

It has been shown that IUdR is incorporated into the DNA of mammalian, bacterial and plant cells and that it specifically replaces the thymidine component. It was concluded from the present experiments that IUdR was incorporated into vaccinia virus DNA, and that 18 per cent of the viral DNA-thymidine was replaced by the analogue. Base ratios and extinction values for vaccinia virus DNA used in the calculations have been reported previously[12]. The significance of the observed incorporation of IUdR into the DNA of vaccinia virus in relation to the anti-viral activity of this analogue remains to be elucidated.

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SOIL SCIENCE

Age of the Youngest Hydrated Halloysite in Kyushu

It has been well established that allophane formed from volcanic ash is followed by crystallization to hydrated halloysite in some circumstances. How many years are needed for the formation of hydrated halloysite in Nature?

A buried surface soil containing hydrated halloysite would offer a suitable sample for dating the mineral by means of radiocarbon measurements, if the soil contains enough organic carbon and has been isolated from biotic activity since the soil was covered with ash. Simultaneously with weathering of volcanic ash, organic matter will start to accumulate in the surface layer of the ash soon after deposition. This accumulation of organic matter would be stopped by any thick cover of ash that later fell, but the weathering of ash would still go on. So the age of volcanic ash in a previous surface layer can be estimated by the content of carbon-14 in the organic matter, provided that the period of time of carbon accumulation is negligibly short as compared with the ash-age.

In this respect, the mantle of volcanic ash on Aso Volcano in Kyushu Island, laid down as a series of showers since the later period of Pleistocene, would offer suitable samples for investigation. The ash-mantle is usually several metres thick, often including former surface soil layers that are easily distinguishable from over- and underlain layers by their high humus content. Clays of upper layers of a profile, less weathered ones, are mostly composed of allophane without kaolin, while those of lower layers, more weathered ones, tend to contain predominant hydrated halloysite with more or less allophane. If the latter layers contain organic matter, such as remains of plants from those days when the layers were on a surface, they would qualify as samples.

Examined samples were collected from a profile which is located on a gentle slope at the western foot of the central cone of Mt. Aso (Choyo, 32° 53′ N., 131° 0′ E.). The profile is about 8 m thick, and 17 layers at least were discernible[1]. X-ray and differential thermal analyses revealed that four upper layers contain predominantly allophane in the clay fraction but with no kaolin, and that the fifth layer (depth: 140–190 cm) is the uppermost one containing hydrated halloysite, the content of which was estimated to be about 33 per cent of the clay fraction (-2μ) by allocation of chemical and dehydration data and cation-exchange capacity delta-values to its constituent minerals (Table 1). This hydrated halloysite is evidently youngest in the Aso district, and probably in Kyushu. The mineral shows similar X-ray diffractive, differential thermal, and electron microscopic properties to the hydrated halloysite derived from volcanic ash and pumice already reported by several workers. That is, the mineral exhibits spherules or curled laths in shape and a low crystallinity. The organic carbon of this layer was calculated to be 8,650 ± 200 years old by measurements of radiocarbon by Prof. Kigoshi at Gakushuin University in Tokyo. This value undoubtedly represents an average of organic carbon accumulated in the layer while it was on the surface.

Table 1

Location	Horizon	Depth (cm)	Organic carbon (%)	In clay fraction: Allophane (%)	In clay fraction: Hydrated halloysite (%)
Choyo	1	0–30	8·2	67	0
	2	30–80	2·4	75	0
	3	80–90	1·2	73	0
	4	90–140	1·7	68	0
	5	140–190	11·0	37	33
Kanoya	1	0–30	5·6	70	0
	2	30–70	12·1	72	0
	3	70–130	1·8	80	0
	4	130–175	7·1	74	0
	5	175–225	2·5	75	0
	6	225–275	4·0	30	35

The period of the carbon accumulation could be roughly estimated from the carbon content of the layer. Since the bulk density of this layer is 0·6, its organic carbon amounts to 330 tons/hectare. Assuming that the carbon was accumulated at 1 ton/hectare/year and that no loss of carbon occurred following annihilation of vegetation due to the ash-cover, the flourishing period of vegetation comes to 330 years. Although the second assumption is not the case in a strict meaning, the decomposition of humus in buried Ando soils is undoubtedly extremely slow. It would be difficult to settle the rate of carbon accumulation in ancient days. However, there is some evidence that 2 tons carbon/hectare/year or even more could be accumulated in volcanic ash soils[2], and consequently it might be probable that 330 tons carbon was accumulated in a hectare even in a shorter time than 330 years. According to the foregoing, it would be concluded that the hydrated halloysite of the fifth layer was derived from the ash that fell between 8,000 and 9,000 years ago, and is the youngest in the Aso district.

In comparison with this, another volcanic ash profile was examined in the same way. The profile is located on the Kasanohara tableland (Kanoya, 31° 24′ N., 130° 52′ E.). Although it has little connexion with the ash of Aso Volcano, origins of ashes and pumices consisting of the profile are still obscure. Three former surface layers are observed in the profile, that is, 2nd, 4th, and 6th in Table 1.

Hydrated halloysite was not found as far as the fifth layer (depth: 225–275 cm), and the sixth layer, the third former surface layer, was the uppermost one containing the mineral. Namely, the hydrated halloysite of this layer is youngest in the profile at Kanoya. However, this layer is beyond the limits of carbon-14 dating, that is, older than 30,000 years. The particles of the mineral are not so different in shape from those of Choyo, and show spherules, curled laths, and short tubes. The particles of Choyo are mostly composed of spherules, while tubes are dominant in the Kanoya mineral. This might explain the metamorphosis of spherules into tubes. Kuwano *et al.*[3] considered that the layer belongs to the lower loam of the Pleistocene Epoch. This would mean that it is about 30,000 years old or more and is consistent with the radiocarbon measurements.

We wish to thank Prof. Kunihiko Kogoshi for dating humus.

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PSYCHOLOGY

Ipsilateral Confusion in 2-Choice and 4-Choice Responses with the Hands and Feet

The experiments to be described here suggest that confusion occurs between ipsilateral responses in certain choice-reaction tasks. This finding has apparently not been reported before.

In the first of the present 4-choice experiments, the subject responded to a positional display consisting of four neon indicator bulbs set in a black screen so as to form a square 4 in. × 4 in. The subject sat facing the screen, and responded by pressing morse keys with the hands and feet. Details of the apparatus will be given in a later communication. The required response to the top right signal was the right hand, to the bottom right signal the right foot, and similarly on the left side. 45 subjects each responded to 80 signals, 20 for each foot and each hand, presented in a random order. 513 errors were made, giving a total error rate of 14·25 per cent. The distribution of these errors is given in the matrix in Table 1. 98·1 per cent of the errors were ipsilateral (for example, right-hand responding to right foot signal, left foot responding to left-hand signal, etc.). There were no diagonally opposite errors (for example, left foot responding to right-hand signal, etc.).

Two further 4-choice experiments were afterwards carried out with slightly altered displays, one with the signals in the form of a square 1¼ in. × 1¼ in., and the other with them in the form of a rectangle, 4 in. (vertical dimension) × 1¼ in. In each experiment 13 subjects each responded to 800 signals, 200 for each foot and each hand, presented in a random order. Results were obtained similar to those of the first experiment. With the small square display, in the first half of the experiment (that is, the first 400 responses for each subject), 97·9 per cent of the errors were ipsilateral (total error rate 11·0 per cent), and in the second half 97·1 per cent were ipsilateral (total error rate 11·3 per cent). With the rectangular display, in the first half 97·6 per cent of the errors were ipsilateral (total error rate 9·6 per cent), and in the second half 85·3 per cent were ipsilateral (total error rate 11·9 per cent).

Table 1. Errors made in Four-Choice Reaction Task with Hands and Feet

		Errors			
		RH	LH	RF	LF
Correct Response	RH		2 (2) 0·2%	105 (42) 11·7%	0
	LH	4 (4) 0·4%		0	139 (42) 15·4%
	RF	116 (38) 12·9%	0		2 (2) 0·2%
	LF	0	143 (42) 15·9%	2 (2) 0·2%	

Down the side of the matrix is indicated the correct response. The columns across show the number of errors made to each signal by each of the other limbs. The figures in parentheses give the number of subjects contributing to the total ($N = 45$). Errors are also expressed as percentages of the total number of responses to each signal.

The same predominance of ipsilateral errors occurred again in a further 4-choice experiment, this time with tactile stimuli. The response movements were the same as in the three previous experiments, and tactile stimulation was provided by screws attached by an adjustable extension to the plunger of a solenoid. 12 subjects each responded to 800 signals, as in the second and third experiments. In the first half of the experiment 98·7 per cent of the errors were ipsilateral (total error rate 8·3 per cent); in the second half 85·5 per cent were ipsilateral (total error rate 6·2 per cent).

Two 2-choice experiments were carried out with the original square display (4 in. × 4 in.). Each was in three parts, with the two responses being either ipsilateral, contralateral or diagonally opposite. In the first, one of the two responses was always the right hand, the alternative being the right foot, left hand, or left foot, respectively. In the second, one of the two responses was the left foot and the alternative was the left hand, right foot, or right hand, respectively.

In each part of each experiment six subjects each responded to 300 signals over three sessions. In each session the three parts were presented in a different order. The mean reaction times for both the right hand and the left foot were least when the alternative response was the contralateral limb, and greatest when it was the ipsilateral (Table 2). In both cases analysis of variance showed that the differences were significant ($D < 0·01$). The number of errors was also greatest when the alternative response was ipsilateral.

Table 2. Variation in Two-Choice Reaction Times according to Alternative Response

	Alternative response		
	Contralateral	Diagonally opposite	Ipsilateral
Exp. 1	(LH)	(LF)	(RF)
Group mean right hand RT (ms)	281	290	316
Mean σ	38	42	**51**
Total errors	14	10	34
Exp. 2	(RF)	(RH)	(LH)
Group mean left foot RT (ms)	301	320	346
Mean σ	40	45	60
Total errors	18	22	56

These findings indicate confusion between ipsilateral hand and foot responses, leading either to errors between them, or to a lengthened reaction time when distinction between them is required. In the only previous experiment on reaction times that appears to be relevant, Davis[1] found no difference in the mean 2-choice reaction time between a situation in which the responses were made by the two hands, and one in which they were made by two fingers on one hand. This gives a warning against generalizing from the present findings to the conclusion that all ipsilateral responses are liable to be confused.

This work was carried out with the help of a research studentship from the Department of Scientific and Industrial Research. I thank Dr. J. A. Leonard, of the Medical Research Council's Applied Psychology Research Unit at Cambridge, for his advice.

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FORTHCOMING EVENTS

(Meeting marked with an asterisk is open to the public)

Tuesday, October 1

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5·30 p.m.—Mr. J. H. Rushton: "Fluid Mixing in Theory and Practice".

Wednesday, October 2

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. S. A. W. Thompson, Mr. S. R. Hills, Mr. M. P. H. Brown and Mr. A. Lewis: "Corrosion of Silver by Aviation Turbine Fuels".

BRITISH INSTITUTION OF RADIO ENGINEERS, MEDICAL ELECTRONICS GROUP (joint meeting with the INSTITUTION OF ELECTRICAL ENGINEERS, at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Electronic Equipment for Medical Research—Build or Buy" opened by Mr. W. J. Perkins and Dr. G. H. Byford.

Thursday, October 3

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Dr. B. Cinader (University of Toronto): "Some Aspects of the Interaction between Enzyme and Antibody"*.

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 7 p.m.—Mr. J. W. Kirk: "Flash Radiography—a Potential Tool for Metallurgy"; Mr. J. C. Needham and Mr. A. A. Smith: "High-Speed Photography of Metal Transfer in Welding".

Friday, October 4

BRITISH MYCOLOGICAL SOCIETY (at The School of Pharmacy, Brunswick Square, London, W.C.1), at 11 a.m.—Scientific Papers.

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "How Should Engineering Education Take Account of Developments in the Newer Materials?—Item 2, Ceramics".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. D. H. R. Barton, F.R.S.: "Photo-Chemical Synthesis".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER or LECTURER IN PURE MATHEMATICS—The Registrar, The University, Hull (October 5).

ASSISTANT LECTURER or LECTURER (with medical qualifications or a degree in physiology) IN PHYSIOLOGY at the University of the West Indies—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (October 7).

LECTURER IN PURE MATHEMATICS—The Secretary, The University, Edinburgh (October 7).

RESEARCH FELLOW (with a good degree, or equivalent, in any branch of science or engineering; a logical outlook, some knowledge of statistics; and preferably research experience) IN INFORMATION SCIENCE IN THE DEPARTMENT OF MANAGEMENT AND SOCIAL SCIENCES, for a four-year investigation of principles of information storage and retrieval—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (October 7).

RESEARCH ASSISTANT (graduate in microbiology or biochemistry, preferably with research experience) IN THE DEPARTMENT OF BOTANY AND MICROBIOLOGY, to work on certain aspects of petroleum microbiology—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (October 11).

CHAIR OF PURE MATHEMATICS—The Registrar, The University, Manchester 13 (October 12).

CHAIR OF PHARMACOLOGY—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne 2 (October 12).

LECTURER or ASSISTANT LECTURER IN CIVIL ENGINEERING—The Registrar, The University, Nottingham (October 12).

LECTURER or ASSISTANT IN PSYCHOLOGY at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (October 12).

ASSISTANT LECTURER (preferably honours graduate in zoology or physiology with suitable postgraduate experience) IN ANATOMY AND HISTOLOGY—The Registrar, The University, Nottingham (October 15).

CHAIR OF MATHEMATICAL STATISTICS—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (October 15).

DEMONSTRATOR (preferably honours graduate in animal physiology or animal husbandry) IN ANIMAL SCIENCE—The Registrar, The University, Nottingham (October 15).

RESEARCH FELLOW IN THE DEPARTMENT OF ELECTRON PHYSICS, to study the Hall Effect in compound semi-conductors above the melting point—The Assistant Registrar (Science), The University, Birmingham 15 (October 15).

ENTOMOLOGIST (with an honours degree in zoology, and preferably previous experience in research on pests of tropical crops), to undertake research on the insect pest problems in coffee throughout Kenya—The Senior Research Officer, Coffee Research Station, P.O. Box 4, Ruiru, Kenya (October 19).

LECTURER (with a degree in science or agricultural science and qualified in biometry, agricultural statistics or mathematical statistics) IN BIOMETRY at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 25).

SENIOR LECTURER IN PHARMACOLOGY at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 25).

ASSISTANT (honours graduate in physics or engineering) IN EXPERIMENTAL PHYSICS, to undertake teaching duties and assist in the planning of a 100-MeV electron linear accelerator facility for nuclear physics research—The Secretary of University Court, The University, Glasgow (October 31).

LECTURER IN PHILOSOPHY at Victoria University of Wellington, New Zealand, The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 31).

LECTURERS or ASSISTANT LECTURERS IN ZOOLOGY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 31).

LECTURERS (2) (with an honours degree in geography or equivalent qualifications) IN GEOGRAPHY at the University of Melbourne, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

SENIOR LECTURER IN PHARMACOLOGY IN THE DEPARTMENT OF PHYSIOLOGY in the Faculty of Medicine, University of Natal/National Provincial Administration—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 31).

SENIOR LECTURER/LECTURER (with an honours degree in physics and evidence of research ability) IN THE SCHOOL OF PHYSICS, University of New South Wales—The Agent-General for New South Wales, 56/57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (October 31).

SENIOR LECTURER IN BACTERIOLOGY at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 1).

CHAIR OF BIOCHEMISTRY at the Royal Free Hospital School of Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 11).

LECTURER and an ASSISTANT LECTURER (with research interests in the high energy physics field) IN THE DEPARTMENT OF PHYSICS in the Faculty of Science—The Registrar, The University, Manchester 13 (November 15).

SENIOR LECTURER IN STATISTICS at the University of Melbourne, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

ASSISTANT PROFESSOR (with postgraduate education equivalent to the Ph.D.) of ANIMAL NUTRITION—Head, Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada (December 15).

ASSISTANT Lecturer (graduate in microbiology)—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

ECOLOGIST (male, between 23 and 35 years of age, with a good honours degree in botany and two years postgraduate experience in ecology) in Western Nigeria, for ecological surveys and assessment of land potential—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S.W.1, quoting Ref. RC. 213/106/01.

HEAD OF THE DEPARTMENT OF ENGINEERING (Grade II)—The Principal, North Devon Technical College and College of Further Education, Sticklepath, Barnstaple, North Devon.

ISOTOPE RADIOGRAPHER/TECHNICIAN (preferably qualified radiographer or candidate with experience of radiochemical laboratories) IN THE ISOTOPE SECTION OF THE PHYSICS DEPARTMENT, to assist with the clinical and laboratory applications of radioactive isotopes to medical problems—The House Governor, The London Hospital, Whitechapel, London, E.1.

PHYSICIST (preferably with experience in radiation dosimetry and/or work with linear accelerators), to join a team (under Prof. J. Rotblat) carrying out research on the effects of radiation—The Secretary, Physics Department, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, E.C.1, quoting Ref. No. AC/288.

POST-DOCTORAL FELLOW, for work in the field of molten salt electrochemistry—Head, Department of Pure and Applied Chemistry, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

RESEARCH ASSISTANT (with a general degree or G.C.E. "A" level in chemistry and/or laboratory experience of analytical biochemistry) AT THE CHESTER BEATTY RESEARCH INSTITUTE, Fulham Road, S.W.3, for work on biochemical characterization of human leukæmia—The Secretary, Institute of Cancer Research, 34 Sumner Place, London, S.W.7, quoting Ref. 301/B/136.

RESEARCH FELLOW (registered medical practitioner or science graduate with an interest in the behaviour and sampling of air-borne particles) IN THE DEPARTMENT OF BACTERIOLOGY AND SURGERY, to work on problems connected with hospital cross-infection—Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. AC/303.

REPORTS and other PUBLICATIONS

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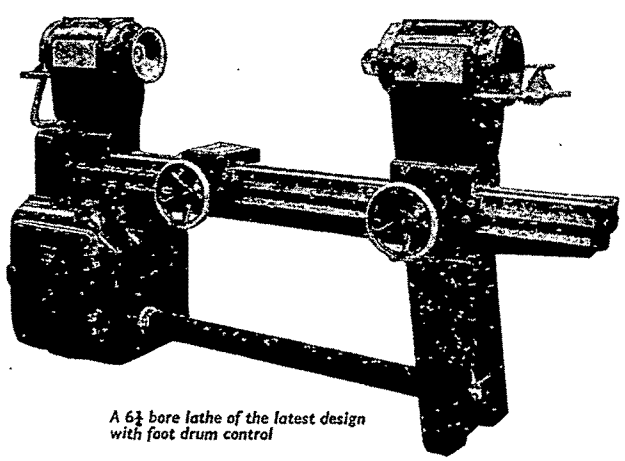
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